WHEAT ROOT ROT PATHOGEN VARIABILITY AND SOIL MOISTURE STRESS EFFECTS ON HOST-PATHOGEN INTERACTIONS

by

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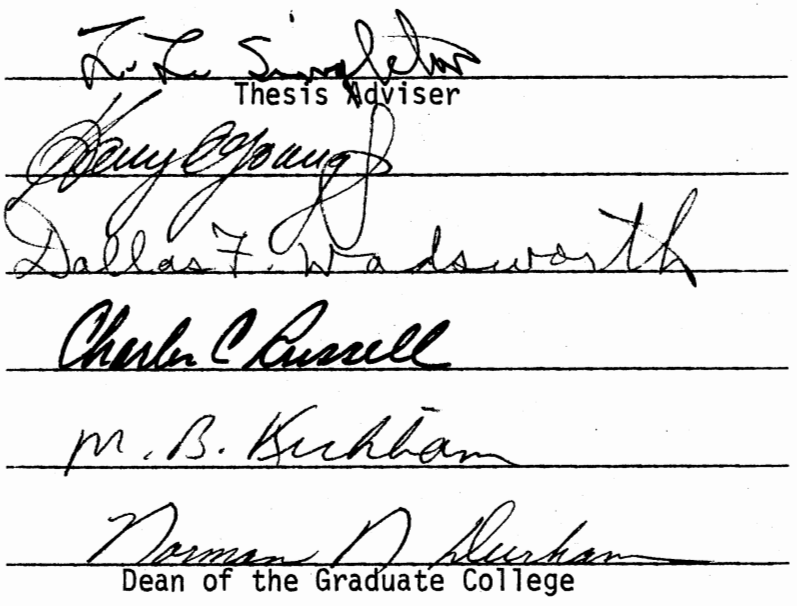

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CHAPTER I

INTRODUCTION

Common wheat, Triticum aestivum L. em. Thell, is a very important food crop in the world and it is the most important of the three great cereal crops of the world. Much of it is grown in semi-arid regions such as the Great Plains of the United States, North and East Africa and India. Drought and root rot diseases are two important major factors that limit maximum wheat production in these areas (14, 18, 57).

In Oklahoma, wheat is an important cash grain crop, and is also important as a forage for livestock. This important crop can be severely damaged by common root rot disease of wheat especially in years of limited moisture. In Oklahoma, this disease seems to be a disease complex involving various fungi such as Helminthosporium sativum Pammel, King, and Bakke; Fusarium spp; Rhizoctonia solani Kuhn; and the nematode, Pratylenchus minyus Sher and Allen. These pathogens are commonly associated with diseased wheat plants collected from infected fields. It appears that the fungi and the nematode combine to produce the destructive dark brown lesion found on the roots of the plants. The main recognizable symptoms on diseased plants in the field are white heads which usually appear in the month of May or June. Diseased plants mature early and produce few tillers and shrivelled seeds.

Water stress can influence plant disease through its effect on host susceptibility to root rot pathogens or through its effect on

host-pathogen interactions (58). Host invasion by the pathogens can occur before or after exposure to water stress, but the effect of water stress is usually the same. Disease development is highly dependent on the influence of water stress.

Much work has been done on the effect of water stress on root diseases (14, 17, 27, 58). Most of the emphases have been on effects of water stress on the pathogens and some other soil micro organisms. Little has been done on the effect of soil moisture stress on host-fungi interactions, the rate and extent of disease establishment and development, and host plant response.

The objectives of this investigation were:

1. To determine the extent of pathogenicity and virulence of several isolates of H. sativum and Fusarium spp. collected from different wheat areas in Oklahoma.

2. To determine the invasion patterns and penetration of wheat seedlings by H. sativum, Fusarium sp. and R. solani.

3. To determine the effects of root rot pathogens and soil moisture stress on growth and forage yield of transplanted and non-transplanted wheat seedlings.

4. To determine the disease reaction of the six hard red winter wheat cultivars: ('Danne,' 'Triumph 64,' 'TAM' 101,' 'Payne,' 'Newton,' and 'Vona') to H. sativum alone, Fusarium sp. alone, or the combination of both pathogens under soil moisture stress conditions.

CHAPTER II

LITERATURE REVIEW

Causal Organisms Associated With Wheat-Root-Rot Disease

Wheat root rot disease is one of the most destructive diseases of wheat in Oklahoma and many wheat growing areas of the world (6, 73). It attacks both seedlings and mature plants and any part of the plant can be attacked. Infected plants usually produce premature white heads and shrivelled seeds (73). In Oklahoma, wheat root rot seems to be a disease-complex associated with the fungi Helminthosporium sativum, Fusarium spp., Rhizoctonia solani and the nematode Pratylenchus minyus.

Losses due to common root rot disease of wheat are very difficult to estimate. However, attempts have been made by some plant pathologists to estimate losses caused by this disease in various ways. Edson et al. (23), in 1935, summarized crop losses from plant diseases in the United States from data they obtained from individual investigators in different parts of the country. They reported losses due to wheat root-rot were as high as 10%. Kansas State had the greatest loss of 4.7 million bushels. No loss estimation was given for the state of Oklahoma. No other reports on losses due to wheat root rot disease in the United States have been found. The need to determine the losses caused by this disease may be important in evaluating

integrated disease control program for wheat.

In Canada, Simmonds (60) reported a reduction in yield of 15 to 20% and 30 to 45% in moderately and severely infected wheat plants, respectively. Machacek (43) estimated losses due to common root rot disease in Canadian Prairies between 1939-41 and reported losses of 8, 16 and 12%, respectively, for the three years. The most recent survey of losses due to this disease in Canada in 1969-71 by Ledingham et al. (37) showed an annual loss of some 30 million bushels for the Prairie Provinces.

Helminthosporium sativum

Helminthosporium sativum Pammel, King, and Bakke causes seedling blight, root rots, basal stem rot, discolored spikes and seed blight, leaf spot, stem lesions and premature death of wheat, barley, rye and many grasses (8). H. sativum under favorable conditions causes severe damage to wheat, barley, rye and grasses, especially as the plants approach maturity.

H. sativum is the name given to the conidial stage of Cochliobolus sativus Ito and Kurib. It is a soil pathogen and its spores can endure adverse environmental conditions. Chinn and Ledingham (6) reported in 1958 that the fungus spores can remain dormant up to 2 years. Boosalis (4) found more than 60% of conidiospores of the fungus remained viable after 490 days in fine sandy loam soil in southern Nebraska but decreased in heavy soil in the same area. The optimum temperature for the development of the fungus is between 24-28 C (43). The range corresponds with the optimum wheat development range of 20-24 C. Disease severity increases in the temperature range of 28-32 C (46).

Christensen (8) studied the pathogenicity and mutation of the fungus and reported the following observations:

1. The fungus has numerous physiological forms. He studied 37 forms in detail. He distinguished these races in culture by their rate of growth, relative amount of submerged and aerial mycelium, nature of mycelial growth, zonation, production of conidia, density of conidial clusters, the color of the mycelium, and the readiness with which the forms mutate.

2. All forms could attack the root and basal stem of wheat and barley. Some forms were very virulent, while other forms were relatively non-virulent.

3. The mutants differed from the parents in their morphological characters and pathogenicity. Some were more virulent than the parents and others were less virulent. He also noted that temperature has a great effect on the frequency of mutation. The optimum temperature for mutation is between 25-27 C. No mutation occurred in cultures grown at 15 C or lower temperature (9).

Dickson (19) reported that seedling and crown infections occur from seed-borne mycelium or from crop residues in the soil. Infection of embryonic tissues is by direct penetration, natural openings, or injuries. Disease development is usually more severe in late sown grain or in a warm soil (19).

Fusarium spp.

Fusarium roseum f. sp. cerealis "Culmorum" (Cke) Snyd. and Hans. is one of the causal agents of wheat root and foot rot diseases and it is favored by dry soil condition (13, 55, 63). Sandy soil favors multiplication

and survival of Fusarium spp. (63). Symptoms of the disease include crown rot, early ripening and a pinkish discoloration inside the leaf sheaths. Diseased crowns are reddish brown and sometimes display a pink color associated with the fungus mycelium (55).

The fungus exists in the soil as single, double and clusters of chlamydospores free or embedded in organic matter. It enters the crown of a cereal plant through infected crown roots and wounds made after crown root emergence, 4-6 weeks after planting (12). Seminal roots and subcrown internodes show comparatively low incidence of infection. Progress of the disease is slow and death of the crowns may not occur until after the plant has headed. Rapid infection of Fusarium spp. is favored by a temperature range of 14-26 C, and 75% atmospheric humidity (74).

Toussoun et al. (65) noted in their studies of Fusarium solani f. sp. phaseoli that the fungus attacks its host in sequential steps when conidia are placed in nutrient drops on excised bean stems in petri dishes in a moist chamber. The fungus spores germinated followed by mycelial growth which penetrated the host tissues and pathogenicity started. They thought that the formation of a thallus by saprophytic growth on the host was a necessary prelude to parasitism irrespective of the nutrients used.

Christou and Snyder (11) reported that F. solani f. sp. phaseoli penetrates both roots and hypocotyls, not forming appressoria, but producing a small thallus. The fungus enters the host directly or through mechanical or natural wounds, but most commonly through the hypocotyl. After direct or wound penetration of the hypocotyls or roots, the fungus invades the whole cortex and grows intercellularly.

Rhizoctonia solani

Rhizoctonia solani infects several hundred plant species including many economic crop plants such as cereals, peanuts, and beans. Usually all principal organs of the plants are infected. Root and stem rots are the common symptoms found on most host plants. The fungus can produce brown to black, flattened, irregular sclerotial structure in or on some host tissues. The sexual stage, Pellicularia filamentosa (Pat.) Rog., develops on the surface of soil or plant parts as a relatively conspicuous white hymenial layer covered with basidia. Two or more basidiospores are borne at the apex of each basidium and these spores can germinate to form thalli of the fungus (32).

Generally, disease development is favored by temperatures between 19 and 35 C and moderate soil moisture, rather than extremely wet soil (32). Blair (2) reported that within a soil moisture range of 30-80% saturation, R. solani grows best at the lowest moisture level.

Flentje (25) found two distinct stages in the infection process of the fungus. The hyphae of the fungus becomes attached to the cuticle of the seedling hypocotyl about the junction of the epidermal cells and grow along these junction lines. This is followed by formation of multicellular infection cushions, each from a hyphal branch in which normal elongation has ceased but in which prolific side branching now takes place. Dodman et al. (20) found in their study of 53 isolates of the fungus that some produce lobed appressoria before penetration instead of the dome-shaped infection cushion described by Flentje (25). Christou (10), in his study of the penetration of the fungus in the bean plant, noted that the fungus produced an infection cushion on the hypocotyl epidermis and penetrated the cuticle directly by means of

an infection peg arising from the infection cushion. Following penetration of cuticle and cell wall, the infection peg in an epidermal cell enlarges to normal hyphal size and the resulting primary hyphae develop a septum a short distance from its point of origin. The hyphae invade the cortex both intracellularly and intercellularly, but more abundantly intercellularly.

The pathogenic effect of R. solani is attributed to its toxic metabolic bi-products. The effect of these toxins has been observed in wheat seedlings (45), soybean seedlings (3), turnip and carrot seedlings (50) and in potato tubers (54). These toxic bi-products are believed to penetrate in advance of the invading hyphae of the fungus.

Pratylenchus spp.

Pratylenchus spp., the root-lesion or meadow nematodes, have been reported to be serious pest of wheat (40, 48). Pratylenchus spp. are migratory endoparasites. According to Linford (42), their favorite place of entrance into the roots is slightly back of the enlongating zone in the piliferous region. Both larvae and adult nematodes enter the roots by forcing their way through or between the epidermal tissues and then feed on the cell contents as they migrate through the tissues.

Research in recent years has thrown more light on the mechanism of injury caused by the nematodes. Mountain and Patrick (49) found that the nematodes would induce necrotic lesions under aseptic conditions. They observed that the nematodes hydrolyzed cyanophoric glucoside and amygdalin in peach root to benzaldehyde and hydrogen cyanide, which are phototoxic to peach roots. They also observed that the nematodes released substances, probably enzymes, in vitro which hydrolyzed

amygdalin. They, therefore, concluded that the lesion in peach roots resulted from the release of phytotoxins through the hydrolysis of amygdalin by the nematodes.

Morgan and McAllen (47) found high concentration of pectinase and cellulose in P. penetrans. They postulated that the high concentration of the enzymes in the nematode could provide rapid hydrolysis of plant tissue and, thereby, provide additional or suitable nutrient substrates, such as glucose and galacturonic acid, upon which root rotting organisms survive. This is an important finding that needs further investigation. It may help in the understanding of the host-fungi-nematode interactions that cause root rot of wheat.

Plant Root Rot Diseases and Water Stress

Levitt (41) defined biological stress as "any environmental factor capable of inducing a potentially injurious strain in living organisms." He classified strain caused by stress into two groups - "plastic strain" and "elastic strain." In "plastic strain," the physical or chemical changes that occur in the living organism are irreversible while in "elastic strain," the physical or chemical changes are reversible, if the strain is removed. Ward (71), on the other hand, considered stress to be any deviation from environmental conditions optimum for expression of disease resistance.

