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Abstract approved [redacted]
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Abundant conidial production occurs from colonized straws and plants infected with Cercosporella herpotrichoides Fron during the cool moist months of the growing season. Experiments were designed to study the survival, inoculum potential and competitive saprophytic ability of conidia in soil.

Laboratory results indicated that cool moist soil favors the saprophytic survival of the fungus in soil. Viable hyphae were observed 16 months after conidia were buried in soil held at 5° and 10°C. Ammonium nitrate inhibited germination and survival of conidia in soil. It also inhibited germination on glass slides not in contact with soil. Ammonium ions were more inhibitory than nitrate ions. Glucose did not influence germination but stimulated saprophytic development and survival of the pathogen in soil.

A marked reduction in survival of C. herpotrichoides after one year occurred in naturally and artificially colonized straws buried

in the soil. Survival was favored in straws placed at the soil surface.

Straws buried in conidial infested soil and incubated in the laboratory or the field were colonized by the pathogen. Colonization was directly related to the inoculum density of the soil and inversely related to time.

Foot rot lesions developed below the soil surface on plants grown in conidial infested soil. Lesion incidence was directly related to the inoculum density of the soil. Based on mathematical models it was concluded that conidia were influenced by host exudates only at the host surface and a rhizosphere was not operative. The fungus was able to grow up to 10 mm from a food base in the soil and cause below ground lesions. Lesions below the soil surface were observed on plants growing under natural field conditions. It is suggested these below ground infections play an important part in maintaining inoculum levels in fields during years not favorable for foot rot development.

In growth chamber studies, plants developed lesions from single conidium inoculations. Disease development on plants in the growth chamber was influenced by the general susceptibility of the inoculated tissue, the stage of growth when inoculated, the rate of plant growth and the openness of the crown. Green tissues were more resistant to infection than senescent tissues. Infection occurring before the onset of tillering can result in lesion development on each

new tiller produced. Plants growing rapidly in a high nitrogen media were able to slough off infections and escape the disease. Tight crowned plants tended to have a majority of their tillers infected while healthy culms were associated with diseased ones in plants having wide spreading crowns.

Successful penetration and infection of a coleoptile or leaf sheath did not insure disease development. The infected tissues may become separated from the main stem before the fungus has penetrated into adjacent leaf sheaths, thus isolating the pathogen, so that the majority of the plant tissue escapes infection.

The infection process failed at one of three stages on green "resistant" tissues of coleoptiles and leaf sheaths: (1) germination failed to occur, (2) germination occurred but the pathogen failed to attempt penetration, and (3) penetration was attempted but failed. Thickening of host cell walls was associated with the failure of attempted penetrations. Thickened host walls were also associated with containment of the pathogen at the perifery of lesions. These thickened cell walls usually stained heavily with cotton blue and were never associated with senescent tissue.

Results indicate that stubble mulching and deep seeding would favor foot rot development. Chemicals or varieties delaying senescence of leaf sheaths would be expected to reduce foot rot injury.

# Etiological Studies on Foot Rot of Wheat Caused by *Cercosporella herpotrichoides* Fron

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TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ETIOLOGICAL STUDIES ON FOOT ROT OF WHEAT CAUSED BY *CERCOSPORELLA HERPOTRICHOIDES* FRON

## INTRODUCTION

The one and one half million acres of cropland in Oregon's Columbia Basin dryland area annually produces about 22 million bushels of wheat. Production is limited primarily to the soft, white varieties used chiefly for pastries. This non-irrigated wheatland extends from the Cascade Mountains on the west to the Blue Mountains on the east and south east. The Columbia River borders the north. Average annual precipitation over much of the area ranges from 10 to 20 inches, occurring between October and April. Wheat is planted every other year on land which is alternately left to summer fallow.

Soil moisture is the factor most limiting wheat production in eastern Oregon. Since this is non-irrigated cropland, man has little control in maintaining sufficient soil moisture to produce maximum yields. Adequate nitrogen levels, which may also be a limiting factor, can be economically maintained by application of nitrogenous fertilizers. Disease is another factor limiting wheat production in the Columbia Basin which may be controlled by man. Foot rot, also known as Columbia Basin foot rot, eyespot and straw breaker is a soil-borne disease of winter cereals. The causal agent is the fungus *Cercosporella herpotrichoides* Fron. A practical and economic control of this disease is not yet known. Sprague (1948) estimated root

rots account for annual losses of four percent in the Northwest, but emphasized that damage by Cercosporella foot rot can be the most destructive. Losses of 80 to 100 percent have been reported for fields in Germany (Neururer, 1961).

Only limited research has been directed toward this disease in the Pacific Northwest in the last 30 years. Sprague's original disease description and research (1931 to 1937) are the only literature citations from this area from 1931 to 1964. During this period investigators in other parts of the world have studied the disease, a majority of the work being done in Europe. Several papers have also appeared from other sections of the United States and from New Zealand.

In order to develop an effective control in the Columbia Basin, basic information concerning survival, infection and disease development is needed. With this need in mind the Oregon Wheat Commission financially supported a three year basic study to determine some of the factors affecting the disease potential of C. herpotrichoides. This investigation is the result of that research grant. Experiments were designed to examine some of the basic aspects of infection and disease development. Laboratory and field experiments were also conducted to determine if currently used cultural practices may affect disease development.

# REVIEW OF LITERATURE

Cercosporella foot rot (eyespot, straw breaker, Columbia Basin foot rot) of cereals has a world wide distribution. In addition to the United States, the disease has been reported from England (Glynne, 1936), France (Foëx, 1936), Germany (Bockmann, 1952), Australia (Adam, 1940), New Zealand (Blair, 1954), Russia (Dzhumabaev, 1966), Denmark, Belgium, Austria, Hungary, Bohemia and Holland (Oort, 1936). Losses to winter wheat from the disease vary with year, area and varieties grown. In England, the mean loss attributed to severe eyespot lesions from 1942 to 1960 was about 12 percent (Glynne, 1963). During this period the amount of infected straws at harvest varied from two to 99 percent. Neururer (1961) reported exceptionally severe foot rot in Germany in 1961, with losses in some fields reaching 80 to 100 percent. A 25 percent loss in grain yield was attributed to foot rot in Holland (Oort, 1936) and Dickens (1964) indicated losses of more than 50 percent in New York. Sprague (1948) has estimated a four percent loss in the Northwest due to root rots.

## Causal Organism

In 1931 (Sprague), C. herpotrichoides Fron was identified as the causal agent of the Columbia Basin foot rot. The disease itself was

recognized as early as 1911 in France (Dickens, 1964) but due to the sterility of the fungus isolated (Foëx and Rosella, 1930) and the confusion with Leptosphaeria herpotrichoides (Sprague and Fellows, 1934) the French workers were uncertain as to the identity of the causal organism.

According to Sprague's (1931) original description the mycelia are septate and of two kinds. The vegetative hyphae are linear-celled and yellow to dark brown. The stromatic hyphae have medium to very heavy walls and frequently consist of polygon-shaped cells forming charred masses on the outside of stems or sheaths or in cells of infected culms. The conidia are usually curved, obclavate, two to several celled (mostly 5 to 7) and vary in size from 1.5 μ to 3.5 μ by 30 μ to 80 μ. Most of the spores are 40 μ to 60 μ long. Conidiophores are simple and sometimes branched. On potato dextrose agar (PDA) colonies are at first hemispherical mounds of smoke-gray to light grayish-olive velvet-nap mycelium, later growing slowly over the surface (Sprague and Fellows, 1934).

Lange de la Camp (1966a) has recently classified 119 isolates into seven growth types distinguished by colony color (gray to orange), growth characteristics, growth rate and sporulation. Monoconidial lines retained their purity in growth type and virulence after one or more passages through wheat or barley (Lange de la Camp, 1966b).

Generally cultures growing at room temperature remain

vegetative. Sporulation may be induced by several methods. Spores are produced on corn meal cultures incubated at cool temperatures (Sprague and Fellows, 1934). Incubating five-mm diameter disks from PDA cultures at low fluctuating temperatures (-3° to 13°C) in moist chambers stimulated abundant sporulation (Glynne, 1953). Chang and Tyler (1964) later showed that fluctuating temperatures were not essential for sporulation and optimum sporulation from mycelial disks was obtained by incubation at 9°C; water agar was the most suitable medium for culture of mycelial disks. Further, spores spread on nutrient media sporulated most abundantly at 21°C and PDA supported this process better than other media tested. Starch, maltose, glycine and ammonium tartrate were shown by Berger (1965) to favor abundant sporulation as contrasted with glucose, sucrose, lactose, asparagine and urea which did not. A C:N ratio of 5:1 favored sporulation.

Growth of C. herpotrichoides is favored by low temperatures and it has a growth range from as low as 3°C to 30°C. The optimum temperature for linear growth in pure culture is reported to be 20° to 23°C (Oort, 1936; Sprague, 1937; Dickens, 1964). However, when growth was recorded as a function of dry weight increase, 9°C was optimum (Dickens, 1964). Compared with other cereal pathogens, C. herpotrichoides has a very slow linear growth rate; 2.5 mm/24 hr. compared with Fusarium culmorum 21.5 mm/24 hr. at 22.5°C

(Macer, 1961a).

Cercosporella herpotrichoides utilized $NO_3$-N, $NH_4$-N, hydrolysed casein and peptone equally well at three nitrogen concentrations and C:N ratios of 20, 40 and 80:1 (Macer, 1961a). Blair (1954) on the other hand found the fungus produced best growth on peptone. He also showed an inability of the fungus to utilize inorganic nitrogen at low (below 6) pH levels; $NH_4$-N was more sensitive than $NO_3$-N. Cultures maintained at a pH between six and eight were able to utilize the inorganic nitrogen. Macer (1961a) was also able to demonstrate an absolute requirement for thiamin.

## Host Range

Winter wheat and winter barley are the two economically important host plants (Sprague, 1948). The fungus will also attack rye and oats but to such a low degree that it is not considered an economic problem (Butler, 1961). In most wheat growing areas of the world spring sown grains escape the disease (Sprague and Fellows, 1934). In Ireland, however, Cercosporella foot rot is a problem in spring sown wheat (Cunningham, 1966). Many species of the Gramineae have been shown to be susceptible to C. herpotrichoides. Susceptible grass hosts include species of Agropyron, Koeleria, Bromus, Sitanion, Aegilops (Sprague, 1936), Avena, Lolium, Phleum, Cynosurus, Alopecurus, Poa, Alpera, Festuca and Agrostis (Cunningham, 1965).

## Disease Cycle

Infected stubble residues are considered to be the chief sources of primary inoculum (Sprague and Fellows, 1934; Glynne, 1942; Ponchet, 1959; Cox and Cock, 1962). Volunteer wheat plants and wild grasses have also been implicated by Ponchet (1959). During late autumn, winter and early spring, spores are produced on the stubble lying on the soil surface; spore production is associated with cool damp weather. In England, abundant sporulation has been observed in wet weather from September to April (Glynne, 1953). Bruehl and Nelson (1964) observed spore production on artificially infested oat inoculum from November till early June in fields at Pullman, Washington. Spores are dispersed by water splashing onto the young wheat plants where primary infections occur. Spores have been caught continuously throughout rainy periods regardless of the rain intensity, thus differing from ascospores of Ophiobolus graminis which were activated only for short periods by heavy rains (Gregory and Stedman, 1958). Cox and Cock (1962) demonstrated that the incidence of diseased culms in April was closely correlated to the amount of sporing material on the soil surface in later winter. They indicated the infections were probably primary and resulted from the spores produced from surface material. Others (Oort, 1936; Ponchet, 1959) have suggested wind may also play a role in dissemination of conidia.

Contrary to the above observations, in New York, Dickens (1964) was unable to detect sporulation in the fall or winter; sporulation was generally restricted to the period of mid-April to mid-May. He observed infected plants in the fall, however, and suggested inoculation had possibly taken place by mycelium growing in the proximity of the young plants. Infected straws buried in soil were able to cause infections in greenhouse tests up to eight cm from the plants (Scheinpflug, 1964). He suggested that movement of conidia in the soil water and not mycelial growth was responsible for these infections. Oort (1936) speculated stubble did not play an important role in dissemination of the disease and the primary infections came from the soil. He was able to isolate the fungus from a soil suspension.

In the spring, if weather conditions are favorable, the disease may spread rapidly. Plants having primary infections produce spores in cool damp weather which are splashed onto surrounding plants (Oort, 1936; Glynne, 1965). Production of numerous conidia in the spring were responsible for the destructive secondary spread of foot rot in New York (Dickens, 1964). In the Northwest as early as middle February to late May, foot rot develops rapidly following rains which apparently wash inoculum from plants with early infections (Sprague, 1937). The lag between the date of rainfall and the observable increase in foot rot was only a few days. In England, spores were found on young infected wheat plants consistantly in March, several

times in November and January and usually no later than May (Glynne, 1953). By placing foot rot infected plants in a disease free field in December, Oort (1936) was able to demonstrate basal infections up to 10 meters and small high-placed eyespots at distances up to 70 meters from the inoculum source.

Since the percentage of straws infected in July did not necessarily correlate closely with the amount of winter inoculum, Cox and Cock (1962) suggested secondary infections were more influenced by spring weather than by surface material. In warm dry springs no spores are produced and the disease does not spread; in fact plants already infected may slough off the fungus (Glynne, 1963). In contrast, early primary infections allow time for the fungus to penetrate deeply into the plant and produce severe lesions even during springs unfavorable for the spread of the disease (Glynne, 1963, 1965).

Symptoms appear as eye-shaped lesions on the leaf sheaths and stems at the ground line. In early spring the lesions are fawn-colored to nearly white and are bordered by an amber-brown to chestnut-colored area, hence the name eyespots. A visible dark-olive to black stroma develops between each leaf sheath and over the eyespot lesions, giving the host parts a charred appearance (Sprague and Fellows, 1934; Sprague, 1948).

As the season progresses the fungus penetrates to the stem either directly from one sheath to the next or through ruptures of the

sheath by crown roots. Stem lesions vary from ivory white to black depending on the extent of stroma development and may completely girdle the culms. Tissues in the lesion are firm and brittle. The lumen of the diseased stems often become filled with wefts of smoke-grey mycelium in the late season (Sprague and Fellows, 1934).

Culms weakened at the point of attack tend to bend or twist near the ground line and may buckle and fall as the heads become heavy with grain. Falling may extend over long periods resulting in a criss-crossed or jackstraw lodging pattern due to the various wind directions during this time and the position of the attack. This crisscross lodging distinguishes foot rot lodging from that caused strictly by wind and rain. Extensive lodging may or may not occur depending on the severity of disease, varieties, succulence and occurrence of storms. The term "straggling" is used to denote scattered lodging of individual culms as contrasted with "lodging" where most or all of the culms in an area have fallen (Sprague and Fellows, 1934; Dickens, 1964). Excessive numbers of dead brown tillers, stunting and failure of head development (Sprague and Fellows, 1934), white heads, delay in flowering and growth, and reduction in final height (Glynne, Dion and Weil, 1945) in addition to reduction in yields of straw and grain have been associated with foot rot. Reduced grain yields have been attributed to smaller heads and shriveled grain (Sprague and Fellows, 1934). Culms with severe lesions yielded about half as much grain as

those not infected (Glynne, 1965). Increase in winter killing by autumn infection according to Lange de la Camp (1966c) was the primary cause of reduced grain and straw yields; delay in head emergence and stalk breakage before May were second and third respectively. Ponchet (1959) indicated in France the disease only becomes important when 20 to 25 percent of the culms are seriously infected at harvest. The tangled condition resulting from heavy lodging also increases the difficulty and expense of harvest (Sprague and Fellows, 1934).

The fungus enters the host cells either directly through epidermal cells or through stomatal openings. Presence of abundant inoculum, followed by substantial stroma formation preceded direct penetration by the fungus. In contrast, infection from spores took place through stomata, forming eye-spot lesions and then the stroma developed on the surface (Sprague and Fellows, 1934). Recently, Defosse (1967) observed appressorial formation within 48 hours after inoculation. An intensely stained halo was observed at the site of penetration when stained with cotton blue.

Penetrating hyphae are reduced in diameter as they pass through the epidermal cell wall and increase their diameter after reaching the cell cavity. Bulbous cells and macrohyphae accumulate at resisting host cell walls. Heavily thickened walls (up to 1/3 the width of the cell) develop in the host cells beneath the stroma and in cells

that are being invaded. The diseased tissues become brittle and cell contents may break down during long infection periods. The xylem elements resist invasion and apparently continue to function, allowing the heads to mature. The fungus penetrates radially forming a series of stromata between each of the leaf sheaths. Generally the head has grown past the line of attack before the fungus reaches the inner sheath; if not, the entire tiller is killed (Sprague and Fellows, 1934).

## Factors Affecting Disease Development

According to Glynne (1965), the incidence of eyespot depends on two main factors: the weather and the amount of infectious material left by preceding crops.

### Weather

As previously mentioned, cool rainy weather between November and March favors infection of young plants by stimulating spore production on infested straws lying on the soil or on previously infected plants. Temperature, humidity and soil moisture in addition to being important as stimulants for sporulation, also play a more direct role in disease development.

Effect of Temperature. The optimum temperature for infection of wheat by C. herpotrichoides is 5° to 10°C (Oort, 1936; Sprague, 1937; Dickens, 1964; Lange de la Camp, 1966b). The disease is

retarded above temperatures of 15°C and controlled at temperatures above 25°C (Sprague, 1937; Dickens, 1964). Foot rot occurs in the Columbia Basin where the mean temperature for the growing season is usually between 4° to 7°C (Sprague, 1937). Freezing has also been implicated to increase foot rot injury. Sprague (1937) suggested weakened culms are attacked early in the season, thus increasing the inoculum and further spread of the disease.

Effect of Humidity and Soil Moisture. High humidity apparently favors disease development. Plants placed in front of a fan in order to reduce humidity showed less foot rot than control plants (Sprague, 1937). He also observed that edges of fields which usually dried out sooner after a rain were always free of foot rot. Extreme thinning also inhibited foot rot development, presumably by lowering the relative humidity. His field and laboratory studies showed that high soil moisture (until near the saturation point) favored disease development; 75 percent moisture holding capacity was optimum in greenhouse experiments. Dickens (1964) was unable to show any difference in disease development between high and low soil moisture levels. He suggested that condensation of water on the bases of culms may have masked possible differences due to actual soil moisture.

## Infectious Material

The amount and disease potential of the infectious material left

by the preceding crop will depend on the survival of the fungus in plant debris and on cultural practices.

Survival in Plant Debris. Macer (1961b) has shown that C. herpotrichoides is able to survive in straw buried 15 and 45 cm below the surface for three years. The fungus survived at the surface and 2.5 cm depth for seven and 18 months respectively. In laboratory studies, C. herpotrichoides showed just slightly higher levels of survival in the nitrate enriched soil; whereas O. graminis showed much higher survival levels in the nitrate enriched soil. Blair (1954) suggested that C. herpotrichoides could survive in straw as long as the straw remained undecomposed, and Bruehl and Lai (1966) have shown that prior establishment of the fungus in wheat straw is effective in preventing invasion of the straw by other fungi. Straws colonized by C. herpotrichoides decomposed more slowly in the soil than those uninoculated (Macer, 1961b). Macer suggested, since no antibiotic activity has been observed for the fungus, it may be able to restrict development of competing fungi through prior and continuing utilization of free nutrients. Laboratory investigations indicate that saprophytic survival is limited to previously colonized straws and the fungus shows little or no saprophytic expansion in soil. Macer (1961a), in classifying C. herpotrichoides as a root-inhabiting type fungus, indicated it has an extremely low competitive saprophytic colonizing ability and is unlikely to colonize straw in the soil. Blair (1954) has reported the fungus lacks a capacity for active saprophytic growth in

soil. In contrast, Byther and Powelson (1966) demonstrated the pathogen's ability to germinate, grow and sporulate in field soil suggesting "short-term" saprophytic survival. Attempts to isolate the pathogen directly from soil have usually been unsuccessful (Sprague and Fellows, 1934; Blair, 1954; Dickens, 1964). Dickens (1964), however, was able to isolate the fungus from soil suspensions following periods of weather favorable for sporulation. Oort (1936) reported isolation of C. herpotrichoides from soil suspensions.

Effect of Crop Rotation. Glynne (1965) summarized the results of crop rotation investigations over the last 30 years at Rolhamsted, England. Intervals of three years to grass, lucerne or arable crops excluding wheat and barley were sufficient in reducing eyespot to unimportant levels; however, they did not completely eliminate it. Two year breaks from wheat were almost as effective as the three year rotation, but one year free from a susceptible crop was not enough to control the disease. Application of nitrogen fertilizers during the rotation or to the wheat crop after rotation may reduce the effectiveness of the rotation (Heard, 1965). In the higher rainfall areas of the Northwest, crop rotation may be practical and specific rotations have been recommended (Sprague, 1948).

Effect of Fallow. In the dry regions of the Pacific Northwest a straight wheat-fallow system is used. In England (Glynne, 1965) a one year fallow reduced the severity of eyespot fourfold; however,

no comparable data is available for the Northwest.

Generally, two types of fallow preparations are practiced in the Columbia Basin: trashy (stubble mulching) and clean (moldboard plowing). Trashy fallow reduces evaporation and thus increases soil moisture, lowers soil temperature, reduces erosion and reduces nitrate accumulation (Ramage, 1953). Since harvesting, cultivating, seedbed preparation and planting are all done so as to leave residues from the previous crop on or near the soil surface (Horning and Oveson, 1962), and since it has been shown the number of infections are closely related to the infective straws on the surface (Cox and Cock, 1962), trashy fallow might be expected to favor disease development. However, sun and wind may dry the straw and kill much of the fungus and thus counteract this effect (Sprague, 1948). In England, the pathogen survived longer (three years) in buried straw compared to straw on the surface (seven months) (Macer, 1961b).

Since foot rot infections result from spores produced on surface residues, control of the disease by burning the stubble has been investigated. In limited experiments, Sprague (1937) reported burning of stubble had little effect on disease severity in the subsequent crop. However, by burning straw on the surface of an infested field just prior to seeding, disease incidence was reduced in the burned over area. Burning was more effective in reducing infections than either deep or surface cultivation (Blair, 1954).

Other Factors

In addition to weather and the amount of surface inoculum other factors influence foot rot. These factors are fertility, date of planting, rate of seeding, chemical application and plant resistance.

Effect of Fertility. Many investigators have studied the effects of fertilizers on foot rot, the majority of them being concerned with nitrogen. Generally, field trials indicate nitrogen application will increase the incidence of the disease (Sprague and Fellows, 1934; Glynne, 1951; Dickens, 1964; Cunningham, 1966). Other workers indicate nitrogen had no effect (Glynne, 1963) or decreased eyespot (Oort, 1936; Salt, 1955; Diercks, 1961, 1964). Several factors apparently influence the effect of nitrogen fertilization: date of application, form, previous fertility level, and method of application.

Nitrogen applied in March, April or May had little effect on the percentage of infections at harvest, while October application decreased the disease (Salt, 1955). In spring wheat planted in Holland, Cunningham (1966) observed increases in eyespot with early nitrogen application; late application showed no effect. Fall applications of nitrogen rich fertilizers increased disease in the Northwest while spring application seemed to have no effect (Sprague and Fellows, 1934). Early spring application of $(NH_4)_2SO_4$ increased incidence of eyespot and lodging in England (Glynne, 1951). However, in a later

publication (Glynne, 1963) no consistant effects of fertilizers were observed over a three year period.

In some cases the form of nitrogen may influence foot rot. Both $Ca(NO_3)_2$ (Diercks, 1961) and calcium cyanamide (Diercks, 1964) controlled the severity of foot rot in Germany. Ammonium sulfate (Glynne, 1951) and $NH_4NO_3$ (Diercks, 1964), on the other hand, increased disease severity. Cunningham (1966) indicated the form of nitrogen made little difference in observed increases of eyespot with nitrogen application.

Salt (1955) suggested the effect of nitrogen on eyespot depends on the original fertility level of the soil. Nitrogen had little effect on the percentage infection in highly fertile soil, but increased infection in fields of low fertility. He emphasized, however, that the beneficial effects of fertilizer on a poor crop outweighed the losses due to increased foot rot. Glynne (1945) also indicated the increased yields from nitrogen application more than compensated for its tendency to increase the spread of disease.