Plant water stress or water deficit refers to situations where cells and tissues are less than fully turgid (35). Water stress actually takes place in plants when the rate of transpiration exceeds the rate of absorption. This phenomenon is characterized by decreases

in water content, osmotic potential and total potential of the plant. The plant loses turgor, the stomata close and there is decrease in growth. The plant anatomy, morphology, physiology and biochemistry are modified by water stress (35).

Stress may influence plant disease through its effects on the pathogen, host susceptibility, or on the host-pathogen interactions (58). Invasion of the host plant may occur before or after exposure to stress, but the end result is usually the same (58).

Stress may prevent invasion of obligate foliar pathogens by causing stomatal closure or formation of thicker cuticle (26) but in most cases other pathogens enter regardless of stress effects. Therefore, the influence of stress on disease susceptibility is usually on disease development (14, 70).

Many researchers (29, 39, 56, 58) think that stresses (drought, flooding, freezing, defoliation, and transplanting) can predispose plants toward greater susceptibility to plant diseases. In the United States in the 1930's, many plant diseases such as birch dieback, ash dieback, oak decline, maple decline, dry face of slash pine, and pitch streak of slash pine were associated with extended periods of low precipitation (29, 56). Leophart and Stage (39) reported that the extended drought which occurred in the United States from 1916 to 1940 was a factor in the origin and severity of pole blight disease of western white pines. Couch and Bloom (16) showed that water stress predisposed Kentucky bluegrass to Sclerotinia homeocarpa.

Cook et al. (15) reported that the hyphal growth of F. roseum "Culmorum" was stimulated when the osmotic potential of growth medium they used was decreased from -8 to -10 bars. Cook and Papendick (14)

also reported increased growth of other pathogens on media of low water potential.

Cook et al. (15) noted the effect of soil water stress on the growth and disease severity of soil-borne plant pathogens. The optimum growth range of Phytophthora cinnamoni Rands; Gaeumannomyces graminis (Succ); Arx. and Oliv; Rhizoctonia solani Kuhn; and Thielaviopsis basicola (Berk. and Br.) Fevr. is -5 bars or wetter. They noted also in their studies that, when the water potential was between -20 to -25 bars, their growth rate was reduced to half. At a lower water potential between -50 to -60 bars, there was little or no growth at all. All these pathogens cause greatest damage in wet soils.

In contrast, the root, foot, stem, and seedling diseases caused by Fusarium solani (Mart.) Appel and Wr. emend. Synd. and Hans. are among the few plant pathogens that cause greatest damage in dry soils. Cook et al. (15) showed that the optimum growth of the fungus was between -10 to -30 bars. At water potential between -40 to -60 bars, its growth rate was reduced to half, and was prevented only when the water potential approached or dropped below -100 bars.

Cook (13) thinks that the ability of certain plant pathogens to grow at low water potentials, which inhibits growth of antagonistic organisms, may be a factor in increased incidence of disease under drought conditions. Cook and Papendick (14) stated that the predisposing effect of drought stress generally is not on initial establishment of the pathogen in the host, but rather on development of established infection.

In spite of much work done in this area of research, little is known about the influence of water stress on plants and their pathogens.

Perhaps the main reason is due to the difficulties and limitations of controlling the water potential of the soil.

Wheat and Water Stress

Wheat grows best in cool climates with moderate rainfall, and performs well in heavy loam and clay soils. It requires cool weather throughout the tillering stage. The minimum water content of the seed that is required for active germination varies with environmental conditions. In general, a range of 35 to 40 percent is required for germination (53).

The minimum, optimum, and maximum temperatures required for effective germination also varies with other environmental conditions, as well as with different species, and conditions of the seeds. However, in general, the temperatures for germination ranges are approximately as follows: minimum, 3.5 to 5.5 C; optimum, 20 to 25 C; and, maximum, 35 C (53). High temperature and high humidity limit its production in the tropics. However, the new spring type bread wheat and the rainfed winter wheat, which have a wide range of adaptability, are being grown now in the tropics (18).

Six distinct stages of growth are usually recognized in the growth and development of wheat after germination, namely: emergence, tillering, stem extension, heading, flowering, and ripening (53).

Water-requirement of plants varies from species to species and even in varieties within the same species. Also the water requirement of any crop is not the same under different climatic and soil conditions. Temperature, atmospheric humidity, light, wind, soil water content and available nutrients, increase or decrease the rate of transpiration

and thereby affect the water requirement of the plant. Shantz and Piemeisel (59) tested 17 varieties of common wheat and 5 varieties of Durum wheat between 1911 and 1917 and found their water requirements based on total dry matter by weight average to be 557 and 542 grams respectively. Many wheat breeders expected that the amount of water requirement to produce a grain of dry substance may be useful for breeding drought-resistant wheat, but it has not been very helpful (59).

Water stress and its relationship to plants have been reviewed extensively by many authors (30, 35, 61). Here more attention is paid to water stress effects on wheat growth development. Water stress affects the morphology, physiology and biochemistry of the plant (35). It also predisposes the plant to soil-borne plant pathogens such as Fusarium spp. and H. sativum, which cause greatest damage in dry soil (13, 26). Its overall effects are reduction in growth and yield.

Wheat is grown in most arid and semi-arid regions of Canada, USA, Australia, USSR, and India (5, 18, 58), and, therefore, must have the ability to resist drought. The ability of wheat to resist drought is influenced by high temperature and soil conditions. Drought and disease severity of certain pathogens, such as H. sativum,are usually accompanied by high temperatures. Some wheat varieties, such as Einkorns, are drought resistant because of their morphological characteristics. They have reduced number of stomata when compared with common wheat (52). Wheat varieties also have variable root systems. Those that have deeper and extensive roots can trap accumulated nutrient and moisture in deeper soil profiles (52). Breeding for drought- and disease-resistant wheat has been a great challenge for many plant breeders, plant physiologists, and plant pathologists.

Research on this is still going on. Oggema and Wabwoto (52) suggested the idea of relating drought resistance to the stages of plant development and to previous field-cultural practices. They also suggested the idea of testing drought resistance wheat under dry and irrigated conditions and by varying amount and time of application of water. In addition, the breeder must determine wilting coefficient and also study the varieties under different ecological and geographical conditions. Their reason for doing this was that heavy clay soils may be an asset in making selections. Plants growing in heavy clay soil, though they usually perform well, may be under water stress most days because water movement is slow in such soils (31).

Vogel (69) mentioned the need for breeders to consider the effects of soil moisture stress on soil-borne pathogens in future breeding for increased tolerance to moisture stress. A study by Cook in 1973 (13) showed that Fusarium root rot severity increases as the soil water potential decreases. He also suggested the need for wheat breeders to work hand-in-hand with soil microbiologists, soil physicists, and plant pathologists. Their attempts to relate plant-water relations to disease development by monitoring both leaf-water potentials and soil-water extraction for different varieties under different systems of management may result in the development of new and more reliable screening procedures for wheat breeders (52).

Bozzini (5) suggested the need to analyze, as carefully as possible, the climatic and soil conditions prevalent in the area concerned and their impact on crop development and crop production.

A better understanding of wheat-water relations and the effects of soil-borne pathogens on stomatal closure and opening will be helpful

is breeding wheat varieties that will be resistant to drought and soil-borne pathogens like Fusarium spp. and H. sativum that cause severe damages to plant under dry soil conditions.

CHAPTER III

PATHOGENICITY STUDY OF DIFFERENT ISOLATES OF *HELMINTHOSPORIUM SATIVUM* AND *FUSARIUM* SPP. ON HARD RED WINTER WHEAT

The purpose of this study was to determine the degree of pathogenicity of different isolates of *H. sativum* and *Fusarium* spp., collected from several wheat fields in Oklahoma, on 'Danne,' a hard red winter wheat cultivar. Relationships between inoculum levels of the pathogens and disease severity were also determined only for selected isolates of *H. sativum* and *Fusarium* spp.

Materials and Methods

Five isolates of *H. sativum* and six isolates of *Fusarium* spp. were evaluated for their pathogenicity and virulence characteristics in greenhouse trials. All isolates were collected from diseased-wheat plants from various areas of Oklahoma. *Helminthosporium sativum* isolates were obtained from wheat fields near: Cherokee (isolate 1) in Alfalfa County, Custer City (isolates 47, 53, 58) in Custer County, and Mangum (isolate 59) in Greer County. *Fusarium* spp. isolates were collected from wheat fields near Cherokee (isolate 13) in Alfalfa County, Custer City (isolate 29) in Custer County, Chattanoga (isolate 30) in Comanche County, Hinton (isolate 31) in Caddo County, Perkins

(isolate 33) in Payne County and Geary (isolate 34) in Blaine County. The isolate numbers correspond to their culture collection numbers in the laboratory of Dr. L. L. Singleton of the Department of Plant Pathology, Oklahoma State University, Stillwater, Oklahoma. For easy reading and understanding of this paper, the isolate numbers will be used to associate the fungal isolates throughout the discussion.

Conidial inoculum of H. sativum and Fusarium spp. isolates were prepared by growing each isolate on oat kernels (oat kernels:water 2:1 v/v) in 250 ml Erlenmeyer flasks. The oat kernels were prepared, autoclaved for 90 mins at 15 psi (1.1 atm) cooled, inoculated with each isolate and incubated at 25 C for 14 days. Conidial spore suspensions were obtained by flooding flasks with sterile distilled water, agitating and filtering through cheesecloth. Clear condial spore suspensions were obtained by repeated flooding of the flasks with sterile distilled water and decanting the upper liquid layer when the spores were settled at the bottom of the flasks. The number of condial/ml of H. sativum was determined by pipetting 0.1 ml of the conidial suspension of each isolate into a nematode counting dish and counted under a stereoscope. The conidia (macroconidia) concentration of Fusarium spp. was determined by direct microscopic count of 0.01 ml aliquot of each isolate using the hemacytometer.

To a known weight of sterile Lincoln sandy soil (94.9% sand and 3% clay) obtained locally, a known volume of conidial suspensions of each fungal isolate was added such that a conidia population of 250 conidia/g of soil on a dry weight basis was obtained. The inoculated soil was mixed thoroughly in Electric Bucket Mixer, No. 7658711 (McMaster-Carr Supply Company, Chicago, Illinois 60680, USA) for 30

minutes to obtain uniform distribution of the conidia in the soil, and water added while mixing to raise the water content of the soil to field capacity (7.5%). The Lincoln fine sand used was previously treated with methyl bromide, air-dried and passed through a 2.0 mm mesh sieve. Conidia-infested soil of respective isolates of H. sativum and Fusarium spp. were put in plastic pots (11.0 x 14.5 cm). Soil temperature in the pots was controlled at 25 C (± 2 C) by using the OSU Soil Temperature Control System developed by Dr. C. C. Russell of the Department of Plant Pathology, Oklahoma State University, Stillwater, Oklahoma. This system consists of "U"-shaped, coated copper heat-sinks inserted in the soil. Desired temperature controlled water obtained from a temperature control water-bath was pumped through the heat-sinks to obtain uniform soil temperature throughout the pots. The heat-sinks-temperature control water-bath is a modification of the "Cornell Temperature Control Baths" (24).

Certified seeds of hard red winter wheat cultivar, 'Danne,' were planted. Five seeds were planted in each pot at a depth of 5.1 cm. Control pots of non-infested soil were prepared and seeds planted also. A completely randomized design was used in the study. There were five replications for each fungal isolate treatment and the experiment was triplicated.

Plants were kept fairly moist and all were fertilized during the fourth week with water soluble fertilizer (15-30-15; 4 g/l). All experiments were terminated at the end of the ninth week. Subcrown internodes with lesions covering 50-100%, 25-49%, 12.5-24%, and 0-12% of their surface areas were classified as severe, moderate, slight, and healthy. Then the disease rated subcrown internodes were clipped off of plants, surface sterilized by agitating them for 2 minutes in 25%

Chlorox solution followed by a rinse in sterile water, and plated on modified Capez-Dextrose Agar (CDA) medium (62) to determine percentages of infected subcrown internodes. Plated subcrown internodes were incubated at room temperature (26-28 C) for 7 days before counts were made to determine percentage recovery of the pathogens from infected internodes.

Inoculum Density Studies With *Helminthosporium Sativum* and *Fusarium* sp.

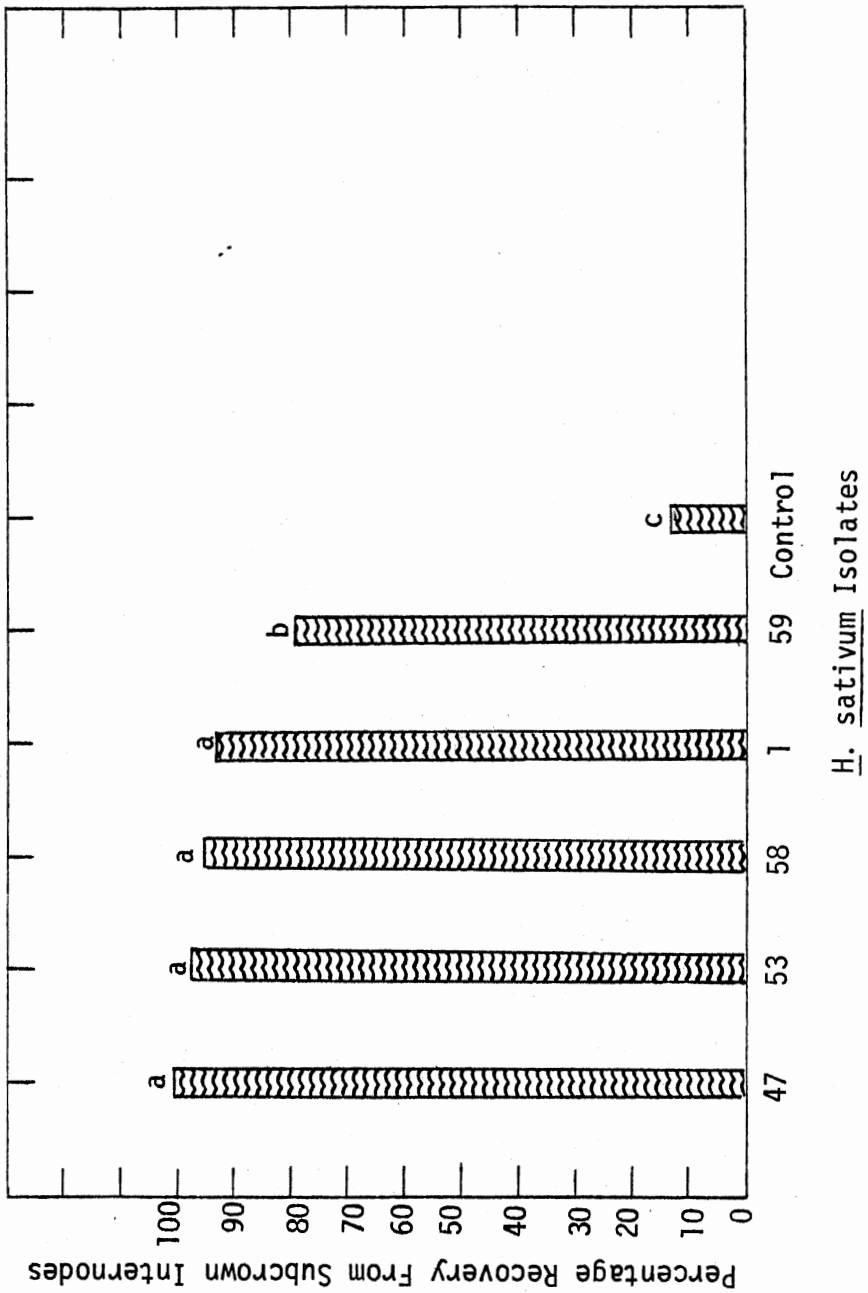
Isolate 47 of *H. sativum* and isolate 34 of *Fusarium* sp. were chosen for further studies of the effects of inoculum density on pathogenicity. They were chosen based on their high degree of pathogencity and virulence observed in previous studies. The materials and methods were the same as described previously, with the exception that the inoculum density levels were adjusted for each isolate at the rate of 1, 10, 250, 500 and 1000 conidia or macroconidia per g/soil on a dry weight basis. The experiment was terminated at the end of nine weeks, and subcrown internodes were rated and plated on Capez-Dextrose Agar as described previously.

Results and Discussion

Helminthosporium sativum Isolates Study

Results of this study showed that all isolates of *H. sativum* were highly pathogenic on the wheat cultivar 'Danne' (Fig. 1). The range in percent recovery for the five isolates from subcrown internodes after 9 weeks was from 79-100% and all were significantly different

Fig. 1. Percentage Recovery of Five H. Sativum Isolates From Subcrown Internodes of Wheat Cultivar, Danne Inoculated at the Rate of 250 Conidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C. Bars having the same letter are not significantly different (P=0.01) according to Duncan's multiple range test. Mean of three replicates.

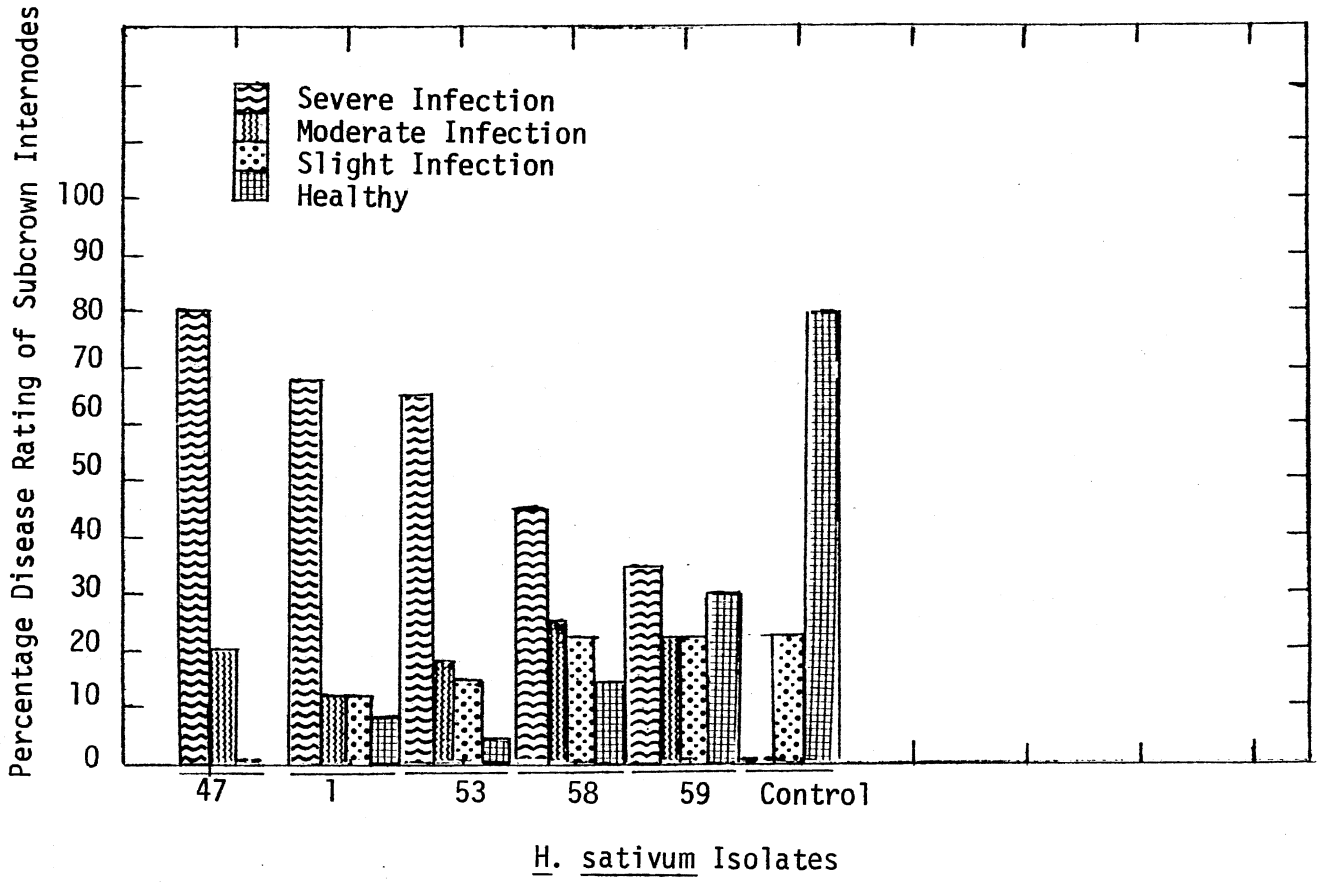

Percentage Recovery From Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
a
a
a
a
b
c
47
53
58
1
59
Control
H. sativum Isolates

from the check. The check plants also showed a mean percent recovery of 12% by the end of the 9 weeks incubation period, possibly because of contamination via watering, air, and/or seed-borne contamination. Results also showed that some isolates were very virulent while other isolates were relatively non-virulent (Fig. 2). The plants inoculated with isolate 47 were more severely attacked than the others. Isolate 47 caused 80% severe infection as compared to isolate 59 that caused only 38% severe infection. This clearly indicates the appreciable differences in isolates degrees of virulence in these tests.

Results of this study also showed variability in virulence among isolates of the fungus from different localities as well as isolates from the same field locations. Isolates 47, 53 and 58 isolated from diseased plants obtained from same field location near Custer City caused mean percentage disease severity of 80%, 68% and 58%, respectively; while isolates 1 and 59 obtained from field locations near Cherokee and Mangum caused mean percentage severe infections of 78% and 38%, respectively (Fig. 2).

The results of this study are in accordance with the findings of Christensen (8) in his study of mutation and pathogenicity of 37 forms of H. sativum. He reported that all the 37 forms of H. sativum could attack the root and basal stem of wheat and barley. He also found that some forms were more virulent. He also found out that mutation is one means that the mutants of the fungus differed from the parents in their morphological characters, pathogenicity, and virulence. Christensen's findings on mutation in H. sativum may account for the variability that occurs in the field.

Fig. 2. Percentage disease Rating of Subcrown Internodes of Wheat Cultivar, Danne Inoculated With Five Isolates of H. sativum at the Rate of 250 Conidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C. Mean of three replicates.


Severe Infection
Moderate Infection
Slight Infection
Healthy
Percentage Disease Rating of Subcrown Internodes
100
90
80
70
60
50
40
30
20
10
0
47
1
53
58
59
Control
H. sativum Isolates

There are possibilities that soil environment such as temperature, moisture, and acidity under field conditions could have affected the growth of both the pathogen and the host plant, thereby influencing disease development and disease severity, since the isolates were collected from different ecological niches. The effects of soil environment on pathogenicity of H. sativum on wheat, as studied by Dosdall (21), showed that the mycelium of H. sativum will grow from 1 C to 37 C; the optimum being near 28 C. Germ tubes penetrated the tissue of both coleoptile and the leaf at from 12 C to 34 C, but severe infection occurred through a narrow range from 22 C to 30 C. Disease development and disease severity were greater at higher temperature than at lower temperature. She (21) found out that the fungal spores germinated better in alkaline solution than in acid solution and the spores could tolerate a high degree of alkalinity. She also noted that wheat plants suffered most from root infection by H. sativum in soils containing both maximum and minimum extremes of moisture. She also noted that disease severity in plants planted in inoculated heavy loam and sandy soils were practically the same and caused less severe effects than the sandy loam and peat soils. Based on her findings on the effects of different soil types inoculated with H. sativum on disease severity in plants, there are possibilities that the isolates used in this study could differ appreciably in virulence if studied in sandy loam and peat soils since the present study was done in sandy soil.

These findings, therefore, suggest the need of investigating and understanding the ecology of the pathogen in major wheat fields in Oklahoma to know factors that influence mutation and pathogenicity of H. sativum and host-pathogen interactions. Information obtained from

the studies will be helpful in developing resistance screening trials against the pathogen and for predicting disease epidemics.

Fusarium Isolates Study

Results of this study showed that all isolates of *Fusarium* were pathogenic but some were more aggressive than others (Fig. 3). Recovery of isolates 29 and 34 from subcrown internodes incubated for 9 weeks ranged from 51-60% and was significantly greater than the percent recovery for the other four isolates (Fig. 3). The lowest mean percentage recovery was 21 and 25% for isolates 13 and 30, as compared to 33 and 36% for isolates 31 and 33, respectively, with the groups (13 and 30 vs. 31 and 33), being significantly different from each other. All isolates were significantly different from the check plants in terms of percent recovery (Fig. 3).

Results also showed that some of the isolates are more virulent than others (Fig. 4). Based on mean percent disease rating of the subcrown internodes of the plants, isolates 33 and 34 caused 16% and 21% severe infection, respectively, while isolates 13 and 30 caused 4% and 0% severe infection, respectively. Thus as with *H. sativum* the *Fusarium* isolates were all pathogenic, but differed in their levels of virulence.

Under the conditions of this experiment, *H. sativum* isolates were more aggressive on Danne wheat seedling than the *Fusarium* isolates with respect to their virulence characteristics. Percentages recovery of *H. sativum* isolates from the subcrown internodes varied from 78-100% (Fig. 1), while percentages recovery of *Fusarium* varied from 21-60% (Fig. 3). Results also showed that *H. sativum* caused more injury on

Fig. 3. Percentage Recovery of Six Isolates of Fusarium spp. From Subcrown Internodes of Wheat Cultivar, Danne Inoculated at the Rate of 250 Macroconidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C. Bars having the same letter are not significantly different (P=0.01) according to Duncan's multiple range test. Mean of three replicates.