Shank application of anhydrous ammonia in the spring increased foot rot in Washington (Bruehl and Nelson, 1964). However, other forms of spring tillage also increased foot rot, indicating that the physical factors involved in the "shanking-in" rather than the nitrogen itself might be responsible for the increase in disease. Sprague and Fellows (1934) also observed if spring harrowing was followed by

rainy weather foot rot increased.

In pot experiments conducted outdoors (Glynne, 1951), early spring application of $(NH_4)_2SO_4$ decreased the severity of eyespot. In contrast, field data indicated nitrogen increased disease incidence. She suggested two means by which nitrogen may effect foot rot development. High nitrogen levels favor the production of a more luxuriant crop thus helping to maintain humid conditions that favor spread and development of the disease in the field. On the other hand, in pot experiments, less humid conditions are maintained. Increased tiller production in the high nitrogen treatments enabled the plants to delay infection of the central straws and discard severely diseased tillers without loss in yields, thus reducing the intensity of infection at harvest.

In greenhouse studies, Dickens (1964) observed the interaction of K, N and P on disease development. A significant interaction between N and K indicated neither element was entirely independent of the other. Generally high nutrient levels favored foot rot. Low level combinations of all three elements were least conducive to disease development. Nutrient combinations promoting luxuriant growth and increased density of tillers promoted foot rot.

Information on the effect of P on disease severity is limited and contradictory. In greenhouse experiments, Sprague and Fellows (1934) observed superphosphate gave good control of foot rot; however,

field data indicated no such effect. Phosphorus deficient soils favored disease according to Oort (1936). No consistent effect of P was observed in Dicken's (1964) greenhouse studies.

Effect of Planting Date. Date of seeding is a very important factor in production of dryland wheat. Adequate leaf and root growth must be achieved to control soil erosion and establish hardiness before the advent of last fall and winter rains. Seeding too early may cause luxuriant growth in the late fall or early spring resulting in exhaustion of available nutrients and soil moisture during grain maturity (Beutler, 1961). No increase in grain yields were reported from seeding prior to about October. Early seeding favors foot rot and losses due to disease may offset the advantages of early seeding (Bruehl and Nelson, 1964).

Increase in disease severity associated with early seeding is well documented (Sprague, 1937; Oort, 1936; Neururer, 1961; Bruehl and Nelson, 1964; Dickens, 1964). Various theories have been put forth to explain this phenomenon. Dickens (1964) suggests that since late seeded plants attain less vigorous growth, the microclimate around the base of plants is less favorable for the pathogen and few primary fall infections occur. Secondary spread of the disease in the spring from lesser numbers of primary infections is then reduced. He also speculated that availability of water and nitrogen at various dates were factors leading to different

physiological stages of growth and ultimately affect the susceptibility of the host. Sprague (1937) has suggested that late seeded plants reach a physiologically susceptible state so late in the season only a few primary fall infections occur. Disease incidence is thus dependent on the secondary spread of pathogens in the spring. In a later publication (1948) he attributed increased foot rot to more abundant growth in early planted fields. Late sowing also may not give the fungus time to spread before sporulation ceases at the onset of drier and warmer weather (Glynne, 1965).

Effect of Seeding Rate. Generally, greater disease severity is associated with densely grown plants which counteracts the benefit of more heads. Sprague (1937) observed no differences in the severity of foot rot in rows 8, 10, 12, or even 14 inches apart during seasons of excessive rainfall characterized by rank growth of the wheat. However, in dry seasons wider spacing reduced disease severity, which Sprague attributed to increased aeration. Lowering the seeding rate from three to one and one half bushels per acre decreased eyespot and increased yields (Salt, 1955). A lower incidence of severe eyespot was also observed by Glynne (1951) at a lower seeding rate, but the lower rate did not necessarily increase yields. In pot experiments, reducing seeding rate increased yields and decreased eyespot severity. Thinner sowing rates are generally recommended on foot rot infested ground (Sprague, 1948). However, Dickens (1964)

suggests that higher rates of planting can be used in late-planted wheat without serious threat of foot rot.

Chemical Control. Several chemicals have been shown to be effective in controlling Cercosporella foot rot; however, no chemicals are in use in the Northwest primarily due to the economic factors involved. Spray application of sulphuric acid was effective in reducing eyespot; inhibition of sporing on surface debris and destruction of outer leaves were according to Glynne (1951), responsible for the control. Defosse (1961) achieved 75 percent reduction in disease by five spray applications of methoxyethylmercury chloride throughout the sporulation period. As mentioned earlier, Diercks was able to partially control eyespot infections by use of $Ca(NO_3)_2$ (1961) and calcium cyanamide (1964). He more recently (1965) reported control of lodging and decreased foot rot incidence by application of chlorcholinchloride immediately after tillering. Fall application of diuron in the Northwest is used to control weeds in winter wheat. Huber, Seely and Watson (1966) observed a reduced incidence and severity of foot rot in diuron treated fields.

Host Resistance. Immunity to Cercosporella foot rot is unknown in commercial varieties of wheat, but some varieties are less susceptible than others. Sprague (1934) tested several hundred varieties and selections for resistance to foot rot. None showed "true resistance" but degrees of susceptibility were noted.

Resistance was generally associated with shallow-seated relatively open crowns, sparse tillering, coarse, short tough leaf sheaths, and very late maturity. Late-maturing varieties have at times appeared resistant in that they escape infection. They reach a susceptible stage late in an average growing season when the fungus is inactivated by dry weather and warm temperatures. Seasons do occur, however, when these late varieties are severely attacked (Sprague, 1948). Bockman (1966) suggests that, since so many environmental factors influence the course of the disease, it is impossible to separate varietal characteristics from environmental influences and he questions the existence of even tolerant varieties. Apparently varieties like 'Cappella Desprez' exhibit fairly consistant low susceptibility to eyespot (Butler, 1961).

# METHODS AND MATERIALS

## General Procedures and Techniques

### Isolation Technique

Although the isolate used throughout most of this study was obtained from Dr. Breuhl (Washington State University), successful isolations were made from diseased materials collected in Oregon. The diseased materials were surface sterilized in a 20 percent Clorox solution for three minutes. Tissue pieces were aseptically cut from the diseased area and plated on water agar (1.5%) containing 100 ppm streptomycin (SWA). Agri-Strep produced by Merck and Company Ind., Rahway, New Jersey was used in these media. The plates were incubated at 10°C for two to three weeks to stimulate sporulation. The tissue pieces were placed in approximately five ml of sterile water containing a trace of Tween-20 and shaken vigorously. A dilution series was made from the resulting spore suspension and each dilution was plated on SWA. The plates were incubated at 20°C for 24 hours. A dilution plate was chosen which would allow a single germinating conidium to be picked off. The conidium was placed on potato dextrose agar containing 100 ppm streptomycin (SPDA) and thereafter maintained on PDA and transferred periodically. This

isolation technique was successful from green plant tissues and dry stubble.

Conidial Production

Two methods were used to obtain conidia. Five-mm disks cut from PDA cultures of C. herpotrichoides were placed on water agar (WA) and incubated at 10°C for two to three weeks to stimulate sporulation. The disks were removed and suspended in distilled water containing a trace of Tween-20. The suspension was agitated and then passed through a glass wool filter to remove mycelial fragments. After centrifugation in a glass centrifuge tube, the conidia were resuspended in distilled water.

The second method was a modification of a procedure described by Chang and Tyler (1964). Greater quantities of conidia could be produced by this second method. A conidial suspension was obtained from PDA culture plugs as outlined in the first method. Instead of using these conidia directly, they were seeded onto plates containing PDA and incubated at 10°C. Vast quantities of conidia could be harvested from these plates one to three weeks later by flooding the plates with distilled water containing a trace of Tween-20. Lightly scraping the surface with a transfer needle facilitated their harvest. After filtration through glass wool and centrifugation, the conidia were resuspended in distilled water. Chang and Tyler (1964) suggested for

maximum conidial production the spores be seeded on full strength PDA and incubated at 20°C. However, I found that conidia produced by this method were atypical, being distorted, swollen and not of uniform size. Incubating the plates at 10°C and reducing the nutrient concentrations in PDA by one half reduced the number of conidia produced; however, they were of more uniform size and had typical conidial morphology.

Soil Type and Manipulation

The soil used was a Walla Walla silt loam obtained from a growers field near Moro, Oregon in Sherman County. The field had a foot rot history. For laboratory studies, the soil was passed through a sieve (16 meshes/inch) and stored in garbage cans in an air dry condition. Desired moisture levels were obtained by atomizing distilled water into the air dry soil. The screened soil had a "field capacity" (FC) of 20.4 percent as determined from a re-packed soil column. The soil was incubated in plastic boxes individually enclosed in polyethylene bags and water loss was periodically checked by weight loss. When experiments were extended beyond one or two months, depending on temperature, it was necessary to add additional water in an attempt to maintain a "constant" soil moisture. The calculated amount of water to be added was atomized onto the soil surface.

In several experiments soluble soil amendments were added to the distilled water at the time the soil was brought to the desired moisture levels. Powdered cellulose (Alphacel, Nutritional Biochemicals Corp., Cleveland, Ohio) amendments were mixed with the air dry soil before the water was added.

Artificially infested soil was obtained by additions of washed (centrifuged) conidia to the soil. Concentration of these conidial suspensions was determined with a haemacytometer and the calculated amounts of these suspensions were added to the distilled water used to moisten the soil.

Growth Chamber and Plant Growth

A Percival growth chamber (bench model E-57) was used in all the laboratory studies. Eight HO 46-watt florescents (F 36T 12/cw/HO) and four 25-watt incadescents yielded a light intensity of about 1000 foot candles at the shelf level. The chamber was programmed for a 10 hour day at 15°C ± 1°C and 14 hour night at 10°C ± 1°C. The relative humidity during the day period fluctuated periodically with the compressor cycles between 58 and 68 percent. Humidity fluctuated between 70 and 90 percent during the night cycle.

The semi-dwarf wheat variety 'Gaines' was used in all laboratory experiments. Plants were grown either in growth pouches or plastic pots. S/P Seed-Pak Growth Pouches, catalog No. B 1220, were

obtained from Scientific Products, 1210 Leon Place, Evanston, Illinois (Figure 1). The seeds were surface sterilized in a 20 percent Clorox solution for two minutes and five seeds were planted per pouch. A non-sterilized Hoagland solution (Machlis and Torrey, 1956) was added to each pouch as needed.

Square (3 3/4") plastic pots filled with vermiculite were also used. Nine plants were grown in each pot. The pots were placed in aluminum foil trays and subirrigated as needed with the Hoagland solution.

## Conidial Germination and Survival in Soil

A conidial suspension was sprayed onto glass slides and allowed to air dry. Immediately after drying the slides were placed in plastic boxes (3 1/4" x 7" x 1 3/4") containing 400 grams of moistened and in some cases amended soil. The boxes were placed in polyethylene bags and incubated at various temperatures.

The slides were periodically removed from the soil and the conidia stained with lactophenol-cotton blue. The percentage germination and the percentage of senescent conidia were determined microscopically by observing 100 conidia. The treatments were replicated three times. Conidia and germ tubes were considered senescent when they were weakly or no longer stained with cotton blue or were visibly being lysed by the soil microflora. The number

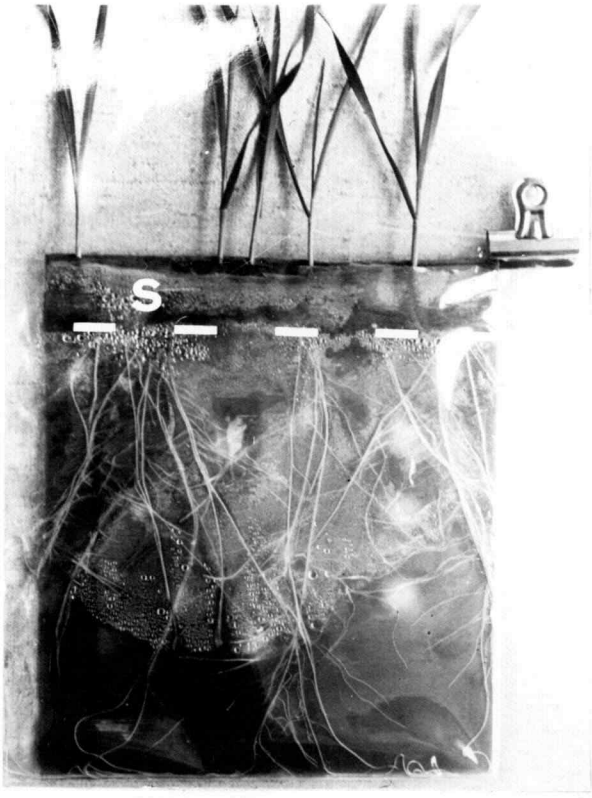



Figure 1. Wheat seedings growing in a S/P Seed-Pak growth pouch. The seed trough is indicated by (S).


Figure 2. Cercosporella herpotrichoides growing from a colonized straw. The straw was recovered from soil and incubated on SWA for three weeks at 10°C.

of hyphal swellings, indicating the relative activity of the fungus after germination was also recorded in several experiments.

Survival curves were determined from observation of senescent conidia. It was assumed that conidia classed as senescent would be completely gone at the time of the next observation and only the viable conidia would be observed on subsequent readings. By using the following formula the percentage of the original population of spores at each time interval could be estimated.

$$Xn = \frac{X_{(n-1)}\ Yn}{100}$$

Xn = Percentage of original population still viable at n observation

$X_{(n-1)}$ = Percentage of original population viable at the previous observation

Y = Percentage of observed viable conidia recorded at n observation

## Competitive Saprophytic Ability of Buried Conidia

### Laboratory Determination

Two hundred and fifty grams of air dry soil for each treatment was moistened to 60 percent FC and incubated at room temperature for five days. On the sixth day calculated proportions of a conidial-water mixture were atomized into the soils to give the desired

inoculum densities and bring the soil to 75 percent FC. Approximately 360 unsterilized straw pieces measuring about 6 mm × 2 mm and weighing about 2 mg were mixed with the infested soil. The straw had been collected from a stubble field and the first internodal stem tissue above the crown was subdivided into the desired sizes.

The soil mixtures were incubated in polyethylene covered plastic boxes at either 10° or 20°C. At one, two and six month intervals a portion of the soil was removed from the boxes and placed onto a Tyler screen (24 meshes/inch). The soil was forcefully washed from the straw pieces with a water jet from a faucet. The straw pieces were placed in nylon mesh bags, washed in running water for 24 hours and then plated on SWA. The plates were incubated for three weeks at 10°C and examined microscopically. When Cercosporella grew from the straw pieces into the agar, it could be easily identified by characteristic sporulation (Figure 2). Since it did not always grow into the agar, it was necessary to place the remaining straws in a drop of water on a glass slide and examine them microscopically. Presence of Cercosporella conidia was considered indicative of colonization.

Field Determination

Field plots were located on fallow land of the Sherman Branch Experiment Station at Moro, Oregon. Two hundred and

twenty grams of moist field soil was screened (16 meshes/inch) and infested with conidia of C. herpotrichoides. Two ml of a conidial suspension having an adjusted concentration were atomized into the soil to give the desired inoculum densities. In the nitrogen amended treatments, $NH_4NO_3$ was added to the conidial suspension to give a rate of 40 lbs of nitrogen per acre. Approximately 180 straw pieces were added to each treatment. Straw pieces were similar to those used in the laboratory study; however, pieces used in half of the treatments were autoclaved for one hour. The infested soils containing the straws were placed in nylon mesh bags and buried six inches below the surface. The treatments were replicated four times. The bags were recovered seven weeks later and the straw pieces screened, washed, plated and examined as in the laboratory experiment.

## Survival in Colonized Straw

### Influence of Burial Depth

Wheat stubble was collected from the field in mid-September. The foot region of stems were cut into five-mm sections and then divided vertically resulting in straw pieces approximately five mm by three mm which weighed about 4.2 mg. Approximately 4,000 straw pieces were added to 2 liters of a supernatant in which 250 grams of fresh suculent wheat plants had been autoclaved for one hour. A

suction was applied for 30 minutes to insure penetration of the liquid into the straw. The straw pieces were allowed to soak an additional 24 hours. Since C. herpotrichoides has only a limited cellulase activity these additional nutrients allowed the fungus to attain a greater and more natural degree of colonization. The liquid was decanted off and the straw pieces autoclaved for one hour. The straw pieces were distributed into six 500 ml flasks containing 150 ml of a one to one cornmeal-sand inoculum. The cornmeal-sand mixture had been inoculated with a spore suspension two weeks prior to introduction of the straw pieces. Following introduction of the straw pieces, the flasks were incubated for two months at room temperature with periodic shaking and mixing. Straw pieces were separated from the inoculum by washing on a Tyler screen (12 meshes/inch) and allowed to dry.

Thirty colonized straw pieces were placed in each nylon mesh bag. Two thirds of the bags were filled with approximately 30 grams of screened (16 meshes/inch) soil collected in the field. Soil and straw pieces were mixed thoroughly and buried in trenches at either three or nine inches below the surface. Wooden stakes were placed near each group of bags to facilitate their recovery. The remaining bags (containing no soil) were held on the soil surface by aid of a wire stake. The treatments were replicated four times. Periodically bags were removed from the soil to determine the proportion of straw

pieces still colonized by C. herpotrichoides. The straw pieces were screened, washed, plated and examined as those in the saprophytic colonization study. Methods used in this experiment are modifications of those used by Macer (1961b) in England.

Dry weight of the recovered straws, soil temperature, and moisture as well as the proportion of straws colonized by Cercosporella were recorded at each sampling period.

Influence of Time of Burial

Naturally infected straw pieces were obtained from the field in June and were stored at room temperature in the laboratory until September. Diseased portions of the stems were cut into pieces approximately six mm by three mm which weighed about four mg. Leaf sheaths with lesions were also cut into pieces about the same size. Twenty-five of these straw pieces were placed in each nylon mesh bag. Half of the bags were filled with screened field soil and buried in trenches six inches below the surface. The remaining bags were held on the surface with wire stakes. In March three fourths of the surface bags were filled with screened field soil and buried six inches below the surface. The treatments were replicated four times. Periodically straw pieces were recovered and the survival of C. herpotrichoides was determined in a manner similar to the previous experiments. Both time and depth survival studies were

carried out in a fallow field at the Sherman Branch Experiment Station at Moro, Oregon.

## Infections Below the Soil Surface in the Laboratory

Two hundred grams of soil were infested with conidia to the desired levels as previously described. A portion of this infested soil was placed in contact with 16 to 24 day old seedlings growing in growth pouches in the growth chamber. The remainder of the soil was divided and incubated at either 10° or 20°C in polyethylene covered plastic boxes. One and two months later, portions of this soil were placed in contact with wheat seedlings. Approximately seven to 10 grams of the infested soil was placed in the seed trough to cover the lower 20 mm of the seedling plumules. Each pouch contained five plants and four pouches were used per treatment.

When the plants were two months old they were removed from the pouches and washed in running water for 24 hours. Lesion development at this time was slight so the lower 15 mm of the plant stems were removed and placed on SWA for three weeks at 10°C. The stems were examined microscopically in a drop of water. The presence of Cercosporella spores was interpreted as being indicative of infection. When this experiment was repeated the plants were harvested when three months old. At this time distinct lesions could be observed and the stems were not plated as in the first experiments.

Five-mm PDA culture plugs and naturally infected wheat stubble were also used as below ground inoculum. These plugs and straw pieces were placed in contact, five mm and 10 mm away from the bases of wheat seedlings growing in pouches. The lower 20 mm of the seedlings plumules were covered with natural field soil.

In order to determine activity of the conidia on the "rhizoplane" of the seedlings, a washed conidial suspension was sprayed onto coleoptiles of 12 day old seedlings and allowed to air dry. The seedlings had been germinated in vermiculite in the growth chamber and rinsed with distilled water before inoculation. The inoculated seedlings were immediately placed in plastic boxes and the lower 20 to 25 mm were covered with soil moistened to 75 percent FC. The boxes were placed in polyethylene bags to help maintain soil moisture and placed in the growth chamber. Additional watering was necessary to maintain the soil moisture. Periodically the seedlings were removed and the lower portion of the stem was dipped into fabil staining solution (Noel, 1964). After 20 to 30 seconds in the stain the coleoptiles were split, removed from the stem and mounted for microscopic examination.

## Influence of Spring Tillage and Nitrogen Application on Foot Rot Incidence

A field plot was established near Moro, Oregon. A skew treader was used by the grower in mid-March as a spring tillage

operation. Urea (40 lbs nitrogen/acre) was applied as a surface spray the last week of March. The plots measured approximately 50 feet long and were 12 rows wide; each treatment was replicated twice. On June 20, five samples totaling at least 350 tillers were randomly collected from each treatment and visually examined for Cercosporella lesions.

## Association of Soil Debris with Foot Rot Incidence

In September of 1964, soil was collected from a growers field near Moro, Oregon. The field had a previous foot rot history. Organic debris was differentially screened from the soil according to size and the various fractions were then added back to the screened soil. Wheat plants were grown in association with these test soils at Corvallis, Oregon and the amount of foot rot was ascertained.

Approximately 700 grams of field soil was passed through a series of Tyler screens (8, 14, 28, and 42 meshes/inch). One fraction was added back to the soil which had passed through all four screens, thus making it necessary to screen five of the 700 gram samples to test the inoculum potential of each organic fraction. Number 10 cans were filled to about three inches of the top with greenhouse potting soil which had been fortified with a 6-10-4 fertilizer at the rate of 400 lbs of nitrogen per acre. Fifteen seeds were placed on the surface and covered with 600 grams of the test

soil. The treatments were replicated five times. Seeds were planted on October 7, the cans were placed outside and were watered as needed. Seedlings were thinned to leave five plants per can. The plants were harvested the following spring on June 15. Tillers having lesions were plated on SWA for positive identification of C. herpotrichoides. Dry weight of the plant material produced in each can was also determined.

# RESULTS

## Conidial Germination and Survival in Soil

Large quantities of conidia are produced from colonized surface debris during the moist damp winter months and from infected plants in the early spring. Experiments were designed to determine the fate of conidia which become buried in the soil due to cultivation and water movement. Germination, anastomisis and hyphal swellings observed by Byther and Powelson (1966) were also found in this study (Figures 3 and 4). Hyphal anastomosis was regularly observed between conidia, germ tubes, and conidia and germ tubes. Anastomosing hyphae were commonly of finer diameter than normal germ tubes and wriggly in their growth patterns. They differed also from germ tubes in that they often originated from intercalary cells of the multicellular conidium, rather than terminal cells.

A majority of the conidia that did not germinate underwent autolysis within 8 to 32 days (Figure 4). Very seldom were conidia lysed by other soil microorganisms. In contrast, germ tubes and hyphae were lysed by bacteria; however, they also underwent autolysis.

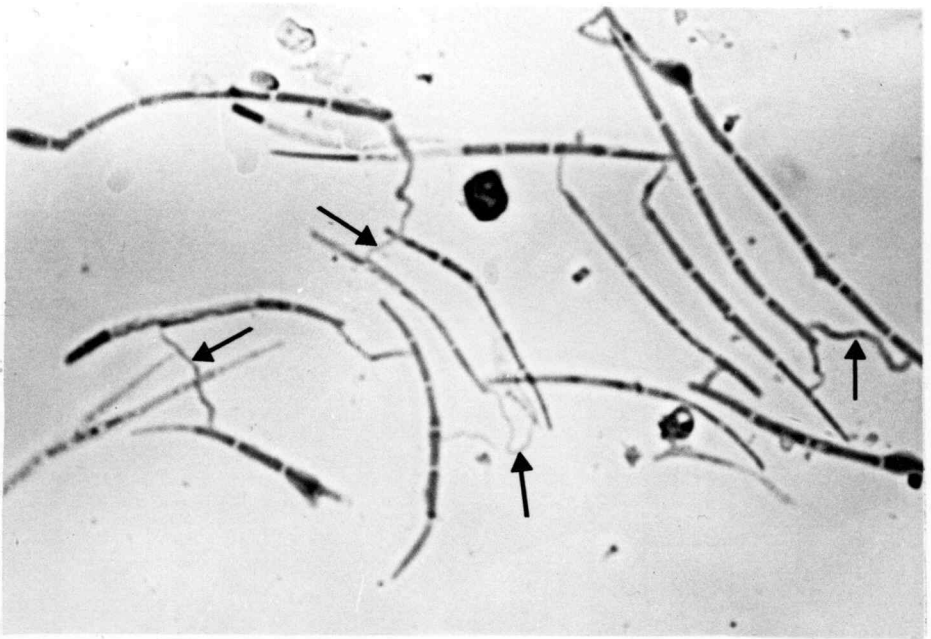


Figure 3. Anastomosing conidia of C. herpotrichoides on glass slides buried in the soil. Arrows indicate some of the anastomosing hyphae.

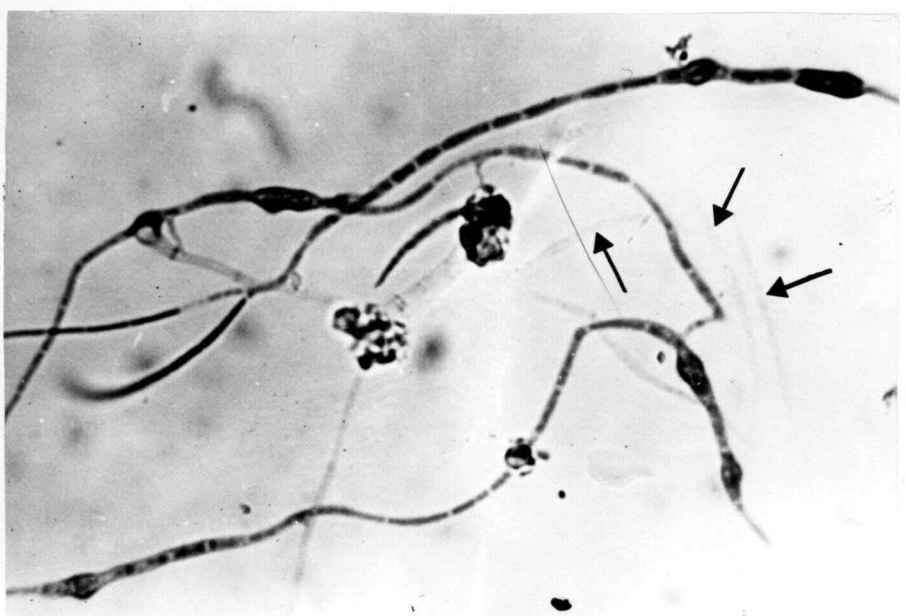


Figure 4. Anastomosis, hyphal swellings and sporulation associated with germinating conidia on glass slides buried in the soil. Arrows indicate conidia which failed to germinate and have undergone autolysis.

Influence of Temperature

Slides sprayed with conidia of C. herpotrichoides were buried in soil moistened to 75 percent FC and incubated at 5°, 10°, 15°, 20°, and 25°C. Incubation at 25°C inhibited germination, at 5°C germination was delayed and conidia at 10°, 15°, and 20°C germinated equally well (Table 1).

Table 1. Influence of temperature on conidial germination in soil.

| | Percentage germination days after burial[a] | | |
|---|---|---|---|
| Temperature | 2 days | 4 days | 8 days |
| °C | % | % | % |
| 5 | 3 | 27 | 48 |
| 10 | 15 | 53 | 52 |
| 15 | 31 | 45 | 45 |
| 20 | 35 | 43 | 51 |
| 25 | 13 | 11 | 13 |

[a] Based on the average of three replications with 100 conidia in each.

Cooler temperatures (5° and 10°C) favored survival of the fungus in soil (Figure 5). Hyphae from germinated conidia were visible on glass slides recovered 16 months after burial (Figure 12). Even though the original conidia had disappeared, the characteristic hyphal swellings made identification possible. Protoplasm, as

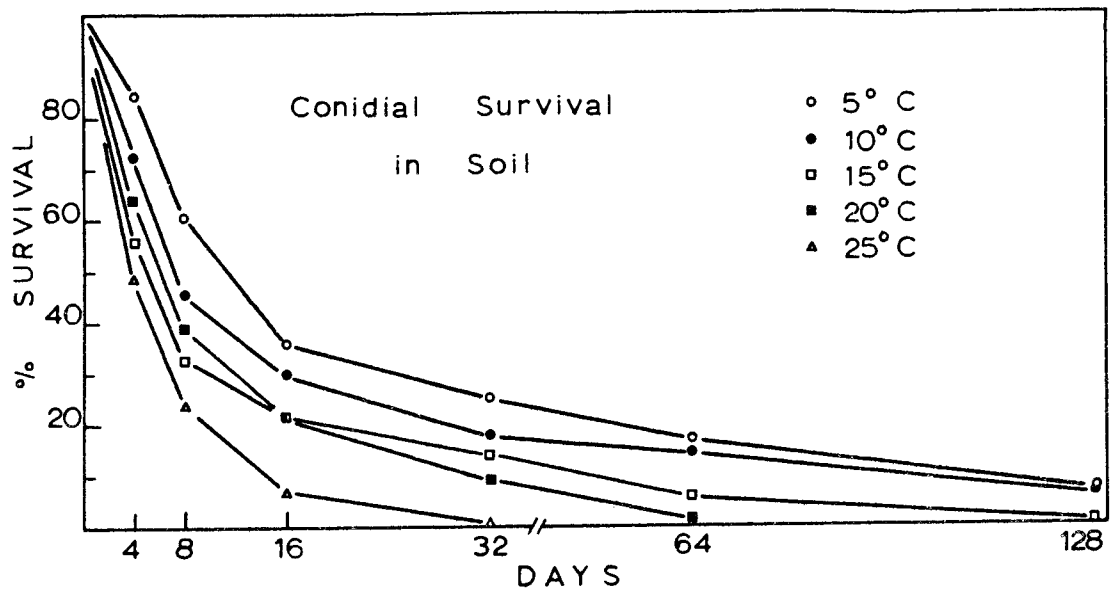



Figure 5. The influence of temperature on survival of C. herpotrichoides in soil determined by direct observation of conidia on glass slides buried in soil. Each point represents the average of three replications with 100 conidia in each.

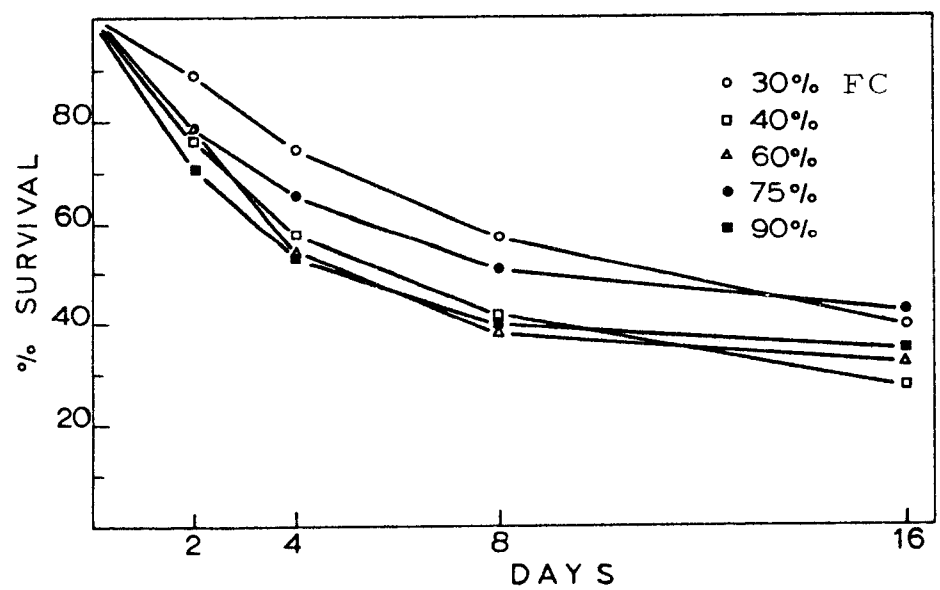



Figure 6. The influence of soil moisture on survival of C. herpotrichoides in soil determined by direct observation of conidia on glass slides buried in soil. Each point represents the average of three replications with 100 conidia in each.

indicated by staining with cotton blue, was limited and confined to a thin layer next to the cell walls. Hyphal swellings showed the most intense staining. The viability of these hyphal fragments was verified by flooding the slides with a glucose solution and incubating them in a moist chamber for one week. New hyphal growth was initiated and in some instances sporulation occurred.

Warmer temperatures hastened senescence of buried conidia. Conidia incubated at 25°, 20° and 15°C had essentially disappeared at 32, 64 and 128 days respectively.

Influence of Soil Moisture

Conidia sprayed on glass slides were buried in soil in which the moisture level had been adjusted to "0", 15, 30, 45, 60, 75 and 90 percent FC; "0" indicated an air dry soil. The soil was incubated at 15°C. Few conidia germinated in the two driest soils (Table 2). No differences in germination were observed after four days in soils maintained between 45 and 90 percent FC; fewer conidia germinated in the soil maintained at 30 percent FC. Since moisture was observed to condense on the slides buried in soil moistened between 45 and 90 percent FC, it is doubtful whether the data can be interpreted as a direct effect of soil moisture on conidial germination. However, it does demonstrate that soil organisms antagonistic to the germination of C. herpotrichoides were not selectively stimulated by various soil

moisture levels.

Table 2. Influence of soil moisture on conidial germination.

| Percentage field capacity of soil | Percentage germination days after burial[a] | |
|---|---|---|
| | 2 days | 4 days |
| % | % | % |
| "0" | 0.9 | 0.6 |
| 15 | 3 | 0.7 |
| 30 | 37 | 31 |
| 45 | 53 | 55 |
| 60 | 47 | 45 |
| 75 | 42 | 64 |
| 90 | 46 | 56 |

[a]Based on the average of three replications with 100 conidia in each.

At 16 days there seemed to be no relationship between soil moisture and survival so the experiment was terminated (Figure 6). Conidia buried in the two drier soils were not lysed nor did they undergo autolysis; however, when the slides were flooded with a nutrient solution and incubated for 48 hours in a moist chamber the conidia failed to germinate.

Influence of Soil Amendments

Soils were amended with various combinations of cellulose, glucose and $NH_4NO_3$ and were moistened to 75 percent FC. The cellulose and glucose were added on an equivalent carbon basis. The one percent cellulose amendment equalled approximately 4,500 ppm carbon in the soil. Nitrogen was added to obtain 150 ppm N and a C/N ratio of 30:1. No net immobilization of nitrogen should occur in the soil at this C/N ratio. Slides sprayed with conidia were buried in the amended soil and incubated at 15°C. Germination, survival and numbers of hyphal swellings was recorded at various intervals throughout a two month incubation period.

Ammonium nitrate, either alone or in combination with glucose or cellulose, inhibited germination (Table 3). Cellulose and glucose alone seemed to have little effect on germination. In combination they had a slight inhibitory action.

Ammonium nitrate also inhibited survival of conidia in soil, as contrasted with glucose and cellulose which prolonged survival (Figure 7). Additions of $NH_4NO_3$ to the glucose and cellulose amended soils nullified their stimulatory effect and caused a rapid initial decline in the conidial population. At the end of 64 days the fungal population in the glucose plus nitrogen and the cellulose plus nitrogen soils was similar to those in the control soil.

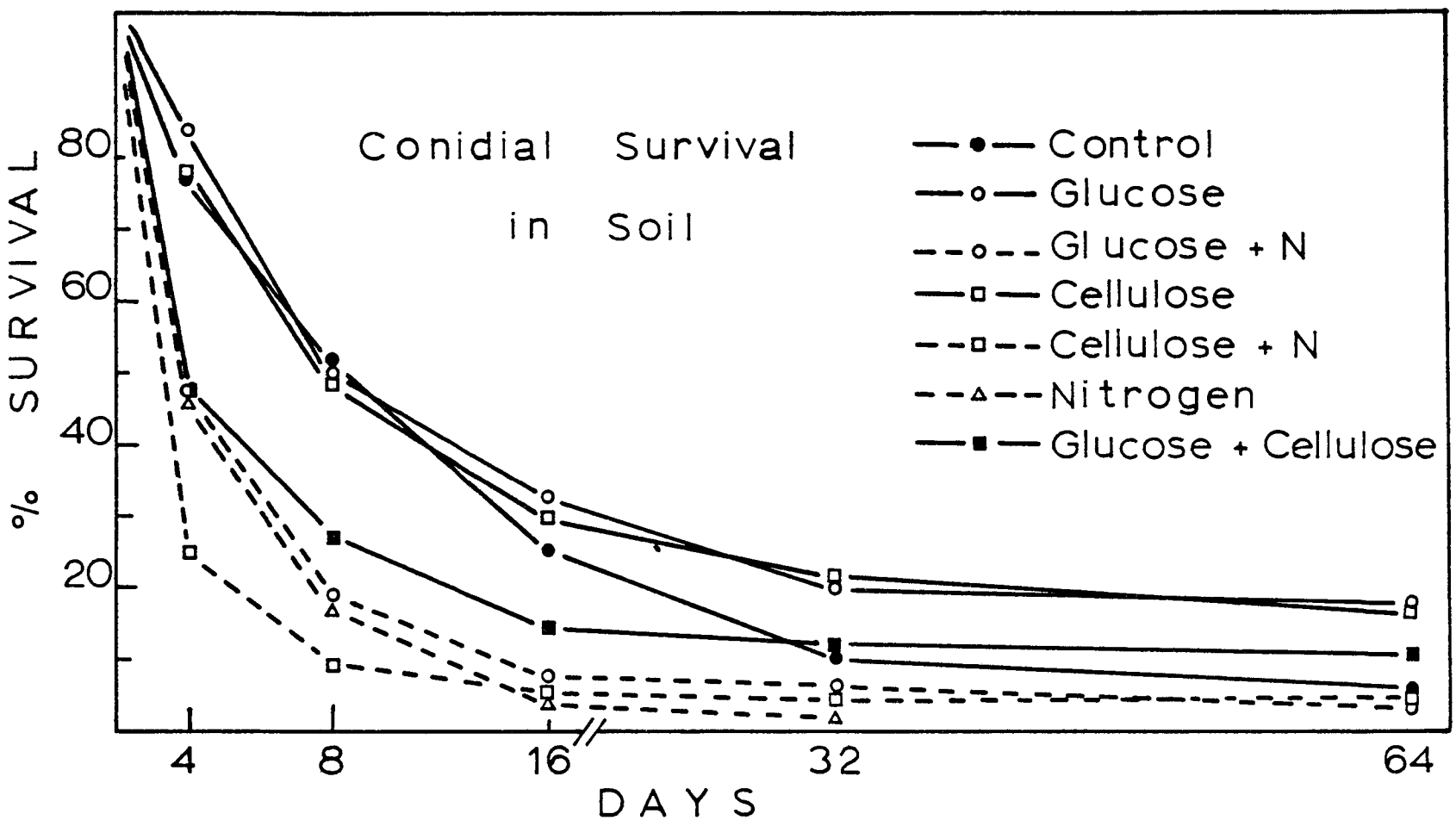



Figure 7. The influence of soil amendments on survival of C. herpotrichoides in soil determined by direct observation of conidia on glass slides buried in soil. Each point represents the average of three replications with 100 conidia in each.

Table 3. Influence of soil amendments on conidial germination. Carbon was added at 4500 ppm (1% cellulose) and $NH_4NO_3$ at 150 ppm N.

| Amendment | Percentage germination days after burial[a] | | |
|---|---|---|---|
| | 2 days | 4 days | 8 days |
| | % | % | % |
| none | 38 | 53 | 54 |
| glucose | 23 | 44 | 50 |
| glucose + nitrogen | 30 | 20 | 22 |
| nitrogen | 26 | 29 | 27 |
| cellulose | 21 | 54 | 49 |
| cellulose + nitrogen | 11 | 18 | 28 |
| cellulose + glucose | 17 | 24 | 45 |

[a]Based on the average of three replications with 100 conidia in each.

The survival curve (Figure 7) only indicated the percentage of the original population which survived and did not indicate the amount of growth resulting from the surviving conidia. Cercosporella characteristically produced hyphal swellings as it grew through the soil. By counting hyphal swellings it was possible to estimate the relative growth of the surviving population. Combining this growth data with the survival data gave a more accurate indication of the fungal density on the slides. The following formula was used to calculate the relative fungus density.

$$\text{Relative fungus density} = \text{Percentage original population surviving} \times \text{Number of hyphal swellings per 100 conidia}$$

Abundant growth and production of hyphal swellings occurred in the glucose and glucose plus cellulose amended soils (Figure 8). The relative fungal density increased during the second month of incubation in both treatments despite the estimated loss of 80 to 85 percent of the original conidial population. The fungal density in the cellulose amended soil was also greater in comparison to the control soil but to a lesser degree. Additions of $NH_4NO_3$ to the glucose and cellulose amended soils negated their stimulatory effect.

The cell walls of the swollen hyphae melaninized as early as eight days in the glucose amended soils and 16 days in the glucose plus cellulose treatment (Figure 10). Similar cells appeared on slides incubated in a moist chamber rather than in soil (Figure 11). Limited melanin formation occurred in the glucose plus nitrogen and cellulose amended soils at 16 and 64 days respectively. Melaninization did not occur in the fungal cells produced in the control soil.

Germination and survival of conidia was minimal in the soils amended with both carbon and nitrogen. However, conidia that did germinate grew more vigorously than those in the control soil. To graphically illustrate this fact a relative growth rate for each germinated conidium was determined by dividing the total number of hyphal swellings observed for one hundred conidia by the percentage

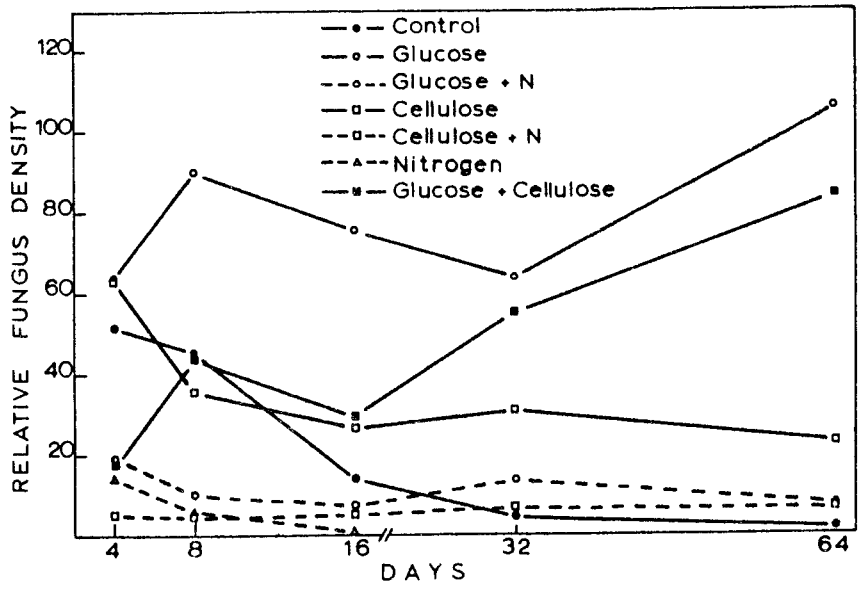



Figure 8. The influence of soil amendments on the saprophytic activity of C. herpotrichoides on glass slides buried in the soil. Each point represents the average of three replications with 100 conidia in each.

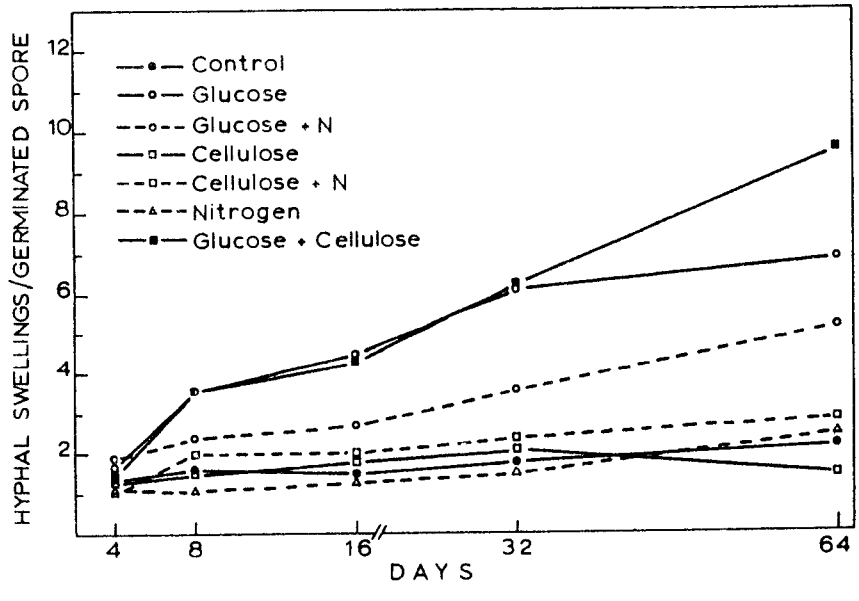



Figure 9. The influence of soil amendments on hyphal growth following germination of conidia of C. herpotrichoides buried on glass slides in the soil. Each point represents the average of three replications with 100 conidia in each.

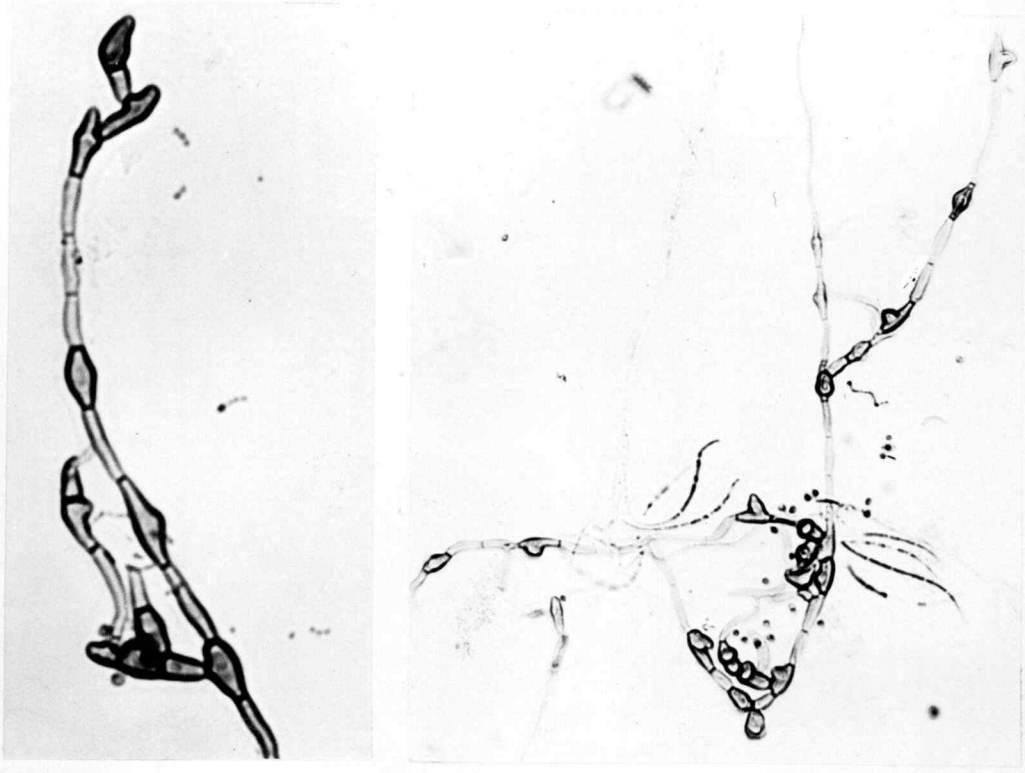


Figure 10. Melanized cell walls of swollen hyphae of C. herpotrichoides in glucose amended soil.