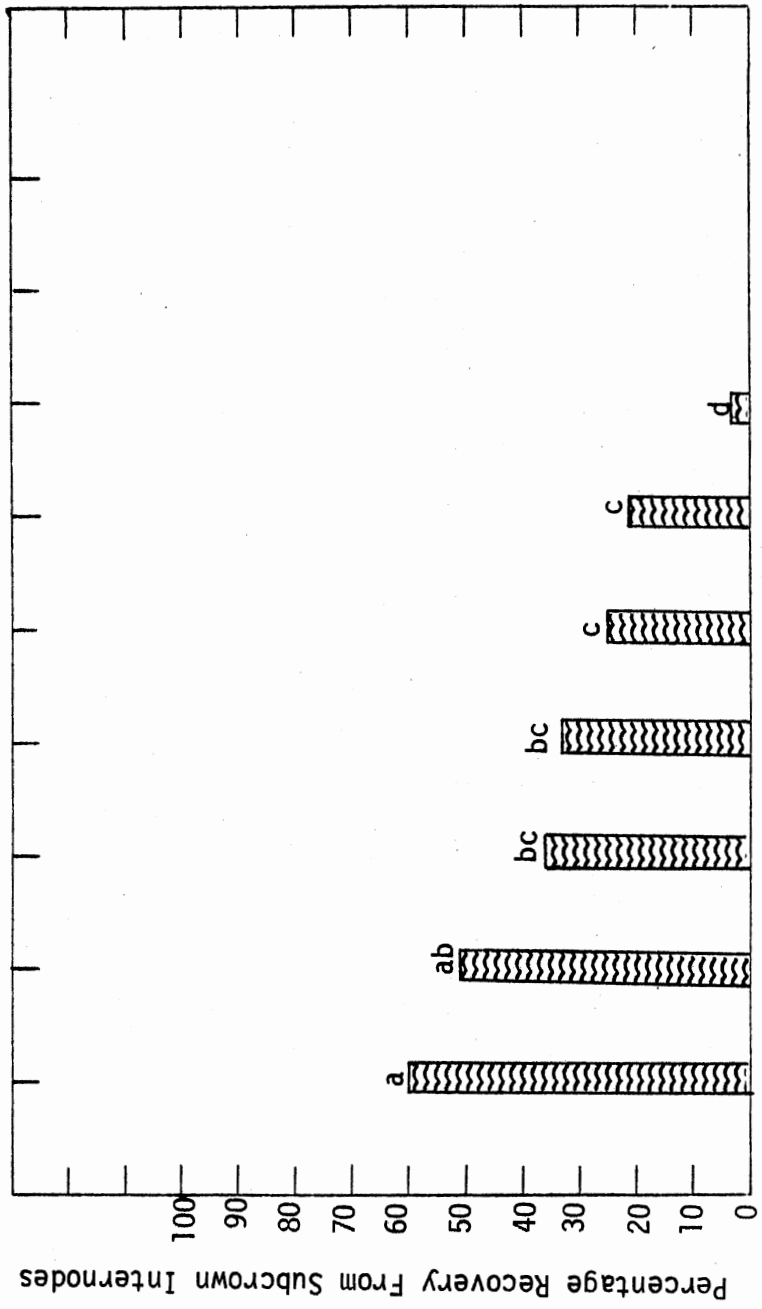
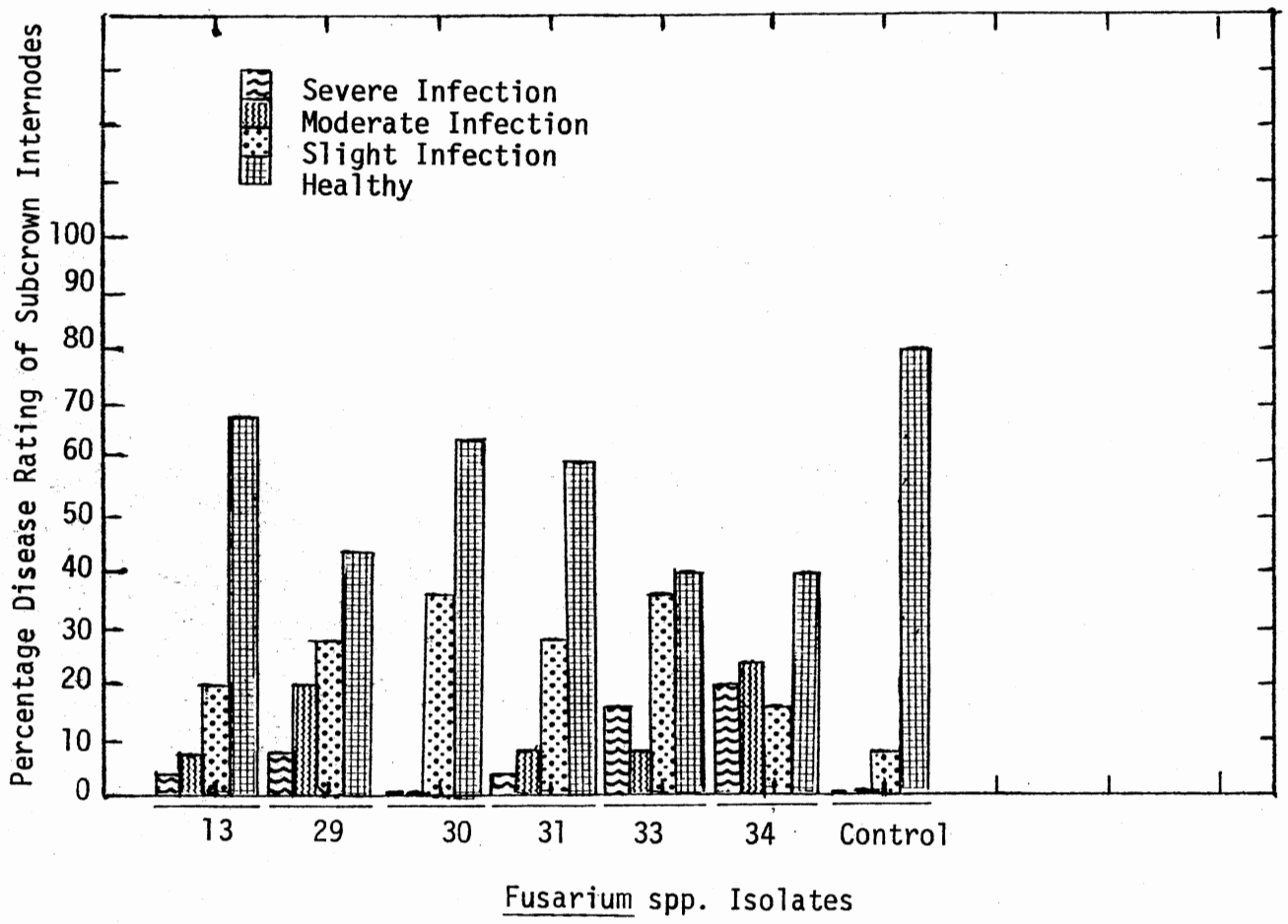

Percentage Recovery From Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
a
ab
bc
bc
c
c
d
34
29
33
31
30
13
Control
Fusarium spp. Isolates

Fig. 4. Percentage Disease Rating of Subcrown Internodes of Wheat Cultivar, Danne Inoculated With Six Isolates of Fusarium spp. at the Rate of 250 Macroconidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C. Mean of three replicates.


Severe Infection
Moderate Infection
Slight Infection
Healthy
Percentage Disease Rating of Subcrown Internodes
100
90
80
70
60
50
40
30
20
10
0
13
29
30
31
33
34
Control
Fusarium spp. Isolates

seedling plants than the Fusarium isolates. Percentages of severe infection of H. sativum isolates on the subcrown internodes varied from 35-80%, while percentages of severe infection by Fusarium isolates on the subcrown internodes varied from 0-21% (Figs. 2 and 4). Thus, this suggests that H. sativum isolates may have greater potentials of damaging the wheat seedlings than Fusarium isolates.

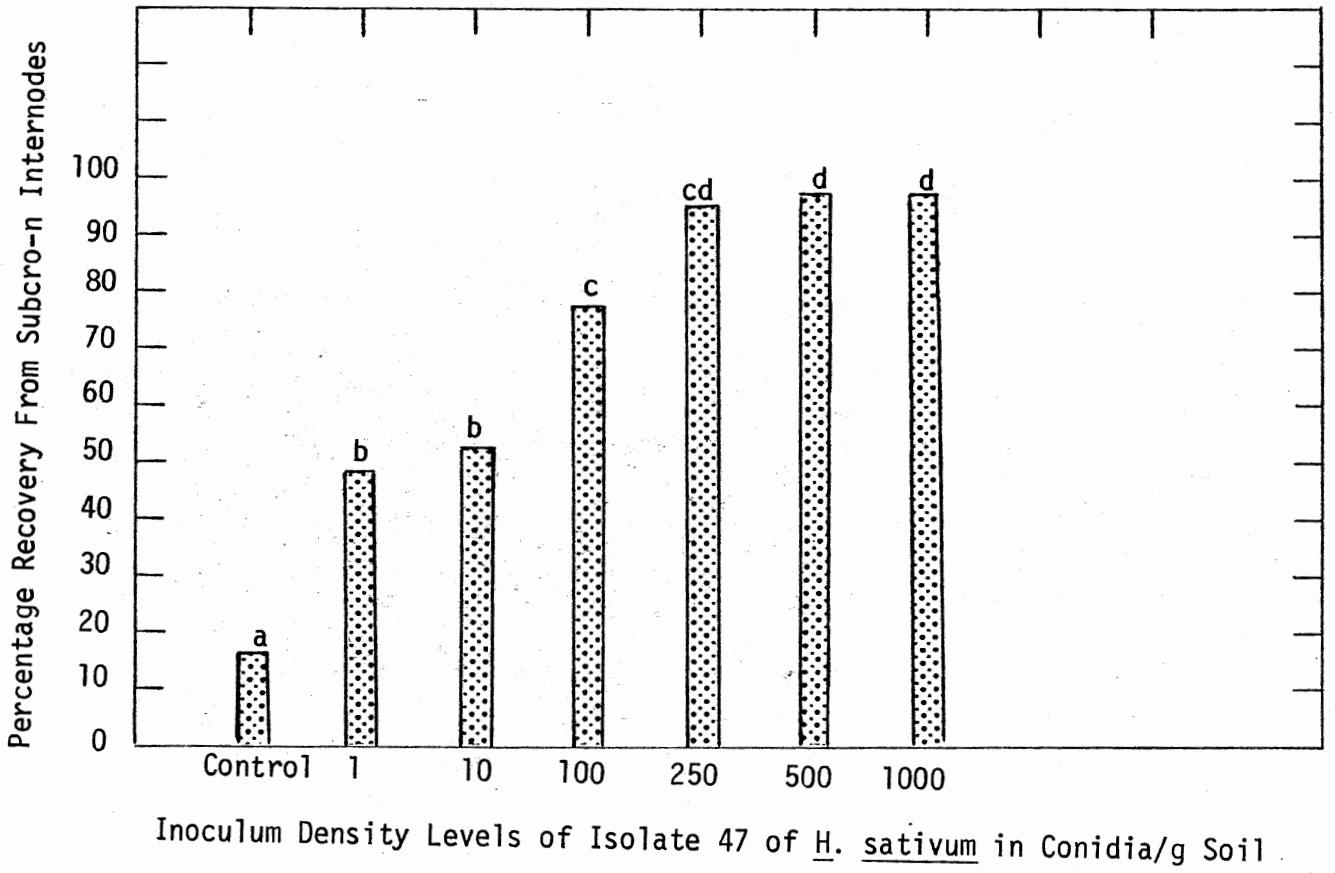
Inoculum Density Level Study

Helminthosporium sativum Inoculum Density Level Study.

Fig. 5 shows mean percentage recovery of isolate 47 of H. sativum from subcrown internodes of Danne wheat seedlings inoculated at the rates of 1, 10, 250, 500 and 1000 conidia per g soil and incubated for 9 weeks. Results showed that H. sativum infected the plants at each inoculum density level. However, percentage infection increased as the inoculum density increased. For instance, mean percentage recovery of H. sativum from subcrown internodes ranged from 48-97% as the inoculum density levels increased from 1-1000 conidia per g soil, respectively (Fig. 5). Percentage infection number plateaued as the inoculum density level increased from 250-1000 conidia per g soil.

Results also showed that mean percentage recovery of H. sativum from plants inoculated at the rate of 1 and 10 conidia per g soil were not significantly different (48% vs. 52%, respectively) (Fig. 5). Also mean percentage recovered of the pathogen in plants inoculated at the rates of 250, 500 and 1000 conidia per g soil were not significantly different (91% vs. 97% vs. 97%, respectively). However, mean percentage recovery of the pathogen from plants inoculated at the rates of 1,10

Fig. 5. Percentage Recovery of Isolate 47 of H. sativum From Subcrown Internodes of Wheat Cultivar, Danne, Inoculated at Different Inoculum Density Levels (1, 10, 100, 250, 500 and 1000 Conidia per g Soil) and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C. Bars having the same letter are not significantly different (P=0.01) according to Duncan's multiple range test. Mean of three replicates.