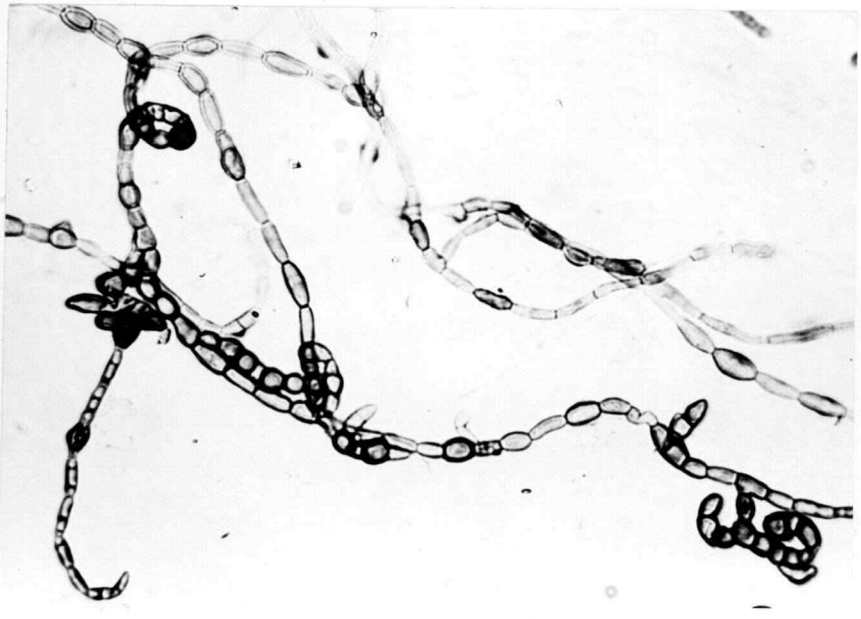


Figure 11. Melaninized cell walls of C. herpotrichoides in a glucose solution incubated in a moist chamber.

of those conidia which germinated. Data processed in this manner indicated the effect of the soil amendment on the growth of individual conidia after they had germinated. This differed from the "relative fungal density" which indicated the effect of the amendment on total fungal growth. Cercosporella herpotrichoides was able to maintain continuous growth throughout the two month period of observation only in glucose amended soils (Figure 9). Apparently utilizable carbon was unavailable in the other soils and the pathogen made very little growth after eight days. Many other soil fungi, bacteria and actinomyces grew on the slides in close association with the pathogen. A majority of those fungi that were initially stimulated by the glucose amendments were lysed or converted to resting structures after one month.

## Competitive Saprophytic Ability of Buried Conidia

While observing the germination and survival of conidia on slides buried in the soil, small pieces of organic debris were observed to be colonized by C. herpotrichoides (Figure 13). The sporulation associated with this debris verified the presence of the pathogen. Two hypotheses could explain the origin of these colonized pieces: previously colonized debris was present in the soil and came in contact with the slides at the time of burial, or the conidia sprayed onto the slides germinated and colonized the debris. The competitive saprophytic colonizing ability of the fungus has been studied by Macer

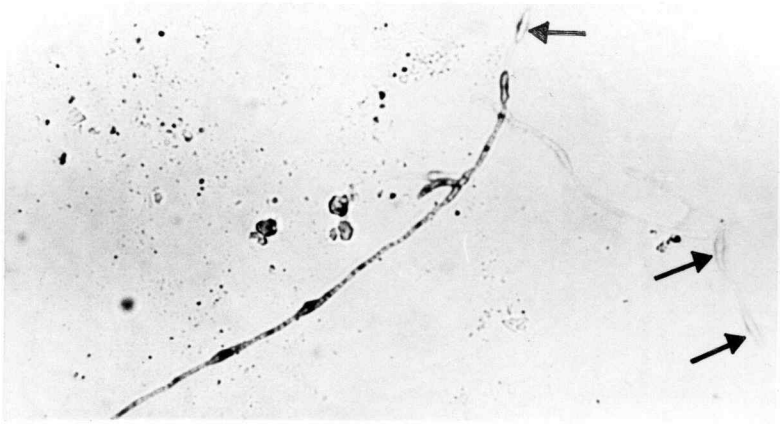


Figure 12. New hyphal growth of C. herpotrichoides arising from hyphae surviving 16 months on a glass slide buried in soil at 5°C. Arrows indicate hyphal swellings of the 16 month old hyphae.

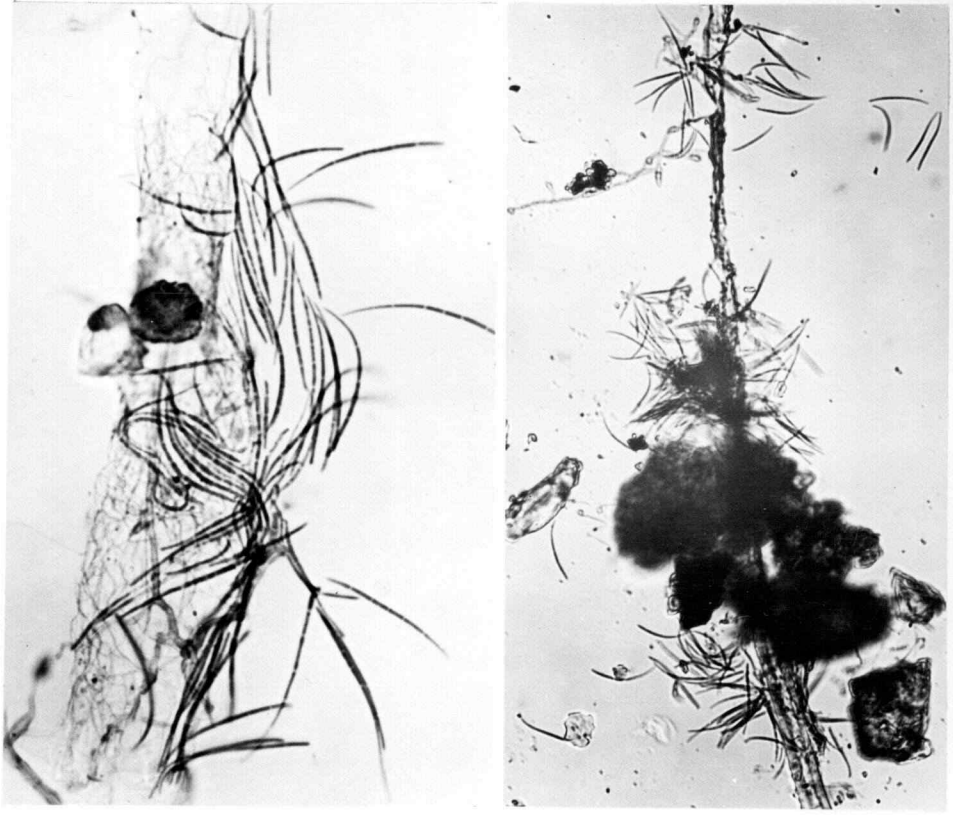


Figure 13. Soil organic debris colonized by C. herpotrichoides observed on glass slides buried in the soil.

(1961a); his data indicated an extremely low saprophytic ability. In his studies the soil was fortified with sand-cornmeal cultures. Sand-cornmeal cultures introduce nutrients into the soil along with the inoculum. Conidial inoculum was used in my study to eliminate the influence of a food base and simulate a more natural inoculum. Laboratory and field experiments were designed to test the competitive saprophytic ability of conidia buried in the soil.

Laboratory Determination

The soil was infested with washed conidia to yield inoculum densities of 500, 5,000, 50,000 and 360,000 propagules per gram of soil. Small straw pieces were added to the soil and incubated for one, two or six months at either 10° or 20°C. The percentage of straw pieces colonized was determined.

The pathogen colonized a portion of the straw pieces. Colonization was directly associated with the inoculum density, the higher the density the greater the number of straws colonized (Table 4). The percentage of straws colonized was inversely related to the length of incubation in the infested soil, suggesting that a portion of the initial colonization was superficial.

Baker, Maurer and Maurer (1967) have suggested that plotting inoculum density versus number of infections on a log-log basis will indicate whether a rhizoplane or rhizosphere effect is operative.

A log-log plot of my experimental data is represented by Figure 14. The percentage colonization was substituted for infections in Baker's scheme. The slopes of these curves (.5) are flatter than a rhizoplane curve theorized by Baker, suggesting the stimulation of fungal activity on the buried straw pieces is less than one might expect on the surface of a actively exudating host plant.

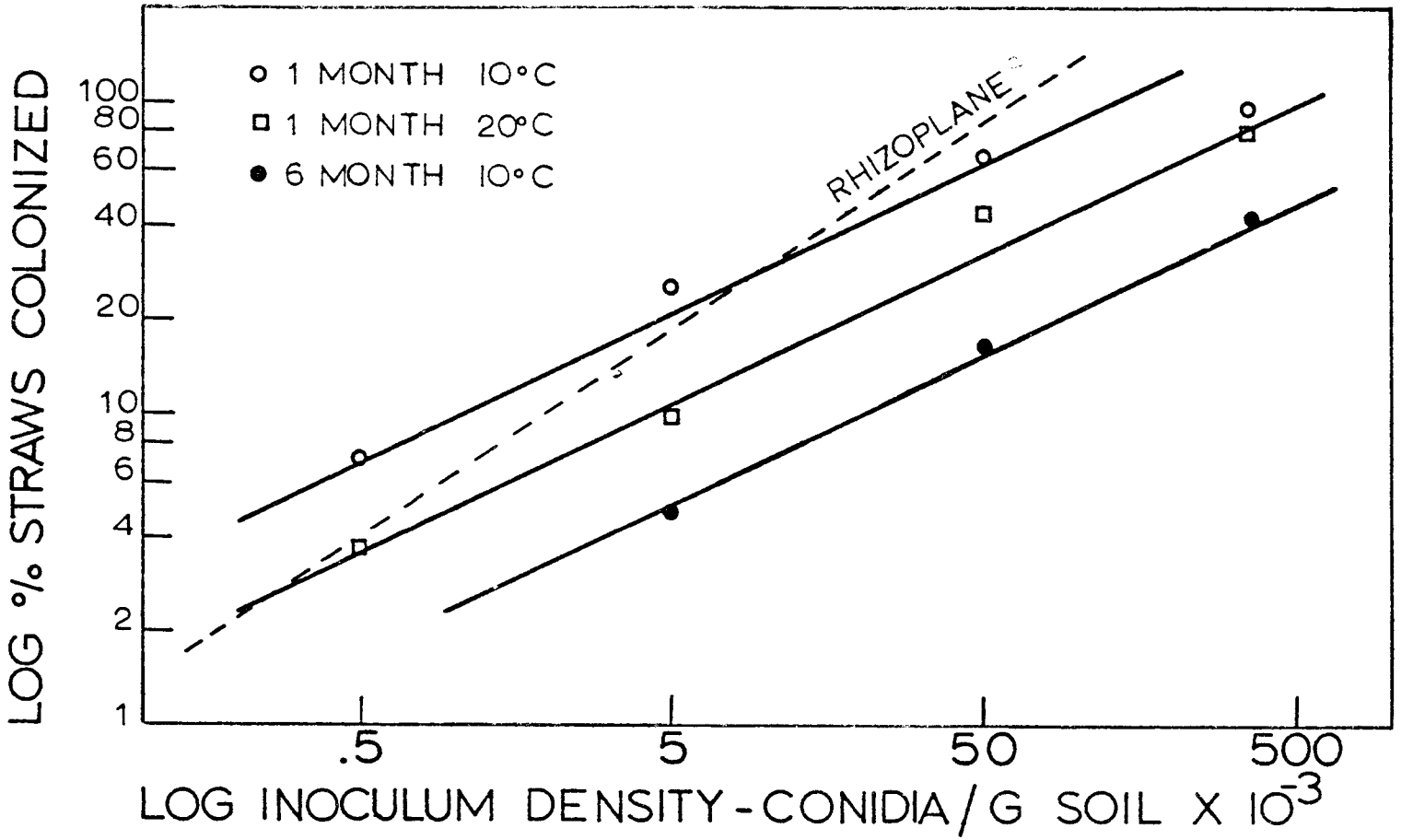
Table 4. Influence of inoculum density on competitive saprophytic ability of conidia of C. herpotrichoides buried in soil.

| Inoculum density (conidia per gram dry soil) | Percentage of straws colonized[a] 1 month | | 2 month | 6 month |
|---|---|---|---|---|
| | 10°C | 20°C | 10°C | 10°C |
| | % | % | % | % |
| 500 | 7.2 | 3.7 | 0 | 0 |
| 5,000 | 25.5 | 9.7 | 11.0 | 4.8 |
| 50,000 | 67.5 | 44.0 | 28.2 | 16.6 |
| 360,000 | 96.0 | 79.8 | 50.0 | 43.0 |

[a]Based on 120 or more recovered straws.

## Field Determination

Field plots for this study were located on fallow land of the Sherman Branch Experiment Station at Moro, Oregon. On March 22, small straw pieces were placed in nylon mesh bags containing field soil artificially infested with conidia of C. herpotrichoides




[a]Rhizoplane postulated by Baker, Maurer and Maurer (1967).

Figure 14. The influence of inoculum density on competitive saprophytic ability of conidia of C. herpotrichoides buried in soil. Each point represents 120 or more recovered straws.

and buried six inches below the surface. They were recovered seven weeks later and the number colonized by C. herpotrichoides was determined. Treatments included various combinations of natural straws, autoclaved straws and additions of $NH_4NO_3$ to the soil. Inoculum densities of approximately 5,000 to 50,000 conidia per gram of dry soil were used.

Autoclaved straw pieces were colonized less frequently by C. herpotrichoides than non-autoclaved (Table 5). Additions of 80 ppm $NH_4NO_3$-N to the soil had little effect on the frequency of successful colonizations by the pathogen. Levels of colonization in the field were comparable to laboratory results in which the soil was incubated at 10°C and the straws recovered two months after burial.

At the time the straws were buried in the field, a sample of the field soil was taken to the laboratory. Straw pieces were placed on slides previously sprayed with conidia. The slides were buried in the soil sample and incubated in the growth chamber. Periodic microscopic examination revealed the straw pieces stimulated germination, growth and sporulation of C. herpotrichoides in their proximity (Figures 15 and 16). Conidia not in contact with the straw were not stimulated in a similar manner. A more frequent and greater stimulation was associated with non-autoclaved straws, agreeing with the results of the field study.

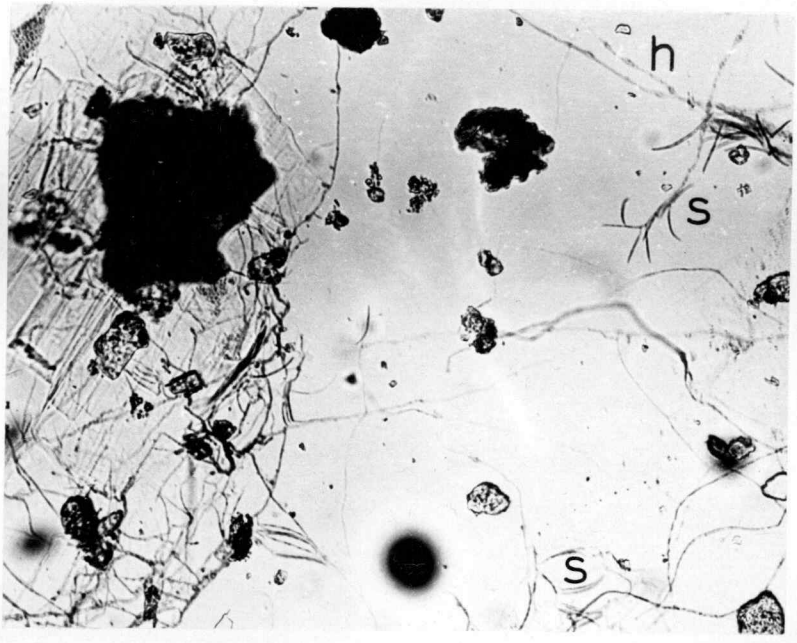



Figure 15. Stimulation of growth, characterized by hyphal swellings (h), and sporulation (s) of C. herpotrichoides on glass slides buried in soil in the proximity of uncolonized straw pieces.

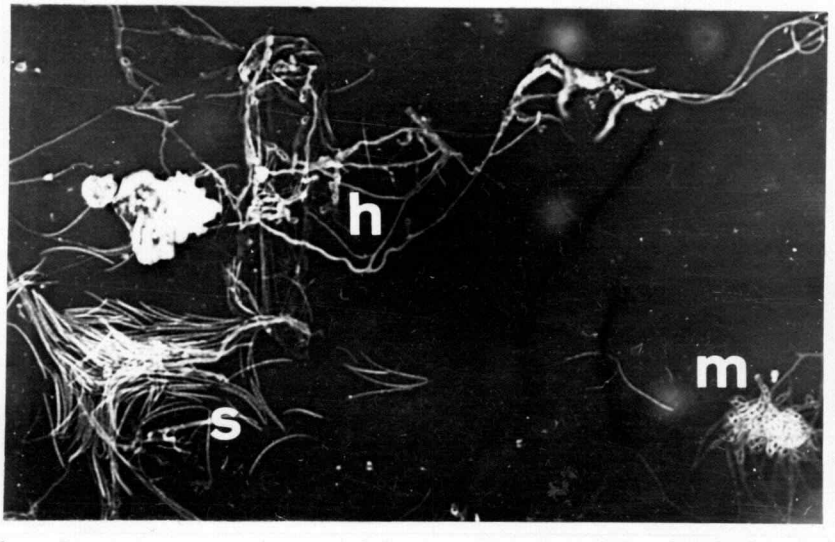



Figure 16. Stimulation of growth, characterized by hyphal swellings (h) and melaninized stroma (m), and sporulation (s) of C. herpotrichoides on glass slides buried in soil in the proximity of uncolonized straw pieces.

Table 5. Field determinations of competitive saprophytic ability of conidia buried in soil.

| Inoculum density (conidia per gram dry soil) | Treatment | Percentage of straws colonized[a] |
|---|---|---|
| | | % |
| 5,000 | | 6.8 |
| 5,000 | autoclaved | 2.3 |
| 5,000 | nitrogen ($NH_4NO_3$) | 7.2 |
| 5,000 | autoclaved + nitrogen ($NH_4NO_3$) | 0.6 |
| 50,000 | | 31.3 |
| 50,000 | autoclaved | 20.0 |
| 50,000 | nitrogen ($NH_4NO_3$) | 25.4 |
| 50,000 | autoclaved + nitrogen ($NH_4NO_3$) | 25.5 |

[a]Based on the average of four replications with 45 or more straws recovered in each.

## Survival in Colonized Straws

### Influence of Burial Depth

Stubble mulching is commonly practiced by growers in the Columbia Basin. Stubble mulching leaves the straw from the previous crop at or near the soil surface during fallow periods. Moldboard plowing in contrast buries the straw below the soil surface. A field experiment was established at the Sherman Branch Experiment Station to determine if different fallow practices might influence survival of the fungus.

To determine the effect of burial and surface exposure on survival of C. herpotrichoides, artificially colonized straw pieces were buried at either three inches, nine inches or placed at the soil surface. The straws were buried in June and samples were taken at three month intervals for 15 months. Decomposition of the straw pieces was also determined by weight loss.

The fungus survived equally well at all three locations for the first nine months (Figure 17). However, at the end of 15 months, 22 percent of the straw pieces placed at the soil surface were still colonized by the pathogen as contrasted with 1.6 and 0.8 percent at the three and nine inch depths respectively. As might be expected the buried straws, decomposed more rapidly than those on the surface (Figure 18). Under the cool, moist soil conditions from September to March (Tables 6 and 7), Cercosporella was able to maintain its population level in the colonized straws. Reduced competition from other soil microorganisms at these cooler temperatures combined with the fact that the fungus attains its maximum growth at these temperatures (Dickens, 1964) probably contributed to its successful survival. Both decomposition of the straws and a declining Cercosporella population were noted in the late spring and early summer. Apparently the warm, moist conditions in the soil during this period favored an increased biological activity in the soil which was detrimental to the pathogen.

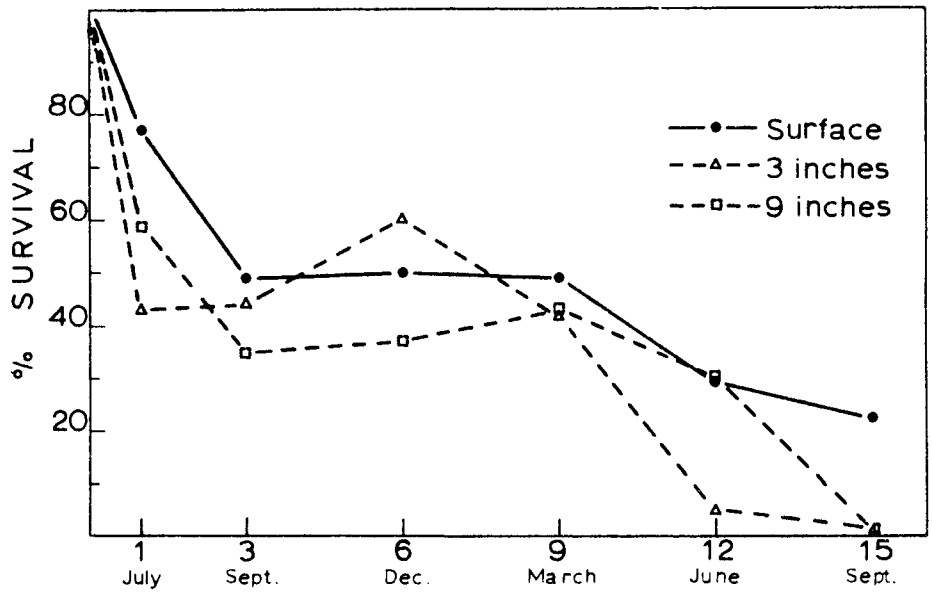



Figure 17. Survival of C. herpotrichoides in artificially colonized straws placed at the soil surface or buried three or nine inches. Each point represents the average of four replications with 30 recovered straws in each.

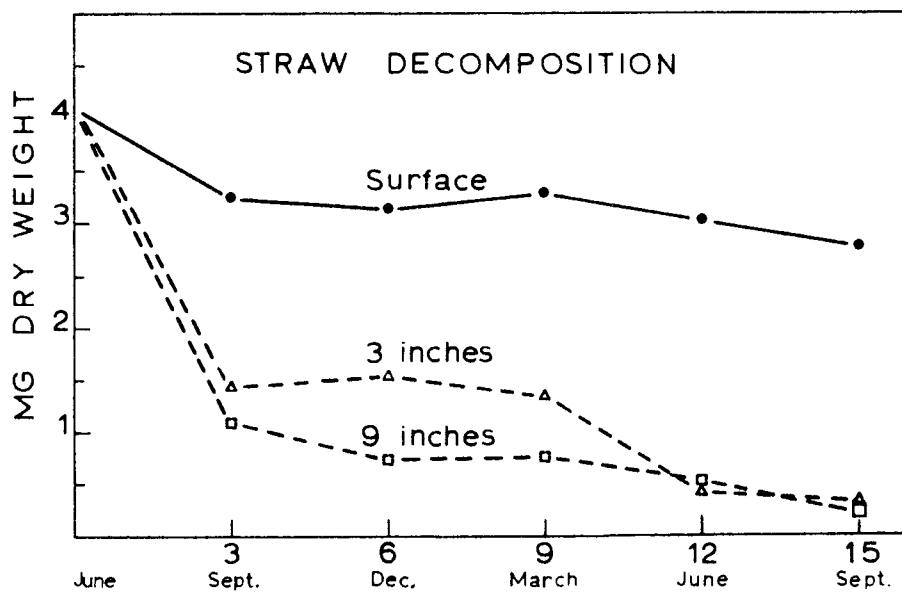



Figure 18. Decomposition of straws artificially colonized with C. herpotrichoides placed at the soil surface or buried three or nine inches. Each point represents the average of four replications with 30 recovered straws in each.

Table 6. Soil temperatures recorded during 1966-67 in field plots at Moro, Oregon.

| Month | Temperature | | | |
|---|---|---|---|---|
| | surface | 3 inches | 6 inches | 9 inches |
| | °C | °C | °C | °C |
| August | 50 | 30 | - | 24 |
| September | 34 | 22 | - | 18 |
| December | 6 | 4 | 4 | 4 |
| March | 25 | 11 | 10 | 8 |
| May | 23 | 13 | 12 | 11 |
| June | 19 | 20 | 21 | 23 |
| July | 37 | 26 | - | 24 |
| September | 23 | 20 | 20 | 21 |

Table 7. Soil moisture recorded during 1966-67 in field plots at Moro, Oregon.

| Month | Percentage moisture | | | |
|---|---|---|---|---|
| | surface | 3 inches | 6 inches | 9 inches |
| | % | % | % | % |
| July | - | 7.5 | - | 8.9 |
| August | - | 6.4 | - | 8.1 |
| September | - | 4.3 | - | 8.9 |
| December | 19.2 | 18.1 | 17.3 | 17.6 |
| March | 5.1 | 13.0 | 12.5 | 14.6 |
| May | 3.6 | 11.2 | 13.5 | 13.6 |
| June | 3.3 | 8.4 | 10.7 | 11.6 |
| July | 1.5 | 5.4 | - | 14.8 |

At the time the colonized straws were buried in the field, colonized straw pieces were also placed in contact with glass slides and buried in soil in the laboratory. Cercosporella herpotrichoides was observed growing from the straws across the glass slides (Figure 19). Growth was characterized by hyphal swellings and formation of melaninized stromatous cells. Abundant sporulation also occurred on the surface of the buried straws (Figure 20).

Influence of Time of Burial

Although spring tillage is more commonly practiced and recommended, some growers perform their initial tillage operation in the fall. Fall plowing is recommended where soil freezing commonly occurs or where weeds and volunteer grain are a severe problem (Horning and Oveson, 1962). A field experiment was established at Moro, Oregon to determine whether fall or spring tillage might influence the survival of the pathogen in colonized straw. In September naturally infected straw pieces were placed at the surface or buried six inches in the soil. Both stem and leaf sheath material were used; they were incubated separately. In March a portion of the surface straws were buried six inches, the remainder were left on the surface. The survival of C. herpotrichoides was determined at three month intervals throughout the year.

Eighty-five percent of the straws left on the soil surface were

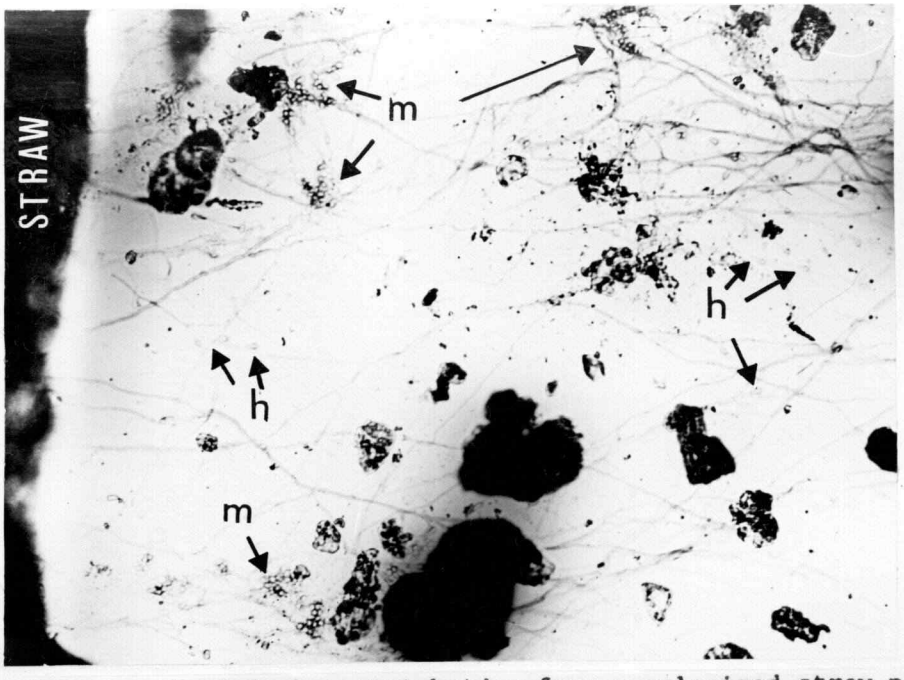



Figure 19. Growth of C. herpotrichoides from a colonized straw placed on a glass slide and buried in the soil. The fungus is characterized by hyphal swellings (h) and melaninized stromatous groups of cells (m).

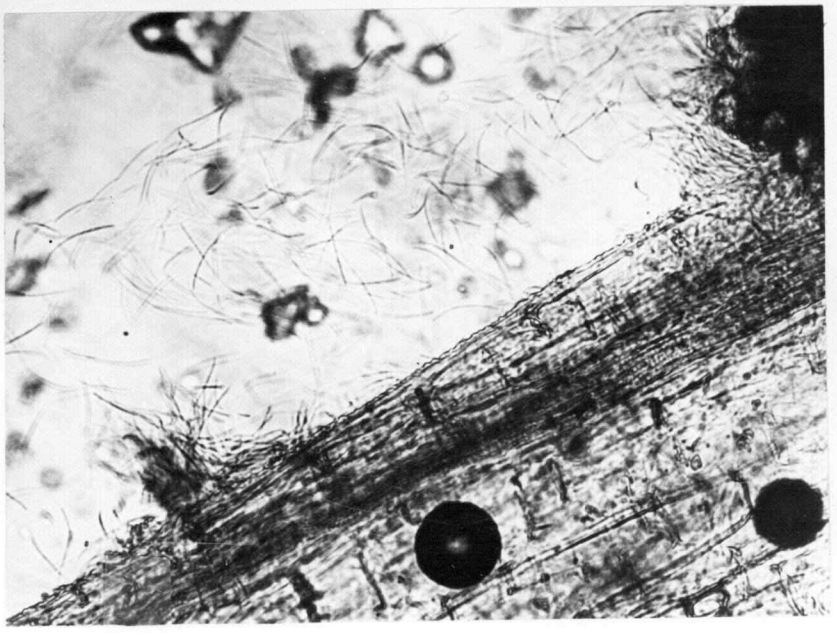


Figure 20. Sporulation of C. herpotrichoides on a colonized straw buried in the soil.

still colonized at the end of one year, compared to 21 percent of straws remaining on the surface during the winter and then buried in the spring (Figure 21). Thirty-five percent of those buried in the fall were colonized after one year. Straw pieces left at the surface during the winter and buried in the spring decomposed at a faster rate when introduced into the soil than those continuously buried (Figure 23). Survival of the pathogen in the leaf sheath material was very similar to that in the stem tissues (Figure 22).

In summary, survival of C. herpotrichoides was favored in straws placed on the surface when compared to those buried in the soil. Studies involving artificially colonized stem tissue, naturally infected stem tissue and naturally infected leaf sheaths were all consistant in this respect. No differences were seen in the survival of pathogen after one year in straws buried either in the spring or fall.

## Disease Development Below the Soil Surface

### Laboratory Demonstration

In the preceding experiments growth of C. herpotrichoides was observed from introduction of conidia and colonized straws into the soil. Experiments were thus designed to test the possibility that conidia and colonized straw pieces buried in the soil could also serve as inoculum sources for infection of stem tissue below the soil

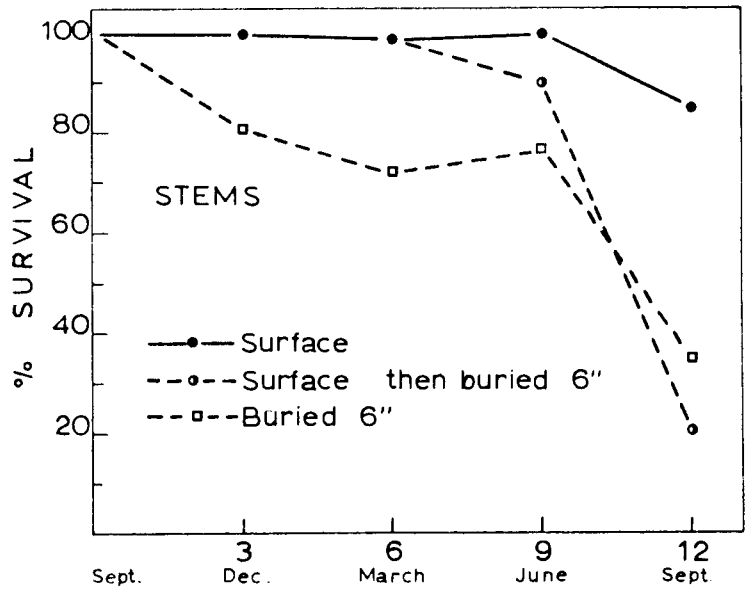



Figure 21. The influence of fall and spring burial on the survival of C. herpotrichoides in naturally infected straws. Each point represents the average of four replications with 25 straws recovered in each.

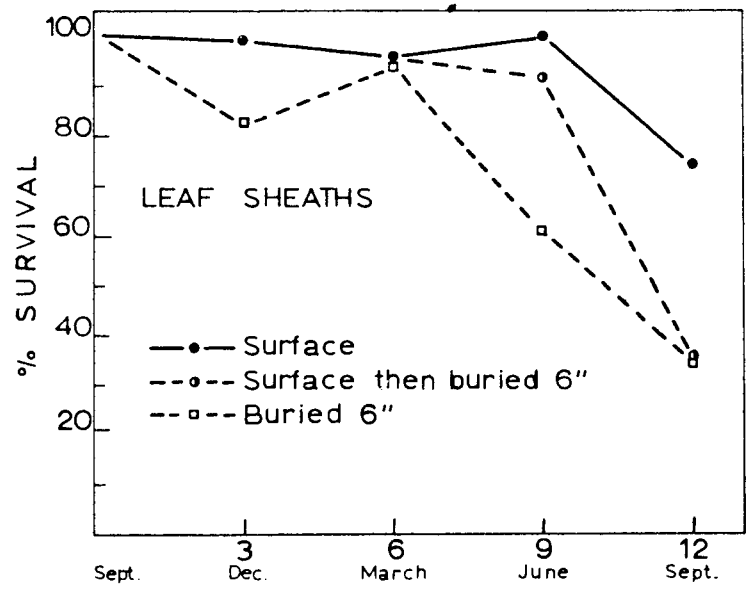



Figure 22. The influence of fall and spring burial on the survival of C. herpotrichoides in naturally infected leaf sheaths. Each point represents the average of four replications with 30 sheaths recovered in each.

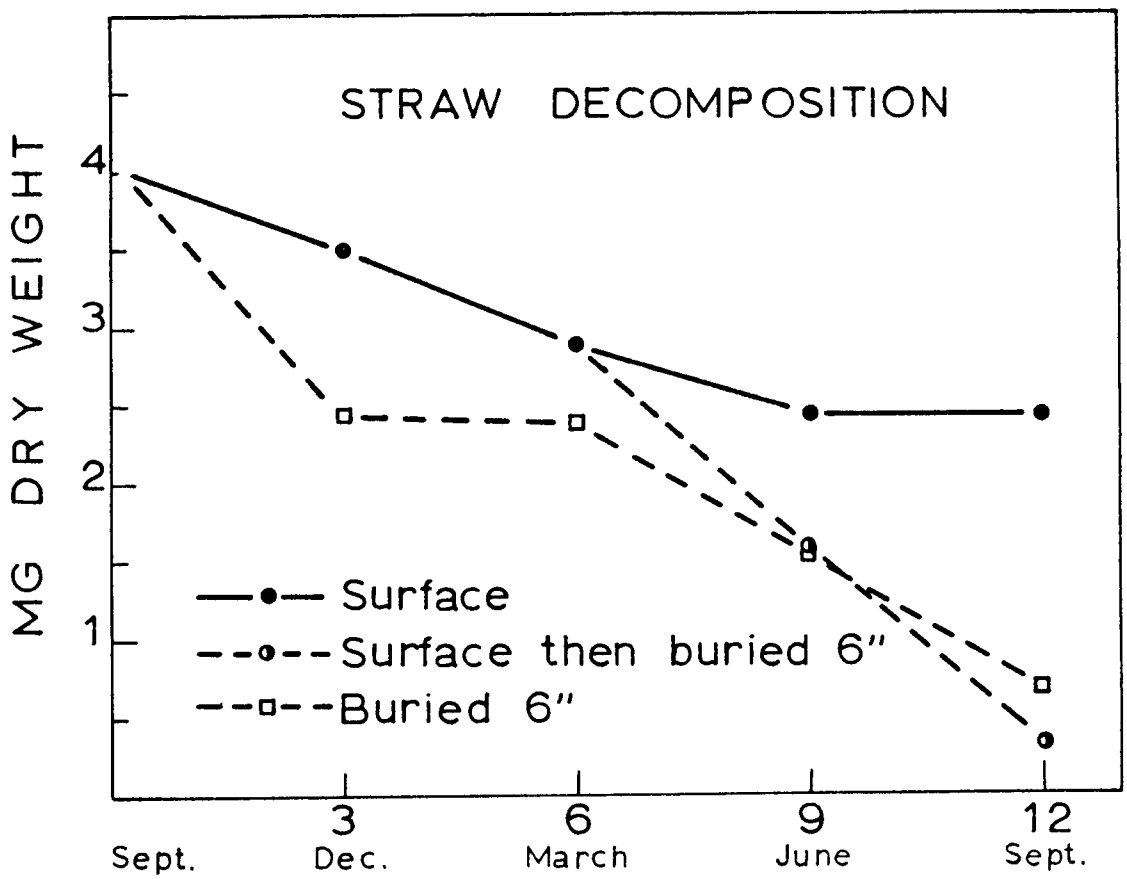



Figure 23. The influence of fall and spring burial on the decomposition of Cercosporella colonized straws.

surface. Sheinpfug (1964) has demonstrated infections resulting from conidia sprayed on the soil surface. He also suggested infections resulting from the burial of infected straws were due to the movement of spores in the soil water from the straw to the host plants.

Conidia were added to the soil to yield inoculum densities of 1,250, 2,500, 5,000, 10,000 and 50,000 propagules per gram of dry soil. A portion of this soil was placed in the seed trough of growth pouches to cover the plumule of 20 day old seedlings. The remaining soil was incubated at either 10° or 20°C for one or two months before being placed in contact with wheat seedlings.

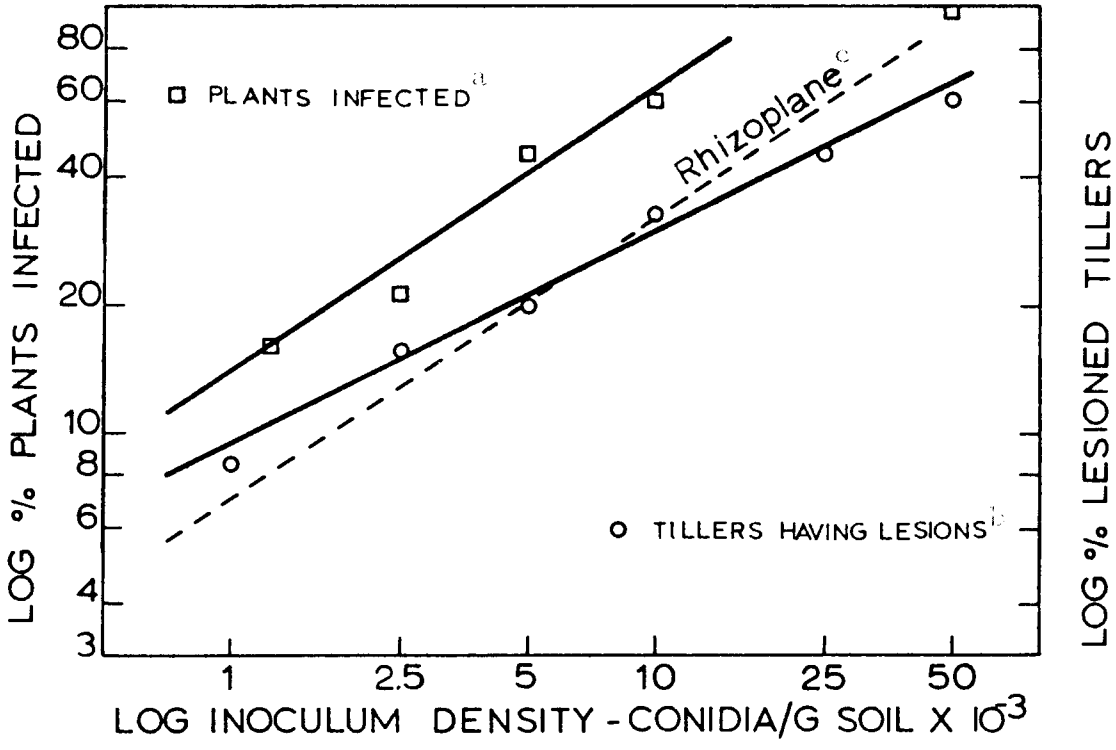
Two months after the seedlings were inoculated lesions were visible on the underground portions of stems. Lesion development at this time was slight, especially at the lower inoculum levels. Infections were thus determined by incubating stem sections on SWA. The number of infected plants was directly related to the inoculum density of the soil (Table 8). The inoculum potential of the pathogen in soil decreased with time, decreasing more rapidly in the soil incubated at 20°C. This agreed with the conidial survival study in that cool temperatures favored the survival of buried conidia (Figure 5). Unfortunately the soils became rather dry several times during their two month incubation which probably limited survival of a portion of the propagules.

Table 8. Influence of the inoculum density of buried conidia of C. herpotrichoides on incidence of infections below the soil surface.

| Inoculum density (conidia per gram dry soil) | Percentage of plants infected after incubating soils various intervals following conidial infestation[a] | | | |
|---|---|---|---|---|
| | 0 | 1 month | | 2 months |
| | | 10°C | 20°C | 10°C |
| | % | % | % | % |
| 0 | 0 | 0 | 0 | 0 |
| 1,250 | 16 | 0 | 0 | 0 |
| 2,500 | 21 | 5 | 0 | 0 |
| 5,000 | 45 | 0 | 0 | 10 |
| 10,000 | 60 | 50 | 0 | 15 |
| 50,000 | 100 | 70 | 10 | 20 |

[a]Based on the average of four replications with five plants in each.

Ploting the number of plants infected versus the inoculum density on a log-log scale resulted in a curve having a slope very similar to the rhizoplane curve (0.67) postulated by Baker, Maurer and Maurer (1967) (Figure 24). A slope of 0.5 rather than 0.67 was determined in a second experiment (Figure 24). Conditions of the second experiment varied slightly from first and may explain this difference. In the second experiment the plants were grown three months following inoculation before they were examined. With this longer incubation, lesions were better developed than in the first experiment. Thus it was possible to record the number of tillers




[a]Each point based on the average of four replications with five plants in each.

[b]Each point based on the average of six replications with five plants in each.

[c]Rhizoplane postulated by Baker, Maurer and Maurer (1967).

Figure 24. The influence of inoculum density on infection incidence and symptom development in soil artificially infested with conidia of C. herpotrichoides.

having visible lesions rather than plating the stems on SWA. Data from the first experiment is more indicative of the number of infections originally influenced by the inoculum densities. The second experiment may be influenced by factors other than the original levels of infection since it was based on symptom development.

Table 9. The incidence of infections below the soil surface from burial of C. herpotrichoides colonized PDA plugs and naturally infected straws.

| Placement of inoculum | Type of inoculum | Percentage plants infected | Percentage tillers infected |
|---|---|---|---|
| | | % | % |
| contact seedling[a] | PDA plug | 100 | |
| 5 mm away | PDA plug | 20 | |
| 10 mm away | PDA plug | 5 | |
| contact seedling[b] | infected straw | 77 | 41 |
| 5 mm away | infected straw | 100 | 79 |

[a]Based on 20 plants. Infections determined on SWA two months after inoculation.

[b]Based on 10 plants. "Infections" were determined by lesion development three months after inoculation.

Five-mm PDA culture plugs of C. herpotrichoides were placed in contact with 5 mm or 10 mm from 20 day old wheat seedlings. Two months after inoculation the number of plants infected was determined by plating stems on SWA. In a second experiment, naturally infected straw pieces were either placed in contact with

wheat seedlings or positioned 5 mm away. Plants were incubated three months and the number of plants and tillers infected was determined by lesion development. Table 10 summarizes both these experiments. The results indicate the pathogen is able to grow through the soil from an adequate food base to the surface of the host where infection of the stems occurs below the surface of the soil.

Table 10. Germination of conidia on seedling coleoptiles and glass slides in the soil.

| Germinating surface | Surface location | Treatment | Percentage germination[a] |
|---|---|---|---|
| | | | % |
| glass slide | in soil | - | 21 |
| coleoptile | in soil | - | 56 |
| coleoptile | in soil | 150 ppm N | 48 |
| coleoptile | in soil | 300 ppm N | 47 |
| coleoptile | above soil | - | 86 |

[a]Based on the average of three replications and 100 conidia in each.

Activity of Conidia on the "Rhizoplane"

Washed conidia of C. herpotrichoides were sprayed on 12 day old wheat seedlings and the lower 20 to 25 mm covered with soil. Their activity was observed periodically. Three days following burial, conidia on the plant surface had a higher percentage germination than those buried on glass slides (Table 10). Additions of $NH_4NO_3$ to the

soil had little effect on germination of conidia on the coleoptiles. In a previous experiment $NH_4NO_3$ strongly inhibited germination of conidia on glass slides buried in the soil (Table 3). A higher percentage of conidia germinated on coleoptiles above the soil surface than on those in contact with the soil. These data suggest that conidia on the "rhizoplane" are affected by the host and the surrounding soil environment.

Growth following germination of the pathogen on the host surface was quite restricted until the host tissue began to senesce about two weeks after inoculation. During the period of restricted growth the fungus apparently made attempts to enter the host. Intercellular appresoria were formed. Host cell walls stained heavily with cotton blue below these swollen hyphae indicating a host reaction to the pathogen (Figure 26). This cell wall reaction was not specific to C. herpotrichoides since many other apparently "non-pathogenic" fungi and bacteria growing on the host surface caused similar reactions. As the host tissue started to senesce, growth of the fungus increased rapidly. Stromatic hyphal cushions were formed (Figure 27) and abundant sporulation occurred (Figure 25). The most abundant activity occurred just below the soil line and gradually decreased with depth in the soil.

Figure 25. Abundant sporulation of C. herpotrichoides on the surface of a coleoptile just below the soil surface.

Figure 26. Intercellular appressoria on a coleoptile below the soil surface.

Figure 27. Melaninized stromatic cushions on a coleoptile below the soil surface.

Field Observations

The preceding laboratory studies demonstrated the pathogen's ability to cause infections below the soil surface; however, any indication that this occurs in the field seems to be lacking in the literature. Field observations were made to determine if infections do occur below the soil line under natural field conditions.

Two miles north of the summit of the Tygh Grade in Wasco County, a field with known Cercosporella history was seeded with 'Omar' wheat in the fall of 1966. Wheat plants showing chlorotic lower leaves were taken to the laboratory and examined for lesions. Isolations were made from lesions below the soil surface on December 13, March 20, May 11 and June 21. Cercosporella was isolated from infections located below the soil surface at every sample period. Figure 28 shows some of the plants collected in March; the crowns of the plants were located 3/4 to one inch below the surface of the soil. The three lesions occurring below 3/4 inch originated from inoculum located below the soil surface as contrasted with the eyespot lesion located at, or just above the soil line. Figure 29 shows a profile through the crownal region of an infected plant collected in June. The severe lesions are obviously located below the soil surface.

## Endemic Levels of Foot Rot

In the spring of 1966 a field plot was established near Moro, Oregon to determine the effects of spring tillage and nitrogen application on foot rot severity. The year 1966 was unfavorable for foot rot development and consequently the incidence of foot rot in the field plots was low. With only two replications, determination of differences between treatments was impossible (Table 11). However, the data does indicate that 5.7 percent (based on 2,800 tillers) of the tillers were infected in a field showing little or no foot rot symptoms. A majority of the infected tillers were severely infected and failed to head, suggesting infection had probably taken place early in the season.

Table 11. Influence of spring tillage and nitrogen application on incidence of foot rot.

| Treatment | Percentage infected tillers[a] | | |
|---|---|---|---|
| | Rep. 1 | Rep. 2 | Average |
| | % | % | % |
| none | 7.5 | 1.3 | 4.4 |
| skew tread | 1.9 | 5.9 | 3.9 |
| nitrogen (urea) | 4.2 | 5.9 | 5.0 |
| skew tread + nitrogen | 8.1 | 10.9 | 9.5 |
| overall average | | | 5.7 |

[a]Based on 350 or more tillers per replication.


Figure 28. Plants with Cercosporella lesions collected in March from a naturally infected field. The three infections below 3/4" were below the soil surface.

Figure 29. Plants with severe lesions below the soil surface. The collection was made in June from a naturally infected field in Wasco County, Oregon.

## Association of Soil Debris with Foot Rot

An experiment was designed to determine if inoculum in a natural field soil was associated only with the plant debris. The plant debris was differentially screened from a soil with a previous foot rot history. These various fractions were then individually added back to the screened soil to determine the inoculum potential of each fraction. Wheat plants were grown in the test soils throughout the winter at Corvallis, Oregon and the amount of foot rot was determined. Disease was associated with the plant debris (Table 12). Fifty-nine percent of the tillers in the unscreened soil had C. herpotrichoides infections compared with three percent in the screened soil. Plants grown in the soil with the larger debris had more foot rot than those growing in the soil containing the smaller sized fractions. The infections in the greenhouse soil probably resulted from contamination by splashing spores.

## Aerial Infection Studies

In the preceding studies, conidia, germ tubes and hyphae were often observed to be interconnected by anastomosing hyphae; this occurred on both glass slides and coleoptiles in the soil. A possibility existed that a single conidium by itself was unable to cause infection, and only when joined with other conidia would their combined inoculum

potential be high enough to cause a successful infection. Theoretically by diluting a conidial suspension, a drop containing a single conidium could be placed on a wheat stem to test this hypothesis. To perform this experiment, it was first necessary to determine conditions needed in the growth chamber for infection to occur from drop inoculation on the aerial portions of stems.