Percentage Recovery From Subcro-n Internodes
100
90
80
70
60
50
40
30
20
10
0
a
b
b
c
cd
d
d
Control
1
10
100
250
500
1000
Inoculum Density Levels of Isolate 47 of H. sativum in Conidia/g Soil

conidia per g soil were significantly different from plants inoculated at the rate of 100 conidia per g soil (48%, 52% vs. 77%, respectively). Mean percentage recovery of H. sativum from plants inoculated at the rate of 100 conidia per g soil were significantly different from plants inoculated at the rates of 250, 500 and 1000 conidia per g soil (77% vs. 91%, 97% and 97%, respectively) (Fig. 5).

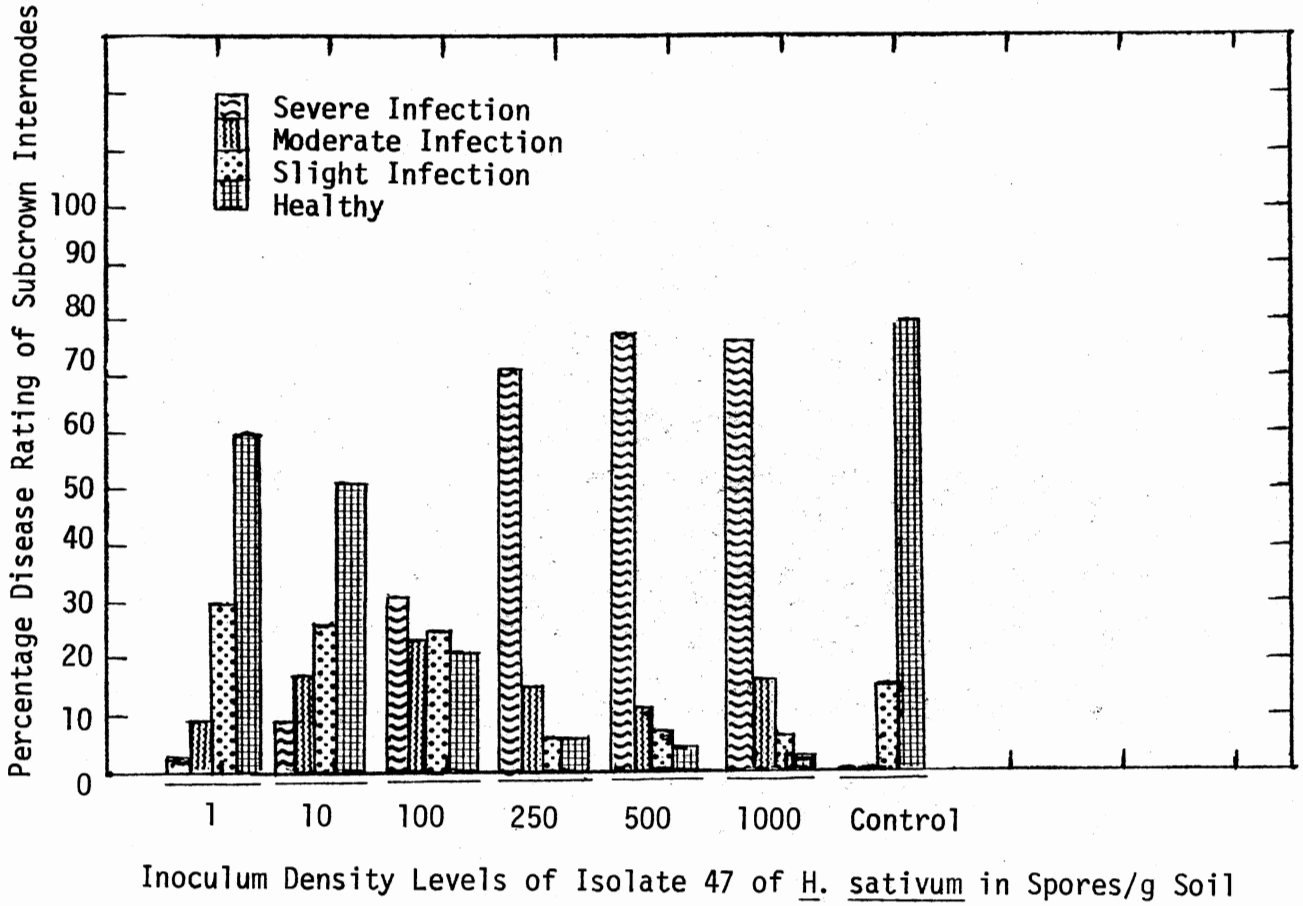
Disease severity/inoculum density level results (Fig. 6) also showed similar trend in infection severity ranging from 5-80% severity for inoculum levels ranging from 1-1000 conidia per g soil, respectively. Also the infection severity rating reached equilibrium in plants inoculated at the rates of 250-1000 conidia per g soil (Fig. 6).

Ledingham and Chinn (38) in 1955 surveyed conidial populations of H. sativum in field soil and studied the relationship of the spore population to disease occurrence on seedlings and mature plants. In field survey of 47 fields, from soil samples they brought in, they found spore population ranging from less than 10 to over 250 viable conidia of H. sativum per gm of soil. Chinn et al. (7) examined spores of H. sativum from 200 soil samples collected from 100 fields within a radius of 85 miles of Saskatoon, Canada, and found conidiospores of H. sativum to number from less than 10 to almost 900 per gram of soil.

Findings of Chinn et al. (7) suggest that conidial populations of H. sativum can range from 1 to 900 per g of soil in wheat fields, depending on conditions of soil environment and that the fungal conidial population may vary seasonally.

Inoculum density levels used in this study are, therefore, typical of what can be found in the field. Results of this study showed that about 5-80% severe infection can be obtained for inoculum density

Fig. 6. Percentage Disease Rating of Subcrown Internodes of Wheat Cultivar, Danne, Inoculated With Isolate 47 of H. sativum at Different Inoculum Density Levels (1, 10, 100, 250, 500 and 1000 Conidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C). Mean of three replicates.


Severe Infection
Moderate Infection
Slight Infection
Healthy
Percentage Disease Rating of Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
1
10
100
250
500
1000
Control
Inoculum Density Levels of Isolate 47 of H. sativum in Spores/g Soil

levels of H. sativum ranging from 1-1000 conidia per g soil, and that disease severity increased with increase in inoculum density level until equilibrium was reached at 250-1000 conidia per g soil. This information will be helpful in host-resistance screening trials because inoculum density levels of the pathogen which can cause economic injury to host plant is already known and can be utilized in the screening program.

Fusarium Inoculum Density Level Study. Results of this study showed that the plants could be infected in each inoculum density level used (Fig. 7). Results also showed that the mean percentage recovery of Fusarium from the subcrown internodes increased from 41-57% as the inoculum density level increased from 1-1000 macroconidia per g soil, then plateaued between 100 and 500 macroconidia per g soil, and declined to 48% at 1000 macroconidia per g soil. The overall results of inoculum density level/infection number for Fusarium under conditions of this study suggest antagonism as described by Van der Plank (68). That is, there is a competitive displacement among the spores for limited infection sites on host plant as the pathogen population density reaches 1000 macroconidia per soil.

Mean percent disease severity rating of the subcrown internodes inoculated with Fusarium showed that 50-85% were healthy, 8-25% slightly infected, 8-20% moderately infected and 0-15% severely infected (Fig. 8). Under the conditions of this experiment, H. sativum caused more injury than Fusarium when the same inoculum density levels are compared.

Wiese (73) reported that under favorable environmental conditions, the threshold population of Fusarium culmorum necessary to cause a

Fig. 7. Percentage Recovery of Isolate 34 of Fusarium spp. From Subcrown Internodes of Wheat Cultivar, Danne, Inoculated at Different Inoculum Density Levels (1, 10, 100, 250, 500 and 1000 Macroconidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C). Bars having the same letter are not significantly different (P=0.01) according to Duncan's multiple test range. Mean of three replicates.

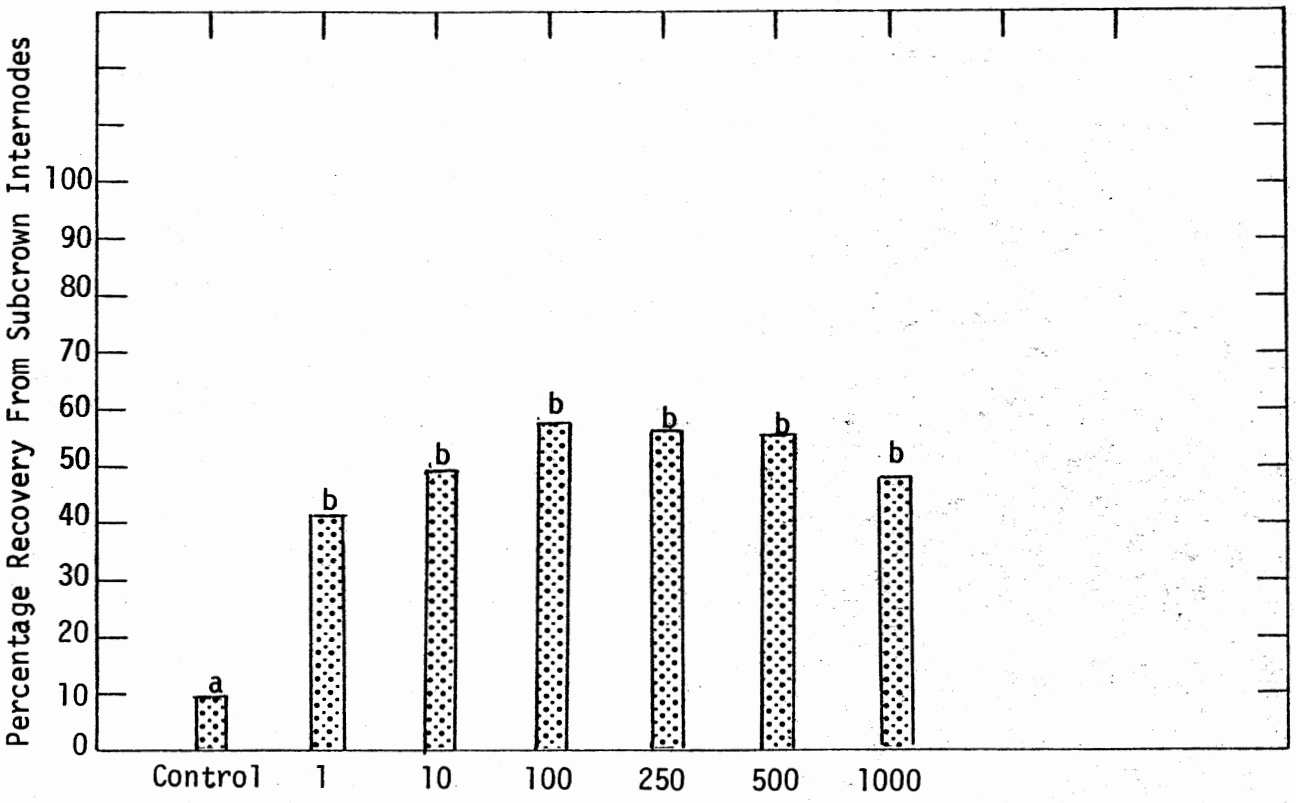
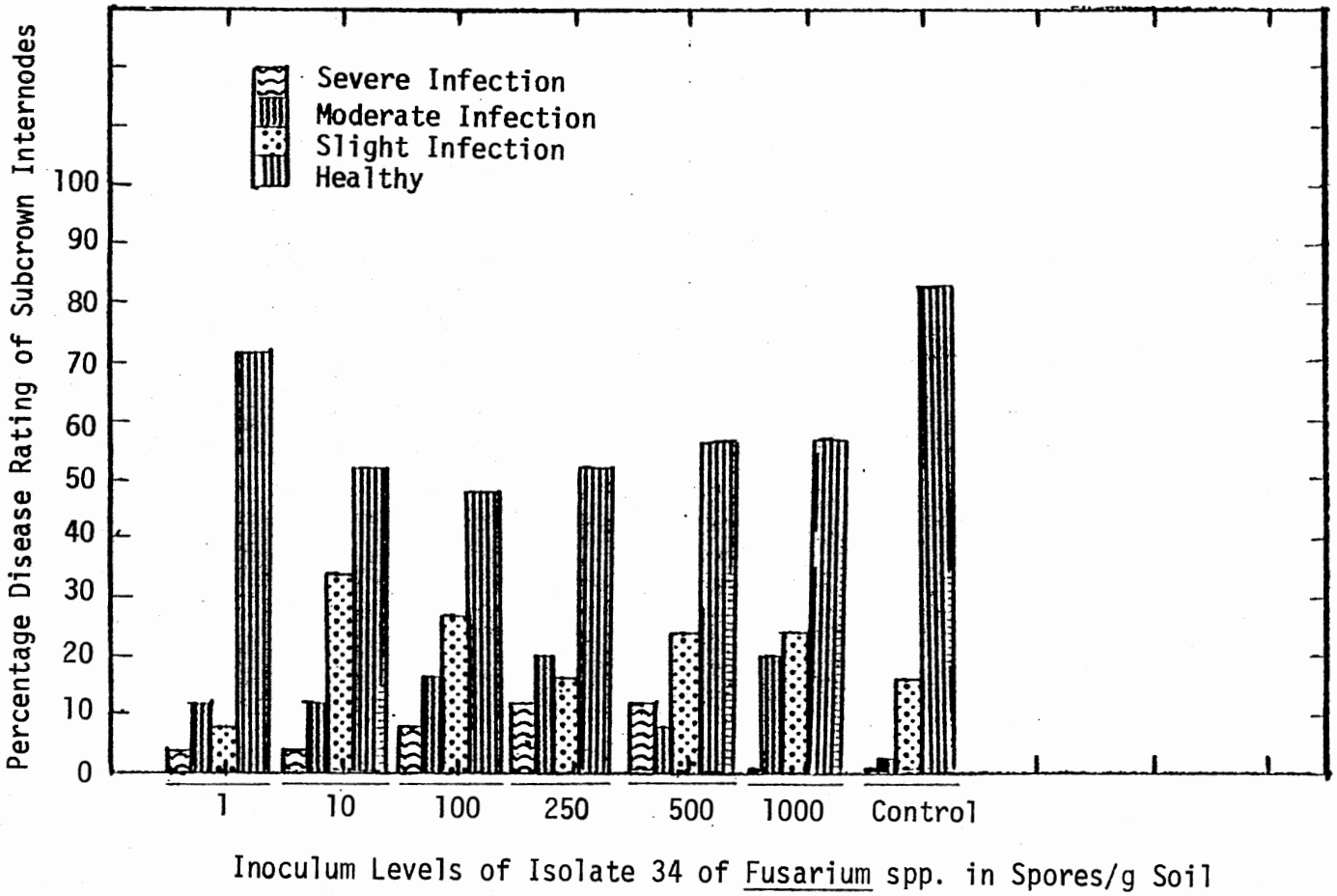

Percentage Recovery From Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
a
b
b
b
b
b
b
Control
1
10
100
250
500
1000
Inoculum Density Levels of Isolate 34 of Fusarium spp. in Macroconidia/g Soil

Fig. 8. Percentage Disease Rating of Subcrown Internodes of Wheat Cultivar, Danne, as Caused by Isolate 34 of Fusarium spp. at Different Inoculum Density Levels (1, 10, 100, 250, 500 and 1000 Macroconidia per g Soil and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C). Mean of three replicates.

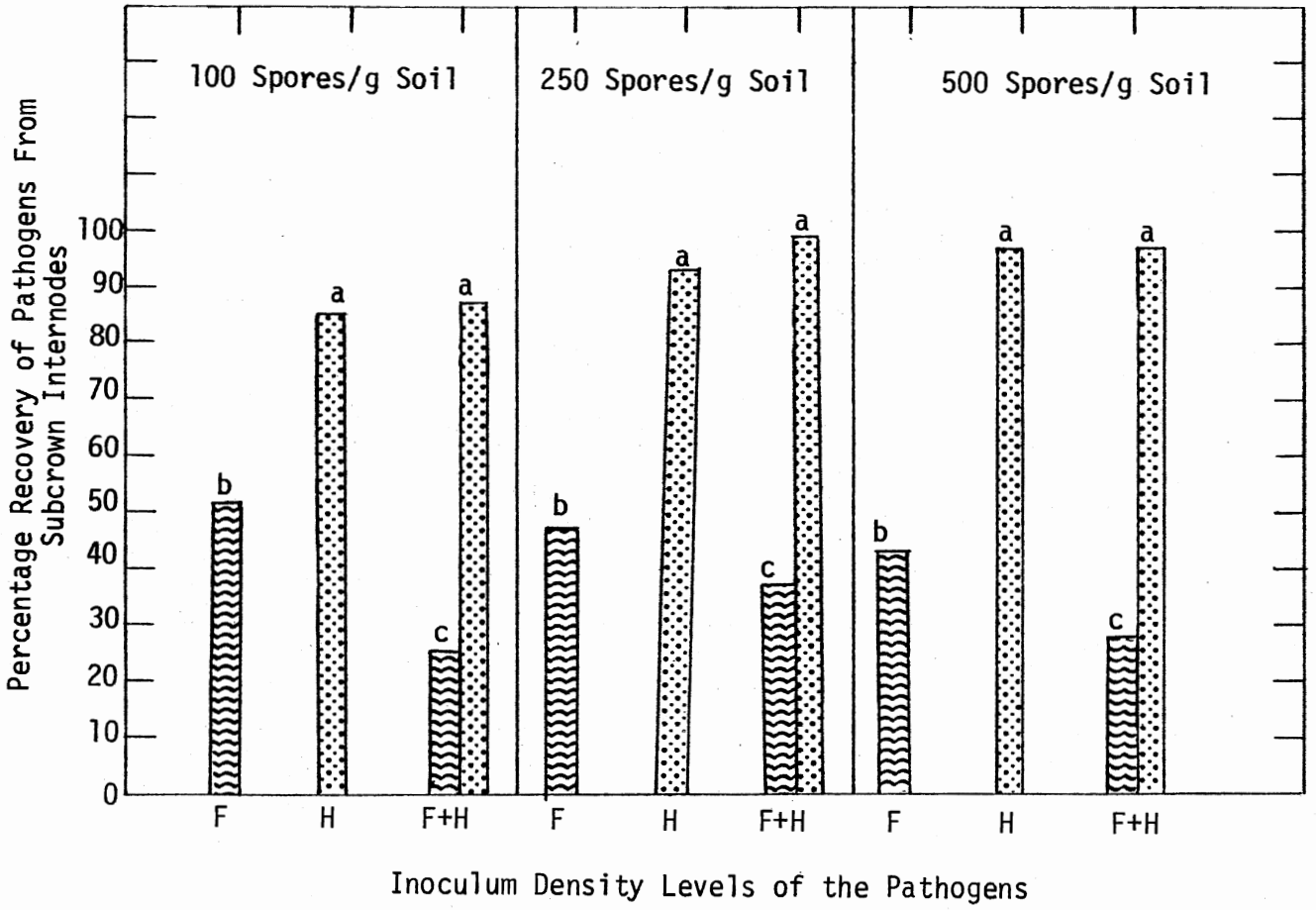

Severe Infection
Moderate Infection
Slight Infection
Healthy
Percentage Disease Rating of Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
1
10
100
250
500
1000
Control
Inoculum Levels of Isolate 34 of Fusarium spp. in Spores/g Soil

detectable yield reduction in approximately 100 propagules (Chlamydospores) per g of soil. Under the conditions of this study, results showed that plants inoculated even at the rate of 1 macroconidium per g soil caused severe infection which might cause a detectable yield reduction. Results also showed that maximum severe infections were obtained in plants inoculated at inoculum density range of 100-500 macroconidia per g soil. There were no plants severely infected when plants were inoculated at the rate of 1000 macroconidia per g soil.

Results of this investigation have identified the inoculum density level of Fusarium capable of causing economic injury on host plant which can be utilized in host resistance screening trials in Oklahoma.

Helminthosporium sativum, Fusarium Each Alone and in Combination. Comparative study of plants inoculated with H. sativum, Fusarium each alone and both combined at three inoculum density levels (100, 250, and 500 conidia for H. sativum and macroconidia for Fusarium), showed that H. sativum's ability to attack the plants did not differ significantly (in all inoculum levels used except in 100 conidia per g soil) whether it was alone or in combination with Fusarium (Fig. 9). This was not the case with Fusarium. Fusarium ability to attack the plants when alone was significantly different from that when it was in combination with H. sativum (Fig. 9). This seems to indicate the existence of antagonism between the two pathogens when they are together, as reported in the literature (36, 68). Boosalis (4) reported existence of antagonism between H. sativum and Fusarium when they are together. Young (75) grew H. sativum and Fusarium spp. on artificial medium in the laboratory and observed that H. sativum grew much slower when Fusarium

Fig. 9. Percentage Recovery of Isolate 34 of *Fusarium* spp. and Isolate 47 of *H. sativum* From Subcrown Internodes of Wheat Cultivar, Danne, Inoculated at Different Inoculum Density Levels (100, 250, and 500 Macroconidia and/or Conidia per g Soil for *Fusarium* and *H. sativum*, Respectively, and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C). Bars having the same letter within each inoculum density level are not significantly different (P= 0.01) according to Duncan multiple test range.
F = *Fusarium* inoculated plants.
H = *H. sativum* inoculated plants.
Mean of three replicates.


100 Spores/g Soil
250 Spores/g Soil
500 Spores/g Soil
Percentage Recovery of Pathogens From Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
b
a
c
a
b
a
c
a
b
a
c
a
F
H
F+H
F
H
F+H
F
H
F+H
Inoculum Density Levels of the Pathogens

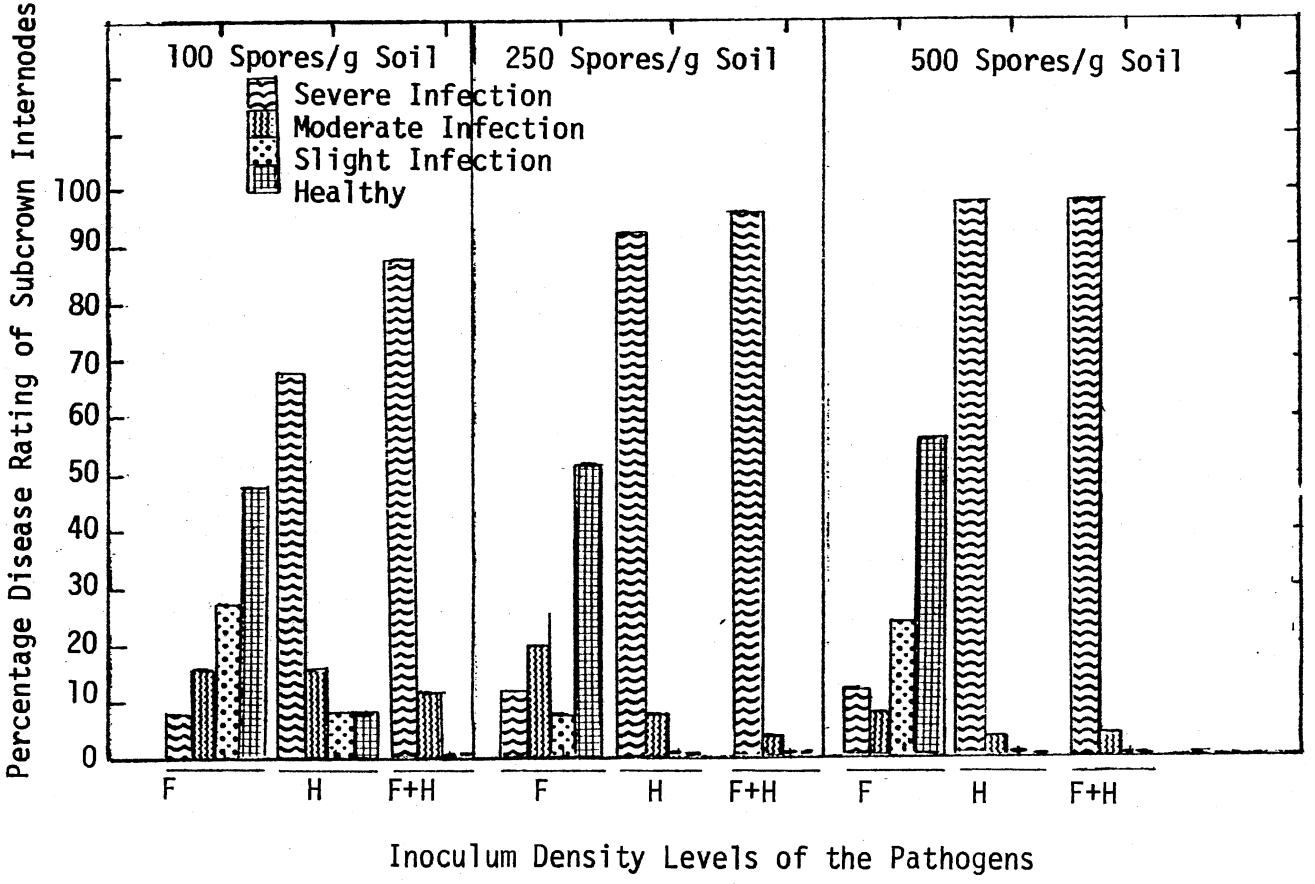
is present. In 1942, Ledingham (36) conducted comparative studies of H. sativum and F. culmorum and reported that the two pathogens showed antagonism toward one another when associated on wheat seed. Greaney and Machacek (28) in 1935 found in their study that the pathogenicity of H. sativum was reduced in the presence of Trichothecium (Cephalothecium) roseum Corda.

Inoculum density level/disease severity rating of the subcrown internodes (Fig. 10) showed no additive effect when both pathogens were combined. The effects of each pathogen alone seemed to be average of the effects of both pathogens together.

Chinn et al. (7) in a comparative study of the role spore population of H. sativum and Fusarium spp. in infected field and greenhouse with naturally infected soils were able to isolate H. sativum from 48 seedlings of the 94 soil samples used and disease ratings ranged from 1 to 46 with a mean of 28 and Fusarium spp. Isolates ranged from 0 to 26 with a mean of 10. Isolation of H. sativum and Fusarium spp. during the seedling stage were made from the basal portions of the seedling. Chinn et al. (7) findings are in agreement with results of this study in that H. sativum alone causes more injury in the seedling stage than Fusarium alone or both together.