Table 12. The association of soil plant debris and foot rot severity.

| Treatment | Weight of fraction from 700 g soil | Tillers infected[a] | Plant weight (g dry wt/can) |
|---|---|---|---|
| | g | % | g |
| unscreened soil | - | 59 | 5.4 |
| screened soil + # 8 fraction | 23 | 48 | 7.9 |
| " " + #14 fraction | 50 | 35 | 10.0 |
| " " + #28 fraction | 65 | 19 | 9.1 |
| " " + #42 fraction | 82 | 8 | 13.6 |
| " " | - | 3 | 9.2 |
| greenhouse soil | - | 7 | 13.5 |

[a]Based on the average of five replications with five plants in each.

Plants were grown in vermiculite in four inch plastic pots and subirrigated as needed with a complete nutrient media. A standard amount of inoculum was placed on each stem by use of a hand drawn micropipet. Approximately one μl of a conidial suspension was placed on the stem just above the surface of the vermiculite.

Requirement for High Humidity

Other workers have indicated a high humidity is conducive to foot rot development (Sprague, 1937; Ponchet, 1959). Since the humidity in the growth chamber was relatively low (60-90%), polyethylene bags were used to raise the humidity in the infection court.

One month old plants were inoculated with a drop containing approximately 1,000 conidia. Each pot contained nine plants; eight were inoculated, leaving one as an uninoculated control. The treatments were replicated three times giving a total of 24 plants in each treatment. After inoculation the plants were incubated one, two, three and four weeks in polyethylene bags. Two months after inoculation the number of plants having foot rot lesions was recorded (Table 13). Plants left in bags three and four weeks had more infections; however, they also were chlorotic and stunted compared to those not covered. Thus, in further experiments plants were incubated in bags for 18 days following inoculation to allow sufficient infection to take place with a minimum of time in the bags.

Age of Plants Versus Susceptibility

An experiment was designed to determine if the age of plants affect their susceptibility to infection. Plants two to 13 weeks old

were inoculated with a water drop containing approximately 900 conidia. Each tiller of the plant was inoculated. Older plants had three to six tillers where as the younger plants had only one. Four pots with eight plants each were used for each age group. One additional plant in each pot was an uninoculated control. The plants were incubated in polyethylene bags for 18 days after inoculation. Visual disease readings were made periodically and when the plants were 20 weeks old a final disease determination was made.

Table 13. Incidence of foot rot influenced by incubation times in polyethylene bags following inoculation.

| Weeks in bags following inoculation | Number of plants observed | Percentage of plants infected |
|---|---|---|
| | | % |
| 0 | 20 | 0 |
| 1 | 24 | 10 |
| 2 | 21 | 29 |
| 3 | 23 | 60 |
| 4 | 23 | 74 |
| uninoculated | 12 | 0 |

Plants four to six weeks old were highly susceptible (Figure 30). A sharp drop in the number of infections occurred in plants seven to nine weeks old. Ten week old plants again increased in susceptibility after which disease incidence gradually declined. By plotting the total number of tillers with lesions versus time, an indication of the progress of the disease was obtained (Figure 31).

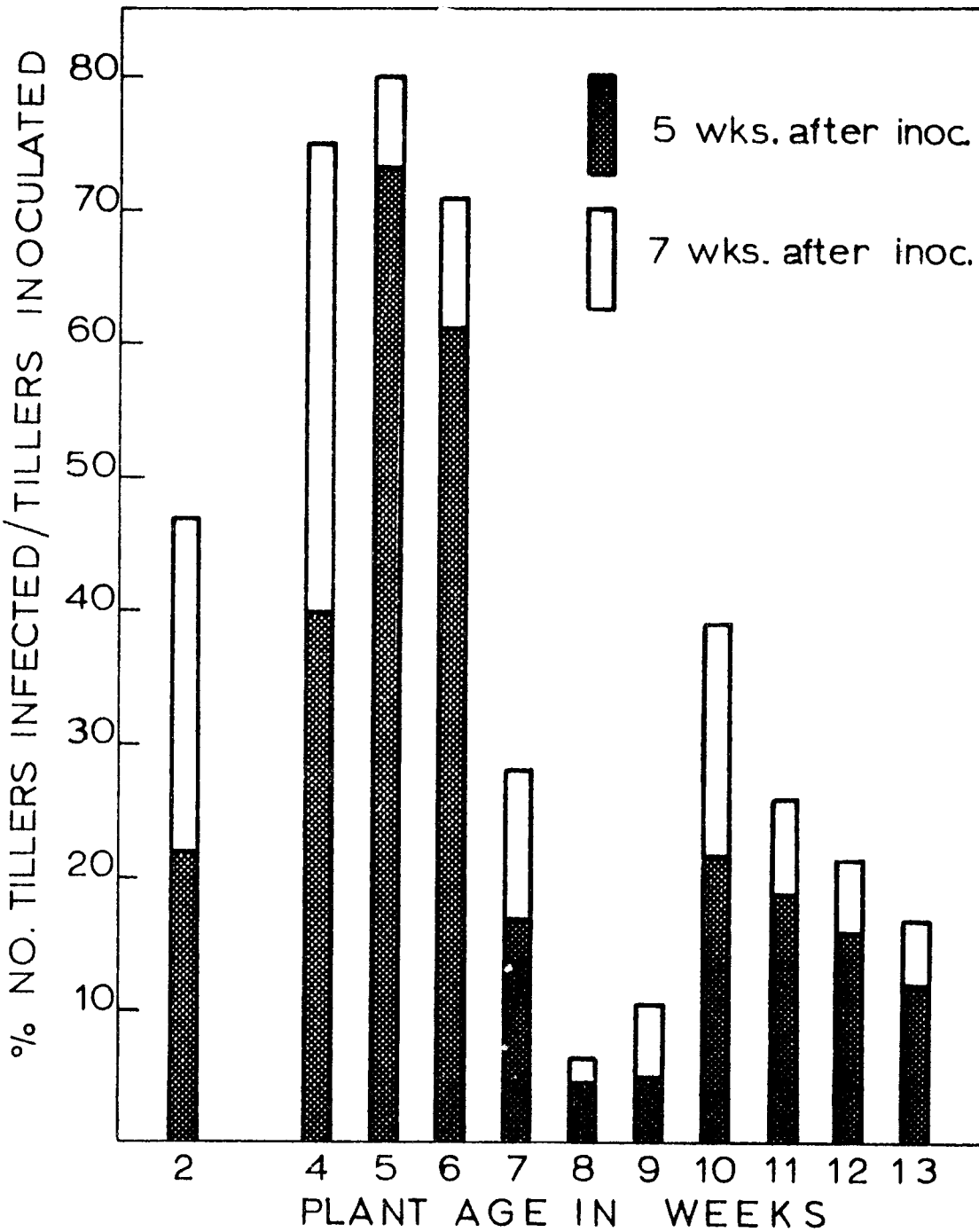



Figure 30. The relative susceptibility of different aged plants five and seven weeks after inoculation. Each point is based on the average of four replications with eight plants in each.

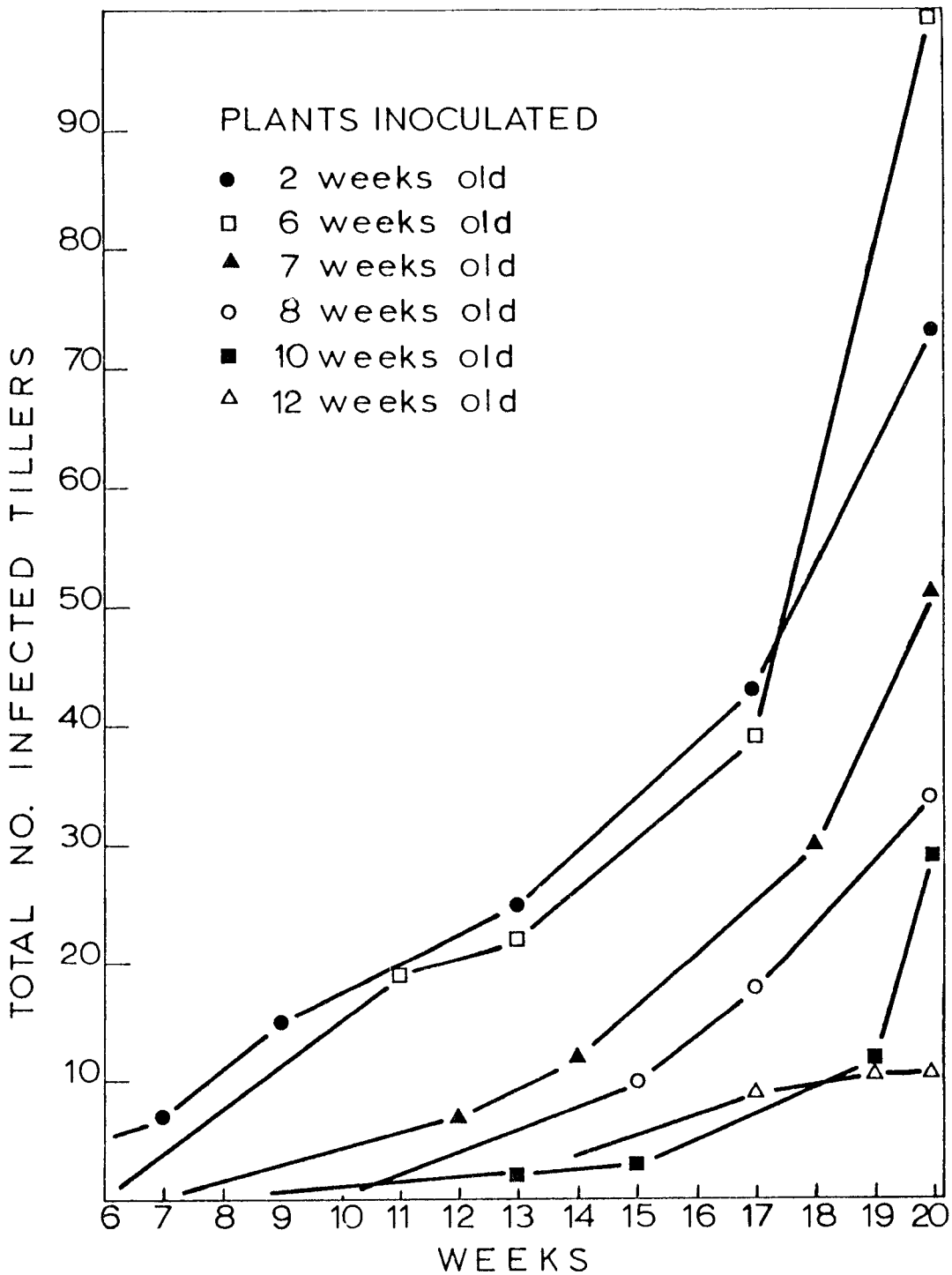



Figure 31. Progressive disease development of plants inoculated at various stages of growth determined by the total number of tillers having lesions in each age group. Each age group is represented by 32 plants.

Disease development depends not only on the original susceptibility of the plant but also on the plants subsequent growth rate and its degree of tillering at the time of inoculation.

Because of the tillering characteristics of wheat, a single infection in a young, untillered plant will result in infection of many or all of the tillers subsequently produced by that plant. New tillers arise between the leaf sheaths of the original culm. Infection is spread to the new tillers by contact with diseased leaf sheaths. If the original infection has not penetrated the sheaths interior to the new tiller, only the new tiller may become infected and the original culm will escape invasion (Figure 32, D). Slow growing and slow tillering plants allow the fungus time to penetrate each new tiller as it emerges beneath the infected leaf sheath (Figure 32, A and C). In rapid tillering plants, the infected leaf sheaths tear apart before the fungus can make contact with some of the new tillers or penetrate deep enough to reach the primary culm. This results in only a portion of the tillers becoming infected (Figure 32, B and D). Thus the final levels of disease observed in the 20 week old plants (Table 14) is the result of not only the original level of susceptibility of the plants but also the factors affecting disease development after the initial infections. Infected plants 20 weeks old, inoculated just prior to tillering (six weeks old) averaged four infected tillers per plant; these four infections resulted from one original infection (Table 14). In contrast,

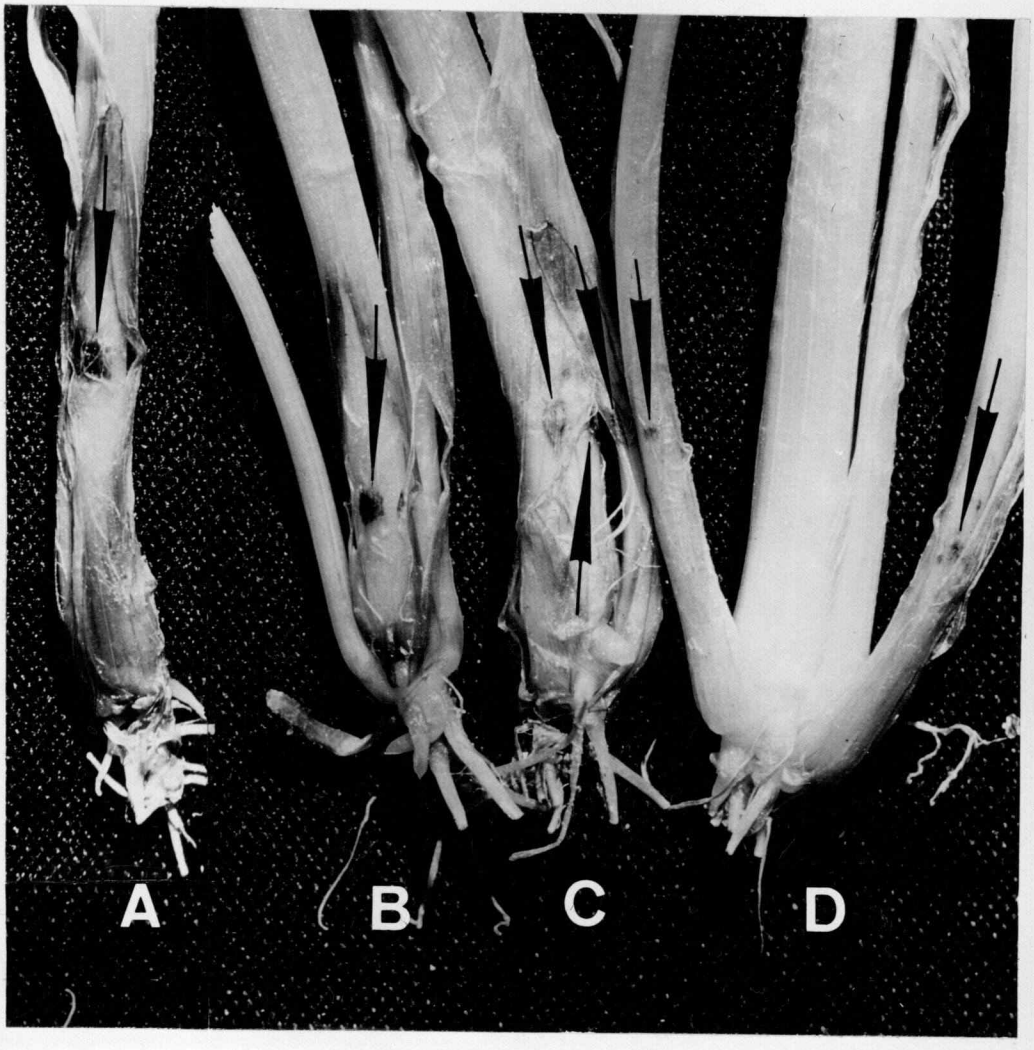



Figure 32. Incidence of diseased tillers in three month old plants. Plants were inoculated when one month old by placing a drop of a conidial suspension on their coleoptile. Arrows indicate stroma development which is indicative of infection.

plants inoculated when they were 11, 12, or 13 weeks old averaged only one infected tiller for each original infection when they were 20 weeks old.

Table 14. Disease severity of 20 week old plants inoculated at various stages of growth.

| Plant age when inoculated | Average no. tillers having lesions per plant | Total tillers having lesions | Total tillers having lesions per tiller inoculated | Plants having lesions |
|---|---|---|---|---|
| weeks | no. | % | % | % |
| 2 | 2.7[a] | 73 | 228 | 81 |
| 3 | - | - | - | - |
| 4 | 2.0 | 73 | 163 | 81 |
| 5 | 2.2 | 68 | 193 | 87 |
| 6 | 4.0 | 62 | 326 | 81 |
| 7 | 2.3 | 47 | 131 | 69 |
| 8 | 2.2 | 22 | 65 | 45 |
| 9 | 1.0 | 13 | 23 | 43 |
| 10 | 1.9 | 22 | 74 | 56 |
| 11 | 1.1 | 12 | 29 | 50 |
| 12 | 1.0 | 12 | 21 | 50 |
| 13 | 1.0 | 9 | 17 | 44 |

[a]Based on the average of four replications with eight plants in each.

Each plant in Figure 32 was the same age and was inoculated at the same time, yet they all show differences in levels of disease. Genetic variation in the host as well as differences in the micro-climate of each plant must also influence the final levels of disease.

## Infectivity of a Single Conidium

Having established that aerial infections could be achieved by microdrop inoculations in the growth chamber it was possible to conduct an experiment to determine if a single conidium is capable of causing an infection resulting in lesion development. A conidial suspension was diluted to obtain one conidium per microdrop. Ten microdrops of the final dilution were microscopically observed on a glass slide; three had no conidia, five had one conidium and two had two conidia. The average number of conidia observed in microdrops of the other dilutions are tabulated in Table 15. One month old plants were inoculated with a single microdrop of the conidial suspension placed on the coleoptile. The plants were incubated for 18 days in polyethylene bags and the number of diseased plants was determined two months after inoculation. Twenty-four percent of the plants inoculated with "one" conidium were diseased compared with 61 to 87 percent in the other higher dilutions. It would appear from this data that under "ideal" conditions it is possible for one conidium to cause infection resulting in lesion development.

## Coleoptile Versus Primary Leaf Susceptibility

A previous experiment determined the influence of pathogen on susceptibility; plants four to six weeks old were highly susceptible

compared to older (seven to nine weeks) plants (Figure 30). The younger and apparently more susceptible plants were inoculated on the coleoptile whereas the older and more resistant plants were inoculated on the primary leaf sheaths since the coleoptiles were dried and withered at this time. An experiment was initiated to determine the susceptibility of plants of the same age, inoculated either on their coleoptile or the primary leaf sheath. The results of this experiment should indicate whether the juvenile susceptibility observed in the previous experiment was due to age of the plant or to inoculation of the coleoptile.

Table 15. Incidence of disease two months after inoculation with microdrops containing various numbers of conidia of C. herpotrichoides.

| Conidia/microdrop[a] | No. of plants observed | Percentage plants infected | Percentage tillers infected |
|---|---|---|---|
| no. | no. | % | % |
| 1019 | 16 | 87 | 75 |
| 305 | 16 | 63 | 69 |
| 63 | 11 | 73 | 52 |
| 26 | 15 | 67 | 71 |
| 4.5 | 13 | 62 | 63 |
| 0.9 | 32 | 24 | 18 |
| uninoculated control | 14 | 0 | 0 |

[a]Average no. determined from microscopic observation of 10 microdrops on glass slides.

Twelve pots containing approximately eight plants in each were grown until one month old. At this time the coleoptiles were peeled back and removed from half of the plants, the coleoptiles were left intact on the remainder of the plants. The plants were inoculated with a microdrop of conidia and incubated for 18 days in polyethylene bags. Fifty-six percent of the plants inoculated on the coleoptile developed lesions three weeks after inoculation compared to only two percent of the plants inoculated on the primary leaf sheaths (Table 16). The inoculated primary leaf sheaths from ten plants were stained in fabil solution and microscopically examined to determine the fate of the conidial inoculum. Conidia had germinated on eight plants, germination varying from 10 to almost 100 percent. Anastomosis and limited growth characterized by hyphal swelling were observed on six of the plants; secondary sporulation occurred on five plants. On three plants the host reacted to the presence of the pathogen as evidenced by the thickening of the host cell walls below appresoria of the pathogen. The thick-cell walls were accompanied by heavy staining in these areas. In one of these plants the fungus successfully penetrated the host. Hyphae were visible extending through several of the subepidermal cells. Each of the host cell walls confronted by the fungus were thickened and heavily stained.

In contrast conidia placed on the coleoptiles resulted in abundant growth and sporulation. Host cell walls beneath the fungus

did not thicken nor stain heavily as they did on the leaf sheaths (Figure 36). Apparently the host offered little resistance to the invading fungal hyphae in the coleoptile tissues. Groups of stromatous cells formed on the primary leaf sheath below the coleoptile. Host cells below these fungal mats also usually failed to visibly react to the presence of the pathogen. However, in cells preceding the invading fungus cellular contents accumulated at the ends of the cells (Figure 34) and their transverse walls began to thicken (Figure 33). Usually six to 12 of these cells in each direction were invaded before the fungus was finally contained in thick-walled cells. The thickened cell walls either stained heavily or were amber-colored and non-staining. These cells formed the extremity of the lesion (Figures 33 and 35).

Table 16. Incidence of disease on plants inoculated on either the coleoptile or the primary leaf when one month old.

| Tissue inoculated | No. plants observed | Percentage of plants showing lesions weeks after inoculation | |
|---|---|---|---|
| | | 3 weeks | 9 weeks |
| | no. | % | % |
| coleoptile | 52 | 56 | 82 |
| primary leaf sheath | 48 | 2 | 28 |

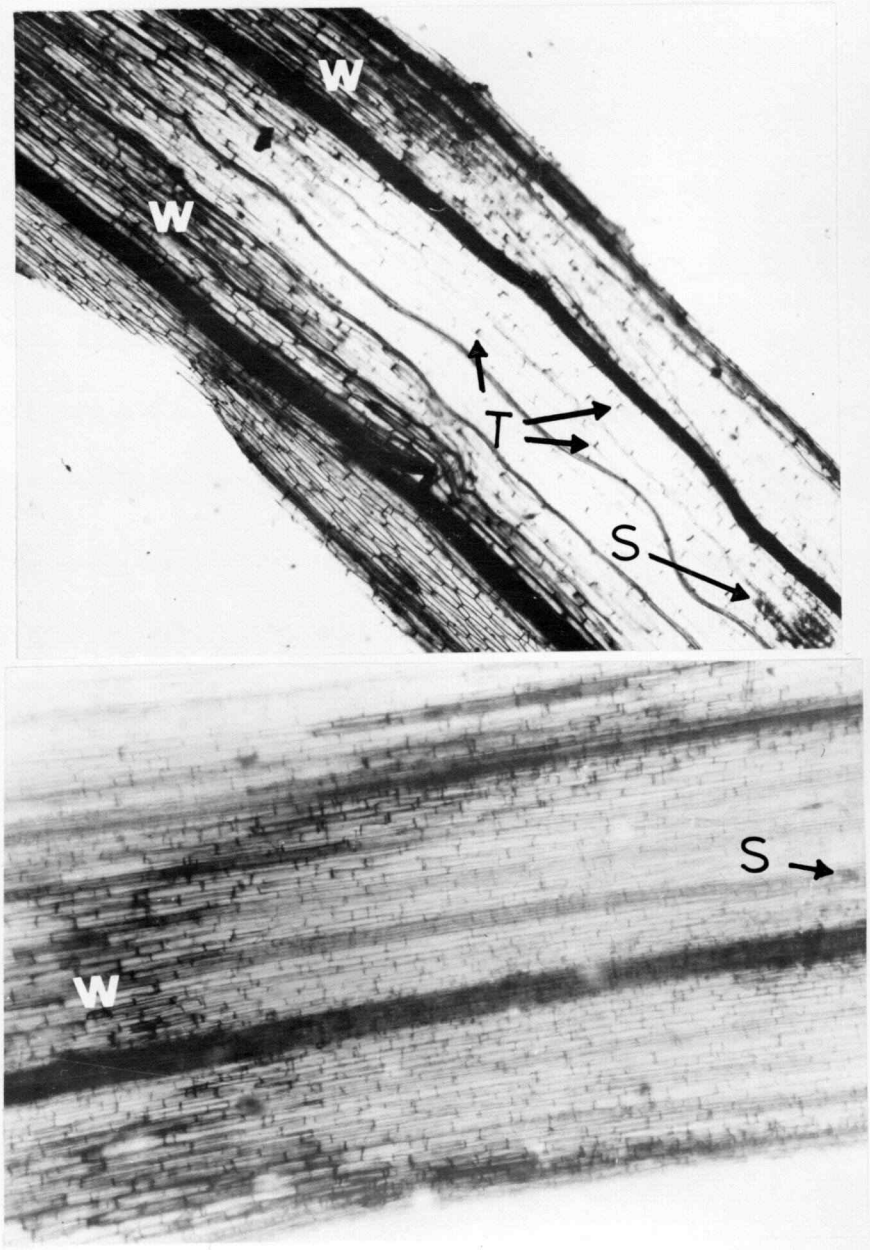



Figure 33. Lesion development on primary leaf sheaths. Only transverse cell walls are thickened (T) between the stroma (S), the site of the original invasion, and the final containment of the pathogen. The invading hyphae are contained in the heavy stained and thick-walled host cells (W) at the perifery of the lesion.

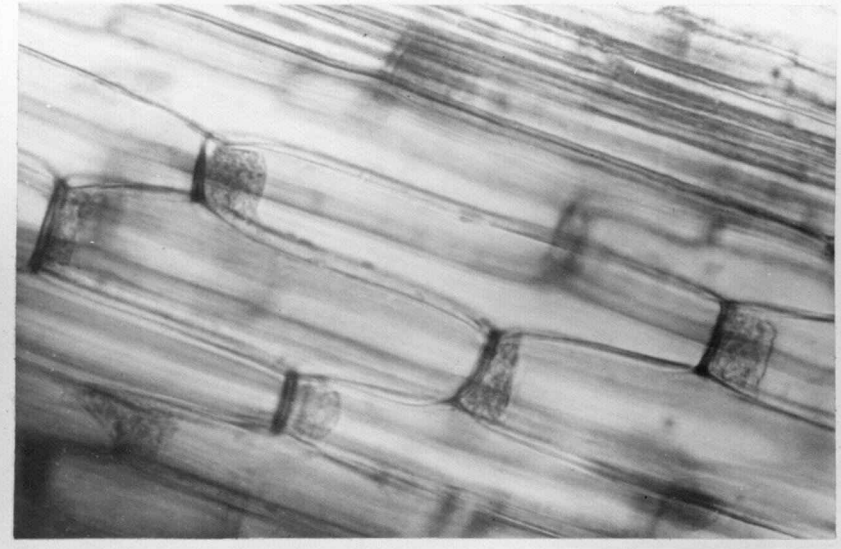


Figure 34. Accumulation of cell contents and thickening of transverse cell walls of host cells in advance of invading hyphae of C. herpotrichoides.

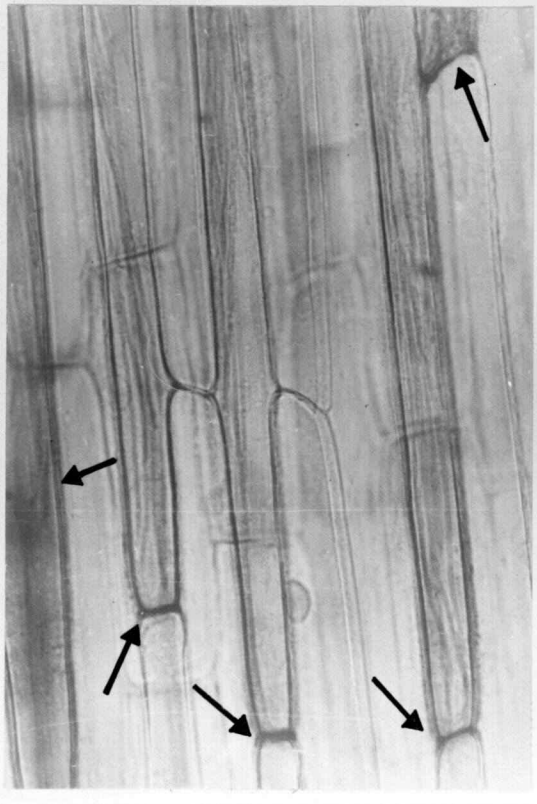


Figure 35. Hyphal containment in thick-walled cells near the perifery of a lesion.

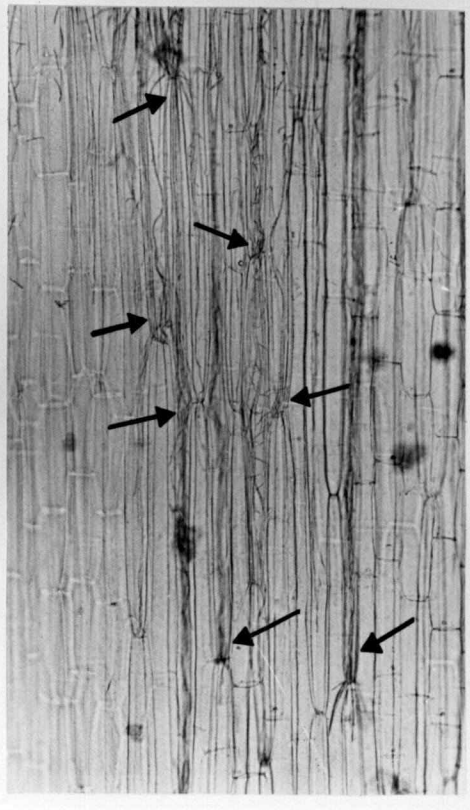


Figure 36. Hyphae ramifying cells of a susceptible coleoptile.

Nine weeks after inoculation 82 and 28 percent of the plants inoculated on the coleoptile and primary leaf sheath respectively showed lesion development. Apparently the incipient infections observed in the primary leaf sheaths were able to overcome the hosts resistance resulting in lesion development.

Influence of Leaf Sheath Senescence on Plant Susceptibility

Having established that juvenile susceptibility was associated with coleoptile inoculations, experiments were conducted to verify the observed increase in susceptibility of 10 week old plants (Figure 30) and possibly determine the reason. Plants five to nine weeks old were inoculated on primary and secondary leaf sheaths by the usual microdrop technique. After inoculation, plants were sacrificed at each age group to determine the fate of the conidia. The percentage of conidia that germinated on the primary leaf sheaths two days after inoculation is indicated in Figure 37. Also included in the figure is the percentage of plants having lesions two months after inoculation. The amount of germination on the primary leaf sheaths was fairly indicative of lesion development two months later.

One week after inoculation the fungus had penetrated into cells of the primary leaf sheaths of the nine week old plants. No visible host reactions to the invasion were seen. Sporulation and abundant growth on the surface also occurred. Cells of primary leaf sheaths

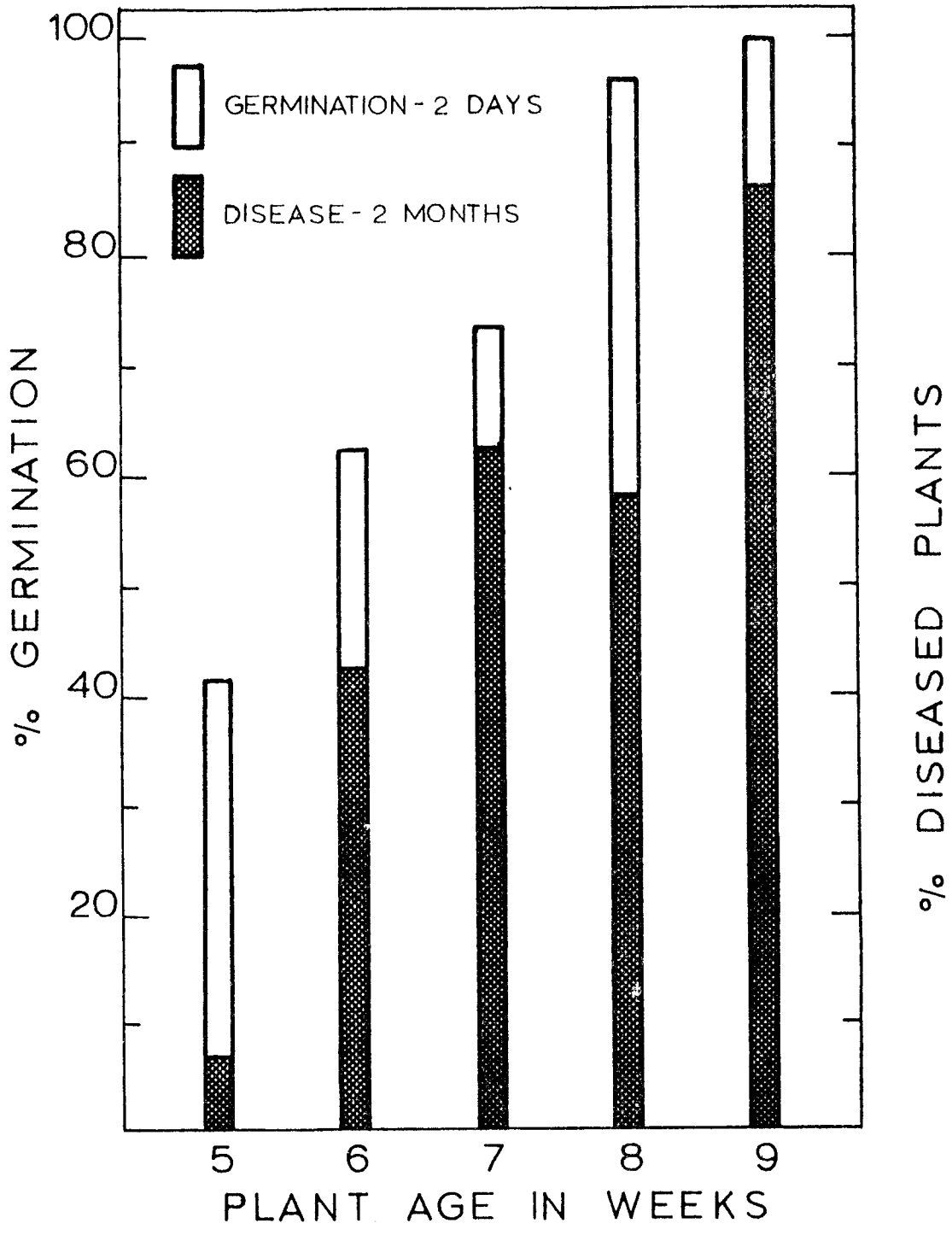



Figure 37. The germination of conidia on primary leaf sheaths of different aged plants compared to the incidence of diseased plants two months after inoculation. Germination was based on the average of three replications with 100 conidia in each. Disease was based on 24 plants.

on eight week old plants had been penetrated at one week, again no visible host reaction was observed. On plants of other ages, surface activity of the fungus was highly variable, generally germination and subsequent growth were very limited. Only occasionally had the fungus attempted to enter host cells as indicated by cell wall reactions.

In a second experiment plants from five to 10 weeks old were inoculated on the primary leaf sheaths only and plants 11 and 12 week old on both primary and secondary leaf sheaths. Disease incidence was determined by lesion development two months after inoculation. A summary of disease incidence in this experiment and the preceding experiment is tabulated in Table 17. It can be concluded that older and senescent leaf sheaths are more susceptible to infection than young green tissues. However, when the tissues become very senile, the plant again becomes more resistant. It would appear the observed increase in susceptibility of 10 week old plants (Figure 30) was due to the inoculation of older and more senescent leaf sheaths.

## Nitrogen and Disease Development

Numerous workers have studied the effects of nitrogen on foot rot development both in the field and in the greenhouse. Generally nitrogen application increased foot rot in the field and decreased it in the greenhouse. Experiments were designed to determine the influence of nitrogen levels on foot rot development under growth

chamber conditions. Plants growing in vermiculite were sub-irrigated with a nutrient solution containing either 0.75, 1.5 or 3.0 M $NH_4NO_3$ as the nitrogen source. Potassium and calcium were added as their chloride salts. The remainder of the nutrient media was similar to that used in the preceding experiments. Plants were inoculated when one or two months old with a microdrop of a conidial suspension and incubated 18 days after inoculation in polyethylene bags. The number of tillers showing foot rot lesions was recorded one month after inoculation and again when the plants were four months old.

Table 17. Incidence of disease in plants of various ages determined two months after inoculation.

| Age of plants when inoculated | No. of plants observed | | Plants infected | | Tillers infected | | Inoculated tillers infected | |
|---|---|---|---|---|---|---|---|---|
| | Expt. 1 | Expt. 2 | Expt. 1 | Expt. 2 | Expt. 1 | Expt. 2 | Expt. 1 | Expt. 2 |
| weeks | no. | no. | % | % | % | % | % | % |
| 5 (G) | 27 | 33 | 7 | 28 | 2 | 12 | 11 | 31 |
| 6 (G) | 26 | 34 | 42 | 58 | 13 | 41 | 70 | 97 |
| 7 (G) | 24 | 35 | 63 | 71 | 23 | 61 | 56 | 114 |
| 8 (GY) | 26 | 32 | 58 | 65 | 17 | 53 | 43 | 87 |
| 9 (GY) | 22 | 33 | 86 | 82 | 33 | 72 | 123 | 191 |
| 10 (Y) | | 30 | | 30 | | 16 | | 40 |
| 11 (Y) | | 33 | | 58 | | 37 | | 25 |
| 12 (Y) | | 34 | | 41 | | 26 | | 23 |

(G) Indicates leaf sheath green in color when inoculated

(GY) Indicates leaf sheath light green to yellow in color when inoculated

(Y) Indicates leaf sheath yellow in color when inoculated

Four month old plants grown in the low nitrogen media developed more lesions than plants grown at either the medium or high levels regardless of inoculation date (Table 18). Plants inoculated when they were one month old had 33, 3 and 4 percent of their tillers infected when grown in the 0.75, 1.5 and 3.0 M $NH_4NO_3$ levels respectively. Plants inoculated when two months old and grown in the low nitrogen medium had 50 percent of their tillers infected; comparable plants at the medium and high nitrogen levels had 10 and 13 percent of their tillers infected respectively.

Table 18. Influence of three nitrogen levels on disease development of plants inoculated when either one or two months old.

| Nitrogen level | Age when inoculated | Plants lesioned 1 month after inoculation | Incidence of disease in 4 month old plants | | |
|---|---|---|---|---|---|
| | | | Plants having lesions | Tillers having lesions | Inoculated tillers having lesions |
| (a) | | % | % | % | % |
| 0.75 M | 1 month | 50[b] | 47 | 33 | 93 |
| 1.50 M | 1 month | 50 | 8 | 3 | 8 |
| 3.00 M | 1 month | 33 | 18 | 4 | 18 |
| 0.75 M | 2 months | 39 | 65 | 50 | 71 |
| 1.50 M | 2 months | 18 | 35 | 10 | 21 |
| 3.00 M | 2 months | 22 | 39 | 13 | 25 |

[a] Indicates Molar concentration of $(NH_4)NO_3$ in nutrient media.

[b] 16 plants were used for each determination.

The level of nitrogen in the media had little effect on the original susceptibility of one month old plants as indicated by the percentage of plants infected one month after inoculation. In contrast the susceptibility of two month old plants was influenced by the nitrogen levels. The plants growing in the two higher nitrogen levels appeared to be more resistant.

Nutrients and Pathogenesis of Excised Coleoptiles

Toussoun, Nash and Snyder (1960) have shown that glucose and nitrogen effects the pathogenesis of Fusarium solani on excised bean hypocotyles with nitrogen favoring pathogenic development and glucose delaying pathogenesis. An attempt was made to determine the effect of glucose, $NH_4NO_3$ and yeast extract on the pathogenesis of C. herpotrichoides on excised wheat coleoptiles. Shoots from 10 day old wheat seedlings were cut 20 mm below the tip of the coleoptile, thus eliminating the growing portion of the shoot. The "shoot tip" which consisted of only the primary leaf and the coleoptile were washed in sterilized distilled water and incubated in a sterilized solution of 60 ppm benzimidazole. They were supported in an upright position by placement in a polyurethane block. The desired amendments supplied at 1,000 ppm were added to the conidial suspension which was placed on the coleoptiles by the micropipet. They were incubated in the growth chamber in polyethylene covered plastic boxes.

The young coleoptiles were resistant to infection and no lesions formed in any of the treatments. Microscopic examination of the coleoptiles revealed the host cell walls reacted to the presence of the fungus (Figures 38 and 39). Similar reactions were observed in other experiments (Figure 26) and have also been recently reported by Defosse (1967). Immediately after staining these reactions are not visible and only become apparent several days after the initial staining process. The cell wall reaction occurred in all treatments. Since yeast extract stimulated growth of the fungus on the host surface, greater numbers of halos occurred but their individual intensity did not seem to differ from the other treatments.

Samples of the conidial suspensions used to inoculate coleoptiles were also placed on slides and incubated over water in polyethylene covered plastic boxes. Ammonium nitrate at 1000 ppm N inhibited germination of conidia on glass slides either alone or in presence of glucose (Table 19). In solutions containing glucose, glucose plus $NH_4NO_3$ and $NH_4NO_3$, 60, 5 and 7 percent of the conidia germinated respectively. Ammonium nitrate also inhibited germination of conidia on coleoptiles but to a lesser degree. Eighty-five, 64 and 32 percent of the conidia germinated on coleoptiles in two days with additions of glucose, glucose plus $NH_4NO_3$ and $NH_4NO_3$ respectively.

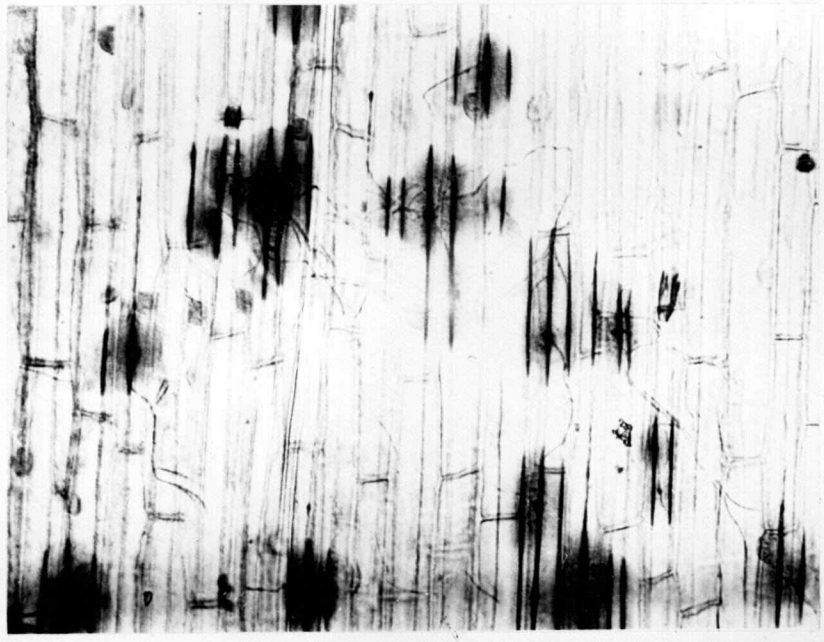


Figure 38. Cell wall reactions of young coleoptiles induced by the presence of C. herpotrichoides.

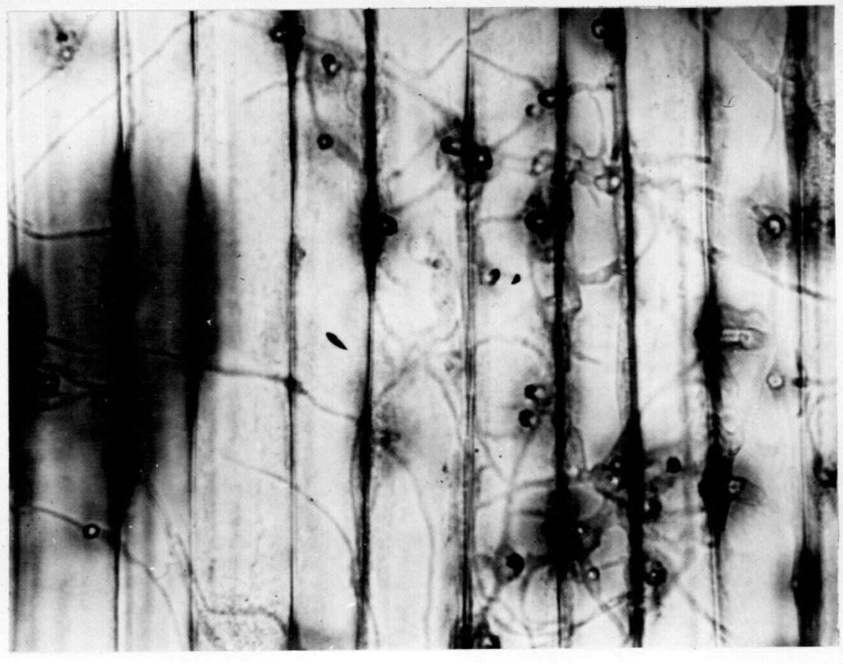


Figure 39. Cell wall reactions of young coleoptiles induced by appresoria of C. herpotrichoides.

Table 19. Influence of nutrients on germination of conidia placed on glass slides or excised coleoptiles.

| Treatment[b] | Germination on glass slide | | | Germination on coleoptile | |
|---|---|---|---|---|---|
| | 1 day | 2 days | 3 days | 2 days | 3 days |
| | % | % | % | % | % |
| 0 | 8[a] | 42 | 65 | 47 | 79 |
| glucose | 11 | 60 | 66 | 85 | 88 |
| $NH_4NO_3$ | 4 | 7 | 4 | 32 | 50 |
| glucose + $NH_4NO_3$ | 12 | 5 | 3 | 64 | 72 |
| yeast extract | 9 | 53 | 92 | 80 | - |

[a]Based on the average of three replications with 100 conidia in each.
[b]Nutrients were supplied at 1000 ppm.

## Influence of Inorganic Nitrogen and Glucose on Germination

Experiments were initiated to determine if the $NH_4NO_3$ inhibition of germination observed in the preceding experiment was due to $NH_4^+$, $NO_3^-$, or possibly to pH since the germination media was not buffered. Ammonium phosphate, $KNO_3$ and $NH_4NO_3$ were used as nitrogen sources and added to a 0.005 M $K_2PO_4$ buffer at either 1000, 100, 10 or 1 ppm-N. Glucose was supplied to each nitrogen treatment at 1,000 ppm-C. The conidial solutions (30,000 conidia/ml) were placed on glass slides over water in polyethylene covered plastic boxes. To check the pH of the germinating media, four ml of these germinating solutions were placed in 125 ml flasks. The pH was

determined at the beginning and termination of the experiments. Germination in these flasks was inhibited (10 to 50%) compared to that on the slides, but showed the same trends.

The ammonium ion was found to be inhibitory to the germination of C. herpotrichoides (Table 20). The nitrate ion also was inhibitory but to a lesser degree. After five days (data not shown), conidia in the $NO_3^-$ solutions showed 90 percent germination. The pH of these germinating solutions does not appear to be responsible for the inhibitory action of these ions. Glucose concentrations of 10 and 100 ppm were inhibitory to conidial germination, while concentrations of 1 and 1000 ppm had a stimulatory effect.

Table 20. Influence of inorganic nitrogen sources and glucose on conidial germination.

| Treatment[a] | Concentrations | Percentage germination[b] | | | pH | |
|---|---|---|---|---|---|---|
| | | 1 day | 2 days | 3 days | Before | After |
| | ppm | % | % | % | | |
| $(NH_4)_2PO_4$ | 1000 | 6 | 2 | 2 | 7.65 | 7.62 |
| | 100 | 3 | 8 | 19 | 7.32 | 7.06 |
| | 10 | 6 | 24 | 53 | 7.14 | 6.81 |
| | 1 | 6 | 37 | 90 | 7.12 | 6.87 |
| $KNO_3$ | 1000 | 3 | 23 | 32 | 6.86 | 6.78 |
| | 100 | 12 | 14 | 42 | | 7.01 |
| | 10 | 9 | 40 | 64 | | 7.00 |
| | 1 | 8 | 56 | 95 | | 7.01 |
| $NH_4NO_3$ | 1000 | 3 | 7 | 19 | 6.88 | 6.71 |
| | 100 | 4 | 10 | 17 | | 6.81 |
| | 10 | 5 | 22 | 60 | | 6.98 |
| | 1 | 2 | 67 | 97 | | 6.97 |
| $(NH_4)_2PO_4$ + $KNO_3$ | 1000 | 5 | 2 | 12 | 7.11 | 6.92 |
| | 100 | 3 | 12 | 16 | | 7.01 |
| | 10 | 6 | 27 | 59 | | 6.97 |
| | 1 | 6 | 72 | 96 | | 7.10 |
| Glucose | 1000 | 14 | 36 | 88 | 7.00 | |
| | 100 | 1 | 8 | 15 | | |
| | 10 | 3 | 21 | 38 | | |
| | 1 | 8 | 75 | 98 | 7.02 | |
| Yeast extract | 1000 | 7 | 89 | 94 | 7.12 | 7.38 |
| none | - | 2 | 13 | 85 | 7.14 | 6.96 |

[a] Glucose was supplied to all nitrogen treatments at 1000 ppm C.
[b] Based on the average of three replications with 100 conidia in each.