Investigation of the ecology and population densities of the pathogens will help to provide a basis for integrated disease management.

Fig. 10. Percentage Disease Rating of Subcrown Internode of Wheat Cultivar, Danne, as Caused by Isolate 34 of *Fusarium* spp. and Isolate 47 of *H. sativum* at Different Inoculum Density Levels (100, 250, and 500 Macroconidia and/or Conidia per g for *Fusarium* and *H. sativum*, Respectively, and Grown for 9 Weeks Under Controlled Soil Temperature of 25 C ± 2 C).
F = *Fusarium* inoculated plants.
H = *H. sativum* inoculated plants.
F+H = *Fusarium* + *H. sativum* inoculated plants.
Mean of three replicates.


100 Spores/g Soil
250 Spores/g Soil
500 Spores/g Soil
Severe Infection
Moderate Infection
Slight Infection
Healthy
Percentage Disease Rating of Subcrown Internodes
0
10
20
30
40
50
60
70
80
90
100
F
H
F+H
F
H
F+H
F
H
F+H
Inoculum Density Levels of the Pathogens

CHAPTER IV

INVASION PATTERNS AND PENETRATION OF WINTER WHEAT SEEDLINGS BY *HELMINTHOSPORIUM SATIVUM*, *FUSARIUM* SP. AND *RHIZOCTONIA SOLANI*

This study was undertaken to determine time of penetration of the first seminal root regions and the first subcrown internode of wheat seedlings by *H. sativum*, *Fusarium* sp. and *R. solani*. In addition, degrees of susceptibility of the seminal root regions (differentiation, elongation, and root cap) and the subcrown internode by the pathogens were determined.

Materials and Methods

General Procedure

Penetration times and regions of first seminal root and subcrown internode of Danne wheat seedling's susceptibility to *H. sativum*, *Fusarium* sp. and *R. solani* were studied. Three methods were used: (a) an *in vitro* agar technique (personal communication with Dr. C. C. Russell, Department of Plant Pathology, Oklahoma State University, Stillwater, Oklahoma); (b) inoculum suspension dip technique, and (c) infested soil technique.

Certified Danne wheat seeds were pregerminated by aerating them in a 0.01% streptomycin sulfate solution until the radicles were about 3-4 mm long. Then 90 healthy seedlings with straight radicles were randomly selected from the pregerminated seedlings for each pathogen study (30 seedlings used for each technique).

Danne wheat seedlings with first subcrown internodes used in the study were obtained by the procedure of Nitsch and Nitsch (51). Ninety healthy seedlings with straight radicles and straight subcrown internodes were used for each pathogen study (30 seedlings used per technique). All seedlings used in the study were blotted on clean Scottie tissues before use in each technique. The experiment was repeated five times.

In Vitro - Agar Technique

For the in vitro agar technique, water agar (0.75%) was prepared and poured into petri dishes just to cover the bottoms of the dishes and allowed to solidify. Pre-cut, 0.47 mil polyethylene kitchen wrapping film discs (0.002 cm/diameter), were placed over the surface of the agar in the dishes and the surfaces were smoothed to remove any air bubbles that might be trapped. A crescentic area of about one-fourth of the agar and film disc in each dish were carefully cut-off with a sharp sterile scalpel and removed. Then each of the film discs on the agar were carefully lifted with sterile forceps and the radicle of the wheat seedling was carefully pushed between the agar surface and the film disc. The surface of the dish was smoothed again to remove any air bubbles. The dishes were taken to a growth compartment in the laboratory (25 C) and placed at approximately 45^{o} from horizontal under

fluorescent lights with the dishes face-down to promote root growth against the film discs.

When the first seminal roots had reached about 1.0 cm in length, the root regions (differentiation, elongation, and root cap) were microscopically identified and located by making marks on the bottom of the dishes. The seedling roots (30 seedlings per pathogen) were then inoculated with the pathogens propagules (conidia for H. sativum, macroconidia for Fusarium sp., and mycelia for R. solani) at the rate of 250 propagules per volume of distilled water by lifting one end of the film disc and placing the inoculum to the external survace of the roots using a graduated syringe. The inoculum was uniformly dispersed on the agar surface by lifting and dropping the film disc with forceps. After inoculation, the cut-off edge of the film disc, as well as the entire edge of film, were sealed with a mixture of petroleum jelly and wax (1:1 ratio), heated to about 50-60 C. This was to prevent contamination and loss of water from the agar. The dishes were then taken to the growth compartment and placed at 45^{o} as previously described.

The subcrown internodes obtained by the procedure of Nitsch and Nitsch (51) were also inoculated and taken to the growth compartment as previously described for the first seminal roots.

At 24 hrs. incubation intervals, 30 seedlings (10 seedlings for each pathogen) were randomly selected, washed with tap water for 2 mins on a 2.0 mesh sieve and blotted on clean Scottie tissues. Then about 1.0 mm of the regions of differentiation, elongation, and root cap (previously identified microscopically and marked at inoculation time) of the first seminal root, as well as the first subcrown internode, were cut out and assayed for the pathogens. The tissue pieces were

surface sterilized by agitating for 2 mins in 25% Chlorox solution, rinsed in sterile water and plated on acidified potato-dextrose-agar (APDA) medium amended with 0.05 g Chloromycin and 0.05 Streptomycin sulfate per liter. Plated tissues were incubated at room temperature for 7 days before counts were taken to determine percentage recovery of the pathogens from infected tissues.

Inoculum-Suspension Dip Technique

The seedlings' first seminal roots (30 seedlings used per pathogen study) were submerged for about 2 mins in a suspension of the pathogens inoculum (conidia for H. sativum, macroconidia for Fusarium sp. and mycelia for R. solani) prepared at the rate of 250 propagules per volume of water and planted in sterile sandy soil in plastic pots (one seedling per pot). Seedling with subcrown internodes, obtained by the procedure of Nitsch and Nitsch (51), were inoculated the same way and planted in sterile soil. All seedlings were incubated in the growth compartment and assayed for the pathogens at 24 hr. incubation intervals as previously described in the in vitro agar technique. The root regions of differentiation, elongation, and root cap, as well as the subcrown internode pieces used for the assay of the pathogens, were cut out using the standardized procedure previously described in the in vitro agar technique. Infected tissues were counted to determine percentage of pathogen recovery 7 days after plating. The experiment was repeated five times.

Infested Soil Technique

Sterile sandy soil was infested with each pathogen at the rate of

250 propagules (conidia for H. sativum, macroconidia for Fusarium sp. and mycelia for R. solani) per g soil and put in clean plastic pots. Selected wheat seedlings obtained as previously described in the general procedure section were planted (one seedling per pot) in the infested soil. Thirty seedlings were used for each pathogen study. Inoculated seedlings were then taken to the growth compartment and at 24 hr incubation intervals 30 seedlings (10 seedlings from each pathogen) were taken and assayed for the pathogens as previously described in the in vitro agar technique. Seedlings with subcrown internodes were inoculated and assayed for the pathogens in the same way. Infected tissues were counted to determine percentage pathogens recovery 7 days after plating. The experiment was repeated five times.

Results and Discussion

Preliminary assay of inoculated seedlings with the three pathogens at zero hour showed no surface carry over of the pathogens in plated tissues after using the surface sterilization technique previously described. But results showed that the pathogens are capable of infecting the first seminal roots and first subcrown internodes of Danne wheat seedlings. Mean percentage recovery of the three pathogens in all the three methods, irrespective of tissue regions, after 24 hours following inoculation were 52.0, 49.0 and 0.0 for Fusarium, H. sativum and R. solani, respectively, and the percentages increased to 70.0, 74.0, and 20.0 by 72 hours, respectively. Thus, this indicates that penetrations of Fusarium and H. sativum occurred most in the first 24 hours after inoculation and penetrations of R. solani were less compared with the other two pathogens.

Results also showed that the mean percentage penetration of Fusarium and H. sativum in the three methods of inoculation with respect to tissue regions did not differ appreciably at each time interval (Figs. 11 and 12). No penetration by R. solani occurred after 24 hours' incubation in any of the three methods of seedling inoculation (Fig. 11). This is possibly because of the time it takes for R. solani to form infection-cushion before it can penetrate (25). Flentje (25) listed four successive stages which may hinder a successful, progressive infection of host plant by R. solani. However, penetrations occurred at 72 hours' incubation in all the methods, but percentage of penetrations obtained were far less than those obtained in plants inoculated with Fusarium or H. sativum at this time interval (Fig. 12).

Results obtained also showed that the subcrown internode and the root region of differentiation were most susceptible to penetration by all the three pathogens with all the three methods and the root cap region was the least susceptible (Fig. 11 and 12). This seems to suggest that the pathogens penetrated the older parts of plants to the younger parts of the plant. This may also account for progressive rotting of roots and subcrown internodes as the growing season progresses (73).

Although Wiese (73) reported that H. sativum is usually the first pathogen to enter the root and is later over-run by Fusarium, as the growing season progressed, it is not possible under the conditions of this experiment to determine the first pathogen to get in since both pathogens were able to penetrate at 24 hours' incubation period. Further studies at smaller incubation times may help throw more light on this.

Fig. 11. Percentage Recovery of *Fusarium* sp., *H. sativum* and *R. solani* From First Seminal Root Regions and Subcrown Internodes of Danne Wheat Seedlings After 24 Hours Incubation in Each of the Three Methods: (A) Russell's *in vitro* Agar Technique, (B) Root Dip, and (C) Infested Soil Method. Seedlings were inoculated with pathogen propagules (conidia for *H. sativum*, macroconidia for *Fusarium* sp., and mycelia for *R. solani*) at the rate of 250 propagules per vol. of water or per g of soil). Incubated at room temperature under fluorescent lights. Mean of five replicates.

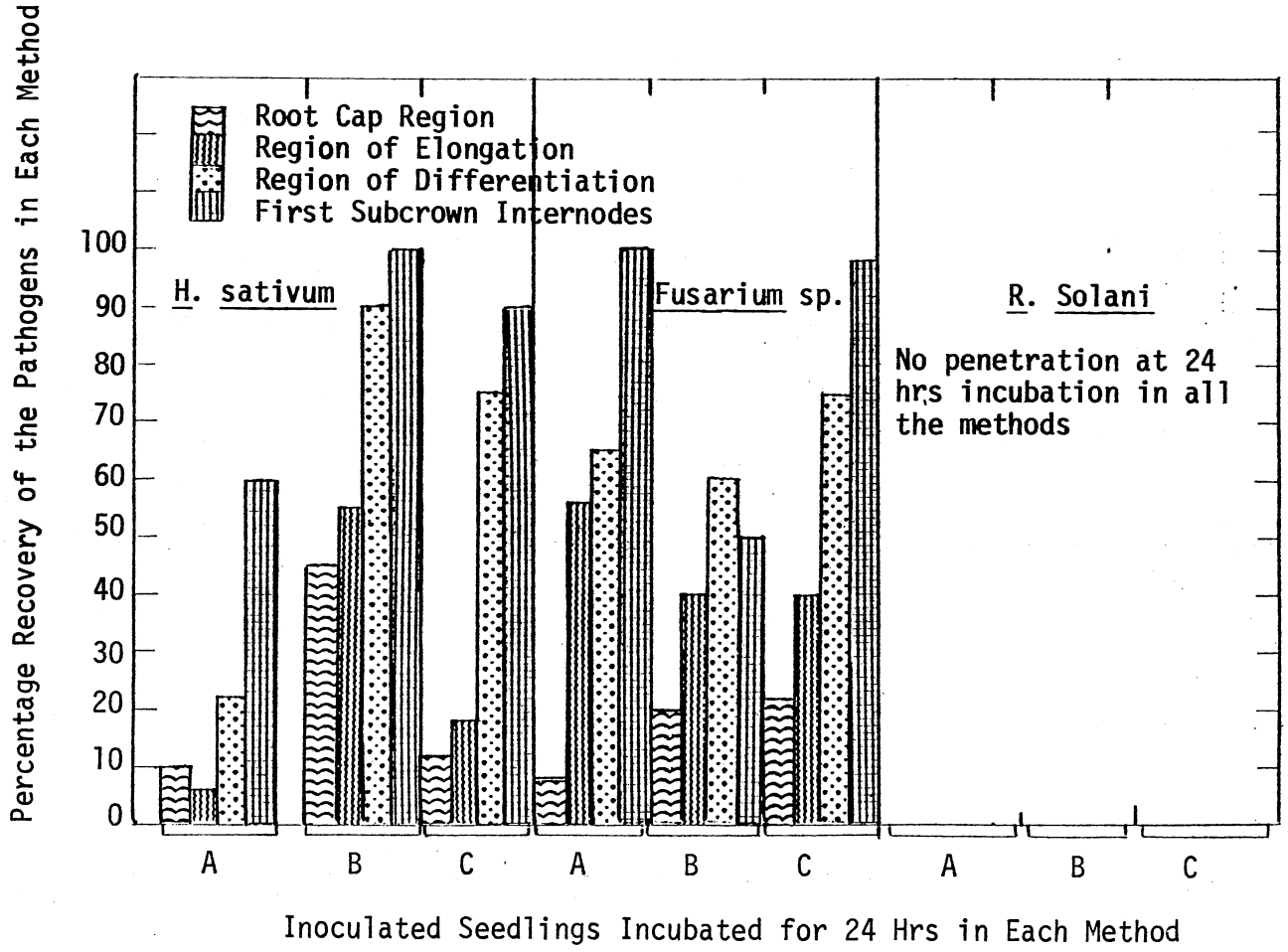
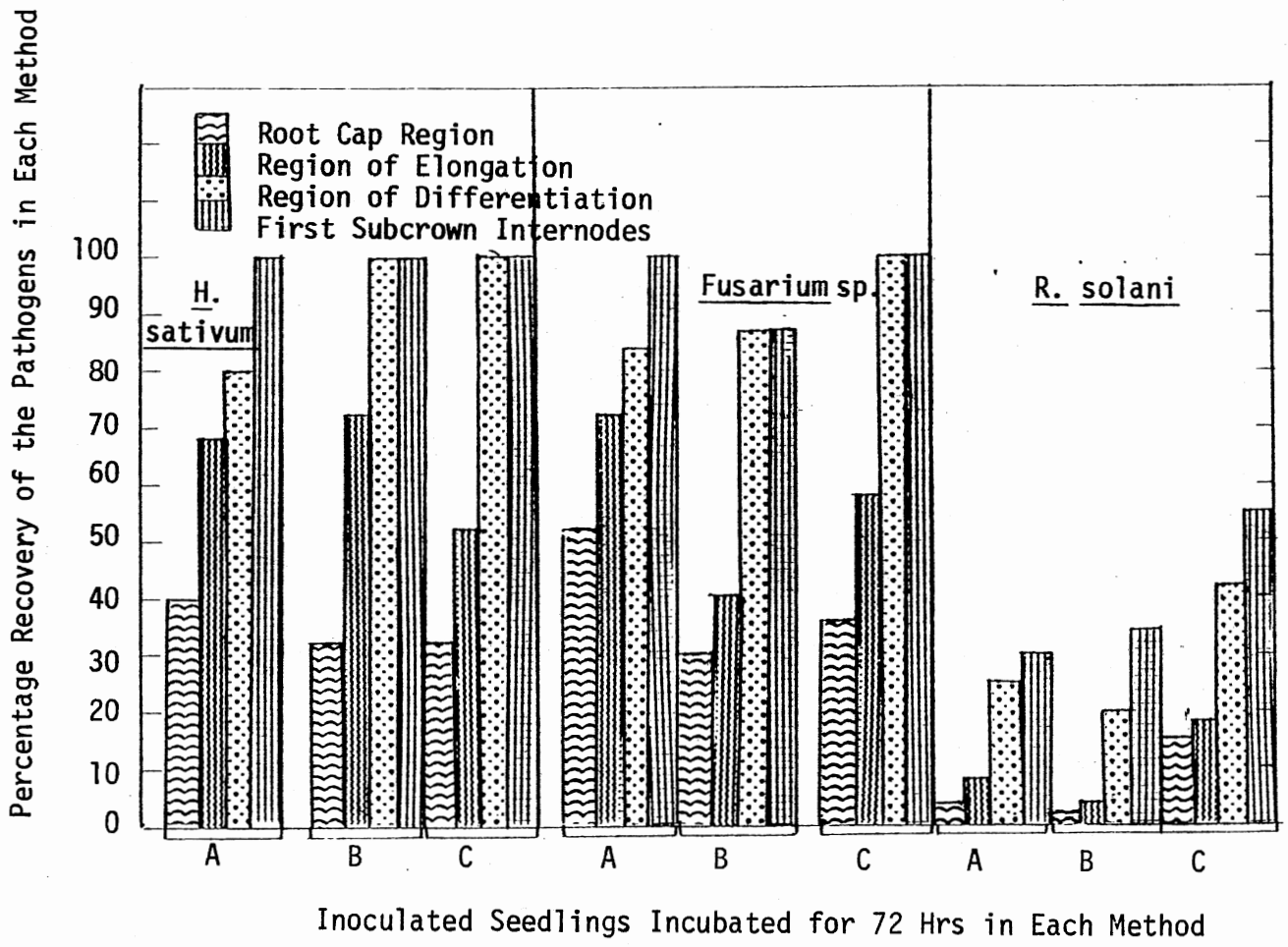

Root Cap Region
Region of Elongation
Region of Differentiation
First Subcrown Internodes
H. sativum
Fusarium sp.
R. Solani
No penetration at 24 hrs incubation in all the methods
Percentage Recovery of the Pathogens in Each Method
0
10
20
30
40
50
60
70
80
90
100
A
B
C
A
B
C
A
B
C
Inoculated Seedlings Incubated for 24 Hrs in Each Method

Fig. 12. Percentage Recovery of *Fusarium* sp., *H. sativum* and *R. solani* From First Seminal Root Regions and Subcrown Internodes of Danne Wheat Seedlings After 72 Hours Incubation in Each of the Three Methods: (A) Russell's *in vitro* Agar Technique, (B) Root Dip, and (C) Infested Soil Method. Seedlings were inoculated with pathogens propagules (conidia for *H. sativum*, macroconidia for *Fusarium*, sp. and *R. solani*) at the rate of 250 propagules per vol. of water or per g of soil. Incubated at room temperature under fluorescent lights. Mean of five replicates.


Root Cap Region
Region of Elongation
Region of Differentiation
First Subcrown Internodes
H. sativum
Fusarium sp.
R. solani
Percentage Recovery of the Pathogens in Each Method
100
90
80
70
60
50
40
30
20
10
0
A
B
C
A
B
C
A
B
C
Inoculated Seedlings Incubated for 72 Hrs in Each Method

Information obtained in this study on plant-sites most susceptible to the pathogen will be of importance in disease ratings and identification of the pathogen(s) concerned and in host resistance screening trials. Wheat cultivar(s) with resistant subcrown internodes and root systems will be needed for good integrated management practices for the wheat root rot disease control.

CHAPTER V

EFFECTS OF WHEAT ROOT ROT FUNGI AND SOIL-MOISTURE-STRESS ON GROWTH AND FORAGE YIELD OF TRANSPLANTED AND NON-TRANSPLANTED WHEAT SEEDLINGS

This investigation was undertaken to determine the effects of H. sativum and Fusarium sp., each alone and in combination, on growth and forage yield of transplanted and non-transplanted Danne wheat seedlings grown under soil-moisture-stress conditions in the greenhouse. Comparisons between transplanted and non-transplanted plants were made to evaluate the effects of intermittent versus continuous exposure of the seedlings to the effects of H. sativum and Fusarium.

Materials and Methods

Certified Danne wheat seeds were planted 5 seeds per plastic pot (11.0 by 14.5 cm) in sandy soil infested with H. sativum and Fusarium, each alone and in combination, at the rate of 250 propagules (conidia for H. sativum and macroconidia for Fusarium) per g soil as described previously in Chapter III. The check plants were set up in the same way, but received no fungal treatments. The pots were arranged in a randomized complete block designed and replicated six times. The seedlings were uniformly watered for the first 13 days. On the 14th day, half of the seedlings were removed from the soil. The transplanted

seedlings were carefully washed with tap water, surface sterilized in 25% Chlorox solution for 2 mins, rinsed in sterile water, and replanted in sterile non-infested soil. After transplanting, the soil was pressed firmly around the seedlings and the seedlings were thoroughly watered and placed in their former position.

When all the plants (transplanted and non-transplanted plants) were 21 days old and transplanted plants re-established, all the pots were thinned to three plants per pot. At the same time, the water-stress treatment began. Plants in replicates 1 and 3 were watered every 3 days; those in replicates 4 and 6 every 6 days; and those in replicates 2 and 5 were watered every 9 days. Approximately 50 ml, 100 ml, and 150 ml of water were added per pot to 3, 6, and 9-day watered plants, respectively. Thus, all plants ultimately received equal amounts of water throughout the experiment, but differed in watering time. Each pot had a plastic container (11.0 x 8.0 cm) into which water was added and allowed to rise into the pots by capillarity. Plants were fertilized once (4 weeks after planting) with water soluble fertilizer (15-30-15; 4 g/L).

Three forage yields were taken when plants were 42, 63, and 84 days old on a per pot basis. The base of the first leaf was cut to obtain fresh weights (nearest 0.01 g) and oven dried (24 hrs; 80 C) and weighed to obtain the dry weights to the nearest 0.01 grams.

Plant height measurements were taken daily for seven days when plants were 35, 56, and 77 days old. Plant height was determined by measuring distance from soil surface to the tip of the longest leaf of every plant.

Roots were clipped off from plants (on a per pot basis) that

survived at the end of the experiment when plants were 84 days old. Soil particles on the roots were cleaned out by washing roots on 2.0 mesh sieve with tap water. Excess water was allowed to drain out and then roots were blotted with Scottie tissues. Root masses were weighed on Mettler Analytical Balance to obtain fresh weights and then oven dried (24 hrs; 80 C) and weighed to obtain dry root weights. The experiment was repeated three times.

Results and Discussion

Effects of the pathogens and soil moisture stress on both transplanted and non-transplanted plants were determined by measuring plant growth, forage and root yields and comparing them to those of check plants.

Effects on Forage Yield

Results showed that dry-forage yield of plants inoculated with *Fusarium* and *H. sativum*, each alone and both combined, differed significantly from check plants in both transplanted and non-transplanted plants (Table I). *Helminthosporium sativum* alone caused the greatest percentage yield reduction in over-all mean, averaged over days of forage harvests and watering regimes, when compared with percentage yield reduction caused by *Fusarium* alone and *Fusarium* and *H. sativum* combined in transplanted plants (38.4% for *H. sativum* vs. 41.6 for *Fusarium*, and 48.9 for both pathogens combined) (Table I). Low percentage yield reduction in plants inoculated with *H. sativum* in the transplanted plants may be due to these two factors: (a) soil moisture stress seems to inhibit the effects of *H. sativum* on wheat plant since

TABLE I

MEAN PERCENTAGE REDUCTION IN DRY FORAGE YIELD CAUSED BY WHEAT ROOT ROT FUNGI AND SOIL STRESS IN TRANSPLANTED AND NON-TRANSPLANTED WHEAT SEEDLINGS CV. 'DANNE'[a]

Treatment at the Rate of 250 Spores/g Soil	Transplanted Plants				Non-Transplanted Plants			
	Three Watering Regimes in Days			Mean of Watering Regimes	Three Watering Regimes in Days			Mean of Watering Regimes
	3	6	9		3	6	9	
Fusarium sp.	37.5**	56.5**	50.0**	47.6	25.0	81.8**	78.6**	51.1
Fusarium sp. + *H. sativum*	40.1**	39.1*	70.0**	48.9	15.6	86.4**	78.6**	53.8
H. sativum	28.1*	39.1*	55.0**	38.4	34.4*	86.4**	92.6**	61.8
CV% (a)	24.8				CV% (a)		31.4	
CV% (b)	19.0				CV% (b)		45.7	

* = significantly different from control at P=0.05.

** = significantly different from control at P=0.01.

[a]Forage harvested 84 days after planting; mean of three replicates.

H. sativum disease development is favored by relative high moisture (21). Dosdall (21) in her study of factors influencing the pathogenicity of H. sativum reported that wheat plants suffer most from root infection by H. sativum in soils containing both maximum and minimum extremes of moisture. Results of present study and previous studies (Chapters III and IV) also indicate that H. sativum causes greatest damage on wheat plants under relatively high or low soil moisture condition. On the other hand, Fusarium caused greatest damages on wheat plants under extremely dry soil moisture conditions (13, 73).

Results also showed that mean percent yield reduction in transplanted plants increased with increase in length of water stress period even though all the plants ultimately received the same amount of water by end of the experiment. The 3-day watered plants gave the lowest yield reduction while the 9-day watered plants gave the highest yield reduction in all the treatments (Table I). Over-all yield reduction caused by the pathogens on transplanted plants were significantly different as compared to control.

A similar trend of results was obtained in non-transplanted plants, except that greater percentage yield reductions were obtained in all treatments as compared to percent yield reductions obtained in transplanted plants (Table I). It is interesting to note here that H. sativum alone caused greater over-all percentage yield reduction in non-transplanted plants than did Fusarium alone and both pathogens combined (61.8% for H. sativum vs. 51.1% for Fusarium and 53.8% for both pathogens combined). This is a reverse of what occurred in transplanted plants. This seems to indicate the fact that H. sativum disease developed and severity is increased by continuous reinfestation of host

plants throughout the plants' development. Plants inoculated with Fusarium alone and combination of Fusarium and H. sativum also caused greater reduction in forage yield in non-transplanted plants as compared to yield reductions obtained in transplanted plants (Table I). In transplanted plants, increase in length of water stress also caused increase in yield reduction, irrespective of amount of water supplied at each stress intervals (Table I).

No additive effects on forage yields were obtained in both transplanted and non-transplanted plants inoculated with Fusarium and H. sativum combined (Table I). Therefore, a competitive or antagonistic