# DISCUSSION

Cercosporella herpotrichoides produces conidia abundantly on colonized straws and infected plants during the cool moist months of the growing season. This study indicates that the pathogen is probably able to survive in the soil throughout the winter and into spring when conidia are washed into or buried in the soil. It seems unlikely, however, that survival would continue through the warm and dry summer since conidia incubated at 25° and 20°C survived only one and two months respectively. Soil temperatures were in the 20s as early as June and continued to be warm through September. Although viable hyphae from germinated conidia were observed on glass slides 16 months after burial, these occurred only in soils incubated at 5° and 10°C.

Nitrogen as $NH_4NO_3$ inhibited the germination and survival of the fungus in soil. Since it also inhibited germination on glass slides not in contact with soil, it may be assumed the inhibition in the soil was due to the amendment and not to stimulation of antagonistic organisms. Glucose did not influence germination but stimulated saprophytic development and survival. Cercosporella herpotrichoides was able to successfully compete with other soil microorganisms for available glucose. This was evident since its growth continued in the glucose amended soils throughout the 64 days of observation. It was

often observed growing in close association with other fungi, actinomycetes and bacteria during this period. The melaninization of swollen hyphal cells was observed only in the glucose amended soils. The survival potential of these cells is not known; however, their apparent resistance to lysis may have been partially responsible for the increased survival of the fungus in the glucose amended soils.

Cellulose-amended soils did not stimulate the saprophytic activity of the fungus as did the glucose. Other workers (Macer, 1961a; Garrett, 1963) have shown C. herpotrichoides exhibits very low cellulase activity; thus it is not surprising the fungus was not able to compete for it. However, the cellulose carbon may have become available to C. herpotrichoides as other cellulitic soil microbes proceeded with its decomposition. After one to two months increased growth of the pathogen was observed in the cellulose plus glucose amended soil compared to soil amended with glucose only. Cellulose-amended soils favored survival of the fungus; this may be attributed to nitrogen immobilization, since nitrogen was found to be inhibitory to germination and survival.

Below ground lesions developed on plants in soil infested with conidia and their incidence was directly related to the inoculum densities of the soil. Based on a model system, it was concluded that conidia were influenced by host exudates only at the host surface and a rhizosphere was not operative under the conditions of this

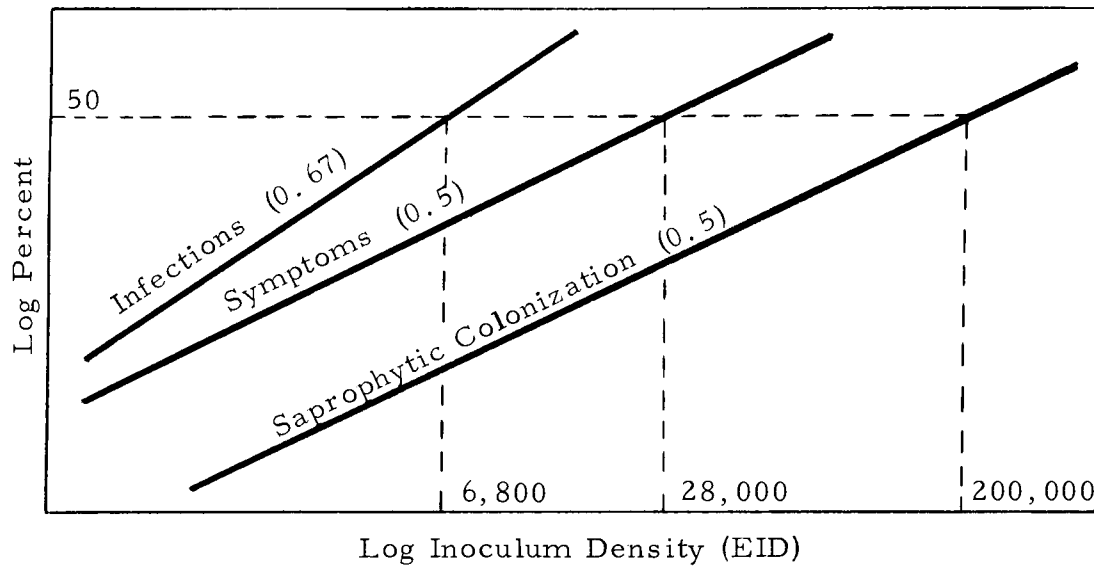
experiment. Using mathematical models, Baker, Maurer and Maurer (1967) have postulated straight line relationships exist where inoculum density and number of infections are plotted on a log-log basis. Furthermore the slopes of the lines indicate either a rhizoplane or rhizosphere effect. Data collected in this study fit these mathematical models in that straight line curves were obtained by a log-log plotting of the experimental data. Where the number of infected plants was determined by plating procedures and plotted against inoculum density, a straight line was obtained having a slope similar to the theoretical rhizoplane curve (0.67).

In contrast when plants were incubated long enough for symptoms to develop, a straight line having a slope of 0.5 was obtained by plotting the percentage of tillers showing lesions versus inoculum density. Factors in addition to the inoculum density may be influencing the slope of a curve when it is based on symptom development rather than infections. Latent infections may be present which are not evident when "infections" are determined by symptom development. It is also possible the plants are able to slough off an initial infection during the extended incubation period. Since both sloughing and latent infections have been observed, either or both could account for the difference in slopes. Latent infections were detected when "resistant" primary leaf sheaths were examined. The number of plants observed with lesions decreased with time in the experiments determining the

influence of nitrogen levels on foot rot development.

Other determinations were made which further characterize the rhizoplane influence on conidial activity. Conidial germination was stimulated on the host surface when compared to germination on glass slides in the soil. Ammonium nitrate added to the soil did not inhibit conidial germination on the rhizoplane as it did on buried glass slides. However, conidia placed on coleoptiles above the ground line germinated better than those on the coleoptiles below the ground. This suggested an antagonistic influence in the soil affected conidia on the rhizoplane. Since growth on the rhizoplane following germination was limited, it would appear the quantity or quality of the host exudates was limited. However, when the tissues became senescent, growth and sporulation of the pathogen was greatly stimulated.

The influence of inoculum densities on saprophytic colonization in soil infested with conidia was also determined. A comparison of the curves from this saprophytic system with those involving parasitic systems could be indicative of the selectivity and stimulatory nature of the plant exudates. Curves can be compared on a basis of their slope and their effective inoculum density value (EID). An $EID_{50}$ value being that inoculum density which will result in either 50 percent of the plants becoming infected or 50 percent of the straws being saprophytically colonized.




This diagram represents data taken from Figures 14 and 24, and Table 4. The "infections" curve was determined from the percentage of plants infected two months after inoculation with conidial infested soils. The "symptoms" curve represents the percentage of lesioned tillers three months after inoculation with conidial infested soils. The percentage of straws colonized two months after introduction into conidial infested soils, is represented by the "saprophytic colonization" curve.

The slope of the saprophytic curve is less than a theoretical rhizoplane curve, indicating not all of the conidia in contact with the straw surface were capable of colonizing the straw. Potential colonizing sites for C. herpotrichoides on a straw surface might be limited by a lack of nutrient availability, competition from other saprophytes or previous colonization by other microbes. In contrast,

since a theoretical rhizoplane slope was observed for initial plant infections, apparently a majority of the conidia on the rhizoplane of the living host are potentially able to cause infections.

It would appear all initial infections do not result in lesion development since the symptoms slope is less than the rhizoplane slope for infections. The slopes based on symptom development and saprophytic colonization are very similar (about 0.5). The pathogens activity on the host was not greatly stimulated until senescence began, thus the pathogen is in competition with other saprophytes and any initial selectivity of the host exudate are somewhat nulified. Cercosporella herpotrichoides may, however, benefit from a "head start" due to its initial stimulation. As will be discussed later, susceptibility was associated with senescent tissues, thus it is not surprising that disease development and saprophytic colonization are represented by curves of similar slopes.

An $EID_{50}$ value of 6,800 was determined from the infection slope. A comparable $EID_{50}$ value from the saprophytic colonization curve was approximately 200,000; indicating a 30-fold increase in inoculum density was necessary for equal numbers of straws to be colonized as plants to become infected. However, the symptom development curve was characterized by an $EID_{50}$ of about 28,000 suggesting only a sevenfold difference between saprophytic colonization and symptom development. A comparison of $EID_{50}$ values of

infection and symptom curves imply that four to five infections were necessary for lesion development on below-ground stems. This contrasts with data obtained in the study of aerial lesion development which indicated a single conidium could result in lesion development. Antagonism from other soil microorganisms may reduce the inoculum potential of the pathogen. This soil antagonistic factor had previously been observed when comparing conidial germination on plants above and below the soil surface.

The above discussion is based on data extracted from several separate experiments and the values indicated should only be interpreted as being representative of trends and not absolutes. Unifying experiments would help to maintain more controlled conditions and yield more positive conclusions. For example, the surface areas of the plants and straw pieces or other substrates to be tested should be approximately equal; they were not in these experiments. Incubation temperatures also varied in these separate studies. Saprophytic colonization was determined at 10°C and disease development under a 15° to 10° C day-night cycle. Age of the conidial inoculum also varied to some degree between experiments. Even with these limitations, the results indicate that model systems and mathematics can be used to help solve the complex problems involving soil-borne pathogens.

Cercosporella herpotrichoides has previously been reported to

exhibit extremely low competitive saprophytic colonizing ability (Macer, 1961a). In his studies the inoculum consisted of maizemeal-sand cultures diluted with varying amounts of soil. The introduction of a food base along with the fungus will effect not only the pathogen's behavior but also the behavior of other soil saprophytic micro-organisms. Possible stimulatory or inhibitory effects may occur which would not be expected under more normal soil conditions. Due to the extreme clumping nature of sand-cornmeal cultures it is also difficult to evenly distribute the inoculum in the soil, especially at the lower inoculum levels. Conidial infested soil was used in both my field and laboratory studies in attempts to simulate more natural conditions. Unexpectedly, high numbers of straw pieces were colonized by C. herpotrichoides when placed into the conidial infested soil both in the laboratory and field. Since colonization was reduced with time and since the pathogen has been demonstrated to have limited cellulase activity (Garrett, 1963) and slow growth rate through straw (Macer, 1961a), it would appear some of the observed colonization was superficial. However, C. herpotrichoides did colonize relatively high numbers of straws and its growth and sporulation were stimulated by straw pieces. In addition, other experiments showed the pathogen was able to successfully compete for glucose amendments and saprophytically survive 16 months in cool soil. It is suggested C. herpotrichoides has a higher degree of saprophytic ability than

previously suspected.

Thus C. herpotrichoides has the ability to saprophytically survive in cool moist soil, cause below ground infections and increase or maintain its inoculum density by saprophytic colonization of plant debris. The saprophytic survival of the fungus in soil will help maintain high inoculum levels throughout the winter and spring months. Erosive movement of soil by water, especially into trenches resulting from deep furrow seeding, could bring conidial infested soil into contact with the host. The increased disease reported as a result of spring tillage practices (Bruehl and Nelson, 1964) may also be explained in part by movement of infested soil against the bases of wheat plants.

The survival of the fungus in straw differed from results reported by Macer (1961b) in England. Differences in climate, size of straw pieces and degree of colonization may account for these differences. Straws used in this study were smaller than those used by Macer. Immediately after burial an initial decline in the percentage of straws colonized by C. herpotrichoides was observed in this study which was not evident in Macer's experiments, suggesting a probable difference in the degree of original colonization.

In both naturally and artificially colonized straws buried in the soil the pathogen showed a marked reduction in survival after one year, contrasting with Macer's (1961b) observation of essentially no

loss after three years. In Macer's experiments the fungus survived for the shortest period in surface straws. However, under the climatological conditions of the Columbia Basin, survival of C. herpotrichoides was favored in straws placed at the surface compared to those buried in the soil.

Stubble mulching is commonly practiced in the Columbia Basin. Maximum amounts of stubble from the preceeding crop are left at the soil surface when this fallow system is used. Results of this study indicate stubble mulching might increase the chance of foot rot injury since survival of the pathogen is favored in surface straws. In addition to favoring better survival of the pathogen, other factors also indicate that stubble mulching practices may increase foot rot problems.

Cox and Cock (1962) have shown the amount of sporing material on the soil surface is directly related to disease incidence. Thus, stubble mulching practices could favor high inoculum levels by allowing the accumulation of potential sporing material at the surface. Deep-furrow drills are recommended for seeding in stubble-mulched fields (Horning and Oveson, 1962). This type of seeding may also increase chances of foot rot damage since more humid conditions favoring foot rot development would be expected to exist in the deep furrows when compared with surface conditions. As previously mentioned, erosive movement of infested soil into the furrows may also increase the

disease potential. Colonized straws buried near the surface in the vacinity of the host are potential inoculum sources for disease development. Naturally colonized straw pieces and PDA disks of C. herpotrichoides buried in the soil caused below ground infections to occur. Infections occurred when the inoculum was placed up to 10 mm from the stems. Since C. herpotrichoides had been previously observed growing from buried colonized straws it was concluded that infections resulted from direct growth of the fungus from the straw to the host. Infections from colonized straws buried up to 8 cm from the stem have been reported by Scheinpfug (1964); these infections were attributed to movement of spores in the soil water.

Conidial inoculum successfully colonized buried straws, thus the plant debris left on or near the soil surface may become saprophytically colonized by C. herpotrichoides. Even though colonization may only be superficial, sporulation is associated with these colonies and more inoculum would become available for the spread of the disease.

Thus colonized stubble left on the surface allows increased survival and increased spore production, and infections may occur directly from the colonized straw pieces at or below the soil surface. These factors, including a possibility of secondary colonization of surface debris, would suggest that stubble mulching potentially favors foot rot development and should not be recommended in fields

where the disease is known to occur. Fields could be stubble mulched until the seed beds were prepared at which time the infectious plant residues could be buried deep enough to allow the new wheat crop to escape contact with the residues.

Foot rot incidence was associated with plant debris in the soil. Cox and Cock (1962) showed incidence of foot rot was associated with the amount of infectious material on the soil surface. It seems possible therefore, that burning of stubble in diseased fields could be effective in reducing the amount of inoculum. Indiscriminate burning of entire fields seems unnecessary and burning should be limited to fields or portions of fields effected by foot rot. Since the fungus survives more than one year in colonized straw, burning should be continued for several consecutive crop years.

Below ground infections resulting either from direct contact or mycelial growth from infected straws in the vicinity of the host may also play a significant role in maintaining endemic levels of disease. Little attention has been paid to endemic disease development. Weather conditions have been shown by many investigators to be a primary factor influencing foot rot epiphytotics. However, infections occurring below the soil surface are much less dependent upon climatic factors and would be expected to occur more consistantly year after year even during "non-foot rot" years. In a year not favorable to foot rot development, about six percent of the tillers in a field

having little foot rot damage (few white heads) were infected with C. herpotrichoides. Many of the lesions were on tillers which failed to head. In heavy tillering varieties such as 'Gaines' a failure of a few tillers to develop generally goes unnoticed by casual observation.

In addition to being less influenced by climate, other factors may contribute to a more consistant level of disease from buried colonized straws. The inoculum is in constant association with the host and therefore present when the host may become most susceptible. The inoculum potential of the pathogen is higher when associated with a food base which may allow the fungus to overcome host resistance (later discussion). Infections may also occur soon after planting giving the fungus time for deep penetration allowing many tillers of the same plant to become infected from only one original infection. The soil will also tend to hold dead and drying leaf sheaths in contact with the main stem, thus averting the sloughing off of shallow infections during dryer periods.

Below ground lesions were observed from December through June on plants growing under natural field conditions. It is suggested these below ground infections play an important part in maintaining inoculum levels in fields during years not favorable for foot rot development and provide the initial inoculum necessary for severe outbreaks of the disease when weather conditions are favorable. Due to the confining limits of the soil, however, sporulation from these

below ground infections is not directly responsible for the rapid spread of disease during epiphytotics; epiphytotics are associated with conidial production from surface debris of the preceeding year's crop and plants having primary aerial infections. Both the primary aerial infections and the secondary spread of the disease are dependent upon favorable weather conditions.

Scheinpflug (1964) observed no differences in susceptibility of plants one to six weeks old. Dickens (1964) also observed no differences in foot rot severity of plants inoculated at emergence or one month later when incubated at 8°C. At 12°C the plants inoculated at the month old stage were more severely affected. Under the growth chamber conditions of this study, disease development was affected by the age of the inoculated plants. General susceptibility of inoculated tissues, degree of tillering at inoculation, tillering characteristics, growth rate of host plants and the amount of nitrogen supplied to the host were some of the noticeable factors determining the total disease development in these studies.

Tissue inoculated and their physiological condition influenced the susceptibility of the plants. Young, green coleoptiles were resistant to infection until they started to undergo senescence. Plants inoculated on "old" coleoptiles were much more susceptible to infection than the same aged plants inoculated on young, green primary leaf sheaths. As the primary leaves became senescent they

also became more susceptible. Thus a cycle occurred in plant susceptibility associated with aging of tissues being inoculated.

Plant susceptibility became more complex as new tillers periodically appeared and the original leaf sheaths withered and died. Inoculation on extremely senescent and dead leaf sheaths usually allowed the plant to escape infection. These leaf sheaths tended to pull away from the main stem after inoculation, resulting in a spacial barrier to the fungus before it had penetrated into adjacent leaf sheaths.

If infection occurs before the plant begins to tiller, a single infection may result in lesion development on each tiller produced. A greater number of infection sites occur as tillers are produced; however, the potential number of infected tillers resulting from one successful infection is reduced. The number of infected tillers resulting from a single infection depends on several additional factors: the relative growth rate of the host and pathogen, and the openness of the crown. Plants growing in the same pots were observed to vary in the number of tillers produced and to their degree of spreading. Tight crowned plants tended to have all their tillers infected, apparently from close contact with infected leaf sheaths and each other. Healthy culms were associated with diseased ones in plants having open crowns. Variations in micro climate and genetic variability of the host determining the tightness of the crown may contribute

to this effect.

Low levels of nitrogen in the culture solution favored disease development of plants inoculated when either one or two months old; however, this effect was the result of two different phenomenon. The initial infection of one month old plants was little affected by the nitrogen level. The subsequent rapidity of growth and tillering of plants after inoculation in the higher nitrogen treatments enabled them to slough off the infection and escape the disease. By contrast the amount of nitrogen in the media affected the initial levels of infection in two month old plants. Apparently the high nitrogen levels delayed senescence which decreased the susceptibility of the older plants. These plants inoculated after tillering had begun were not able to slough off the infections. In fact, more plants having lesions were observed two months after inoculation than one month, suggesting latent infections were present in some of the "resistant" plants.

Limited observations indicated the infection process failed at one of three stages on the young, green, "resistant" tissues: (1) germination failed to occur, (2) germination occurred but the pathogen failed to attempt penetration, and (3) penetration was attempted but failed, failure being associated with the thickening of the host cell walls. The later two stages are similar to those observed by Flentje (1957) in the infection process of Pelliculania filamentosa.

On some resistant tissues conidia either failed to germinate or

germinated poorly. Since conidia on glass slides germinate without the addition of exogenous nutrients it would appear a factor other than nutrient availability is inhibiting their germination. Water availability and the presence of an inhibitor might explain these observations. Brown (1965) has emphasized the importance of water availability in plant disease development. Even in the humid atmosphere of the plastic bags, inoculating drops were usually not visible the day after inoculation, and free moisture on the plant surface may be limiting fungal germination.

Growth after germination was limited on resistant tissues and the fungus often made no attempts to penetrate the cells as evidenced by the lack of any host reaction. When penetration was attempted on resistant tissues, host cells attempted to restrict the pathogen. Host cell walls thickened and stained heavily in areas of attempted penetration. Cell wall reactions of wheat plants have been commonly observed, associated with both pathogenic and non-pathogenic fungi. Flentje (1957) observed thickening of cell walls of wheat coleoptiles at attempted penetration points by the non-pathogen Pellicularia filamentosa. Stevens (1921) observed similar thickenings with a pathogenic Helminthosporium and with a non-pathogenic Alternaria. Ophiobolus graminis, also a pathogen, stimulated cell wall thickening and lignituber formation in wheat (Fellows, 1928). The thickened cell walls showed large amounts of lignin and comparatively little

cellulose. In my study the cell walls of the below ground portion of coleoptiles became thickened and stained heavily with cotton blue when associated with some apparently non-pathogenic fungi and bacteria.

At points of attempted penetration by C. herpotrichoides a halo was observed on the epidermal cell wall when stained with cotton blue. Haloes and cell wall thickening similar to those associated with C. herpotrichoides on wheat have been observed on oat seedlings infected by Fusarium culmorum (Simmods, 1928). Similar reactions have been reported when oidia of Erysiphe graminis were placed on barley leaves. Differential staining indicated either structural or chemical modification of the cellulose wall at the halo (Akai, et al., 1967). The halo staining reaction observed in my study was associated with a response of the "resistant" host cells to the presence of the fungus. If the reaction was due strictly to a breakdown or change in the cell wall due to activity of enzymes secreted by the pathogen, they would also have been observed in the walls of susceptible tissues; they were not. Occasionally C. herpotrichoides penetrated into cells of the "resistant" host tissue. Each cell wall encountered reacted to the fungus and progress of the infection was extremely slow.

The older chlorotic susceptible tissues did not show any of the above responses to infection. Abundant germination occurred and host cells were easily penetrated. These older cells had evidently

lost their capacity to resist the fungal invasion since no cell wall thickening or halo staining was observed. The existing cell walls do slow the fungus down as evidenced by the accumulation of hyphae at the walls before it penetrates into the next cell.

The green "resistant" tissues lying under the older susceptible tissues are unable to resist the invasion of the pathogen from adjacent infected tissues. It would appear that the growth rate of the pathogen when associated with the susceptible tissue (a food base) is rapid enough to allow penetration of the "resistant" cells before they can react to its presence. However, cells preceding the invading fungus do react, cellular contents collect at the ends of the cells and the transverse walls begin to thicken. These cells may become invaded but apparently restrict the fungus so that it is eventually contained in the thick-walled cells which make up the periphery of the lesion. Successful penetration and infection of a coleoptile or leaf sheath does not insure disease development. The infected tissues may pull away from the main stem before the fungus has penetrated into adjacent leaf sheaths, thus isolating the pathogen and the majority of the plant tissues escape infection.

Chemicals which could delay the natural senescence of leaf sheaths might also help to control foot rot since healthy cells are apparently able to resist the pathogen. Also chemicals stimulating resistant reactions similar to the cell wall reactions observed with

both pathogenic and non-pathogenic organisms may also be effective in controlling the disease. The reduced incidence and severity of foot rot noted by applications of diuron (Huber, Seely and Watson, 1966) may be a result of one or both of these modes of action. Diercks (1965) has in fact indicated the resistance imparted by application of chlorcholinchloride was probably due to increased wall thickness of cells at the base of the culms.

Results of these studies suggest possible factors which might be influencing the incidence of foot rot associated with the date of seeding. Increase in disease severity has been associated with early seeding (Sprague, 1937; Dickens, 1964). Late seeded plants may remain "resistant" throughout the late fall and winter due to the resistant nature of their young green tissues. In contrast, early seeded plants would be likely to have senescent and therefore susceptible tissues in the late fall and winter. Plants infected early in their development are potentially exposed to greater disease incidence since multiple infection may occur from a single infection due to the tillering characteristics of wheat plants. Also due to tillering, early seeded plants possess a greater number of potential infection sites during the fall and winter than the late seeded plants. The number of primary infections occurring during the fall and winter may be influenced by the above factors. These primary infections in addition to their own destructive nature are also responsible for the secondary

spread of the disease in the early spring, thus emphasizing their importance in total disease development. The frequency, duration and dates of favorable periods for infection and sporulation in the fall and winter will influence the above factors; however, information concerning these periods is mostly unavailable.

It appears that through proper cultural practices foot rot losses may be held to a minimum until more tolerant varieties can be developed or economic chemical control is achieved.

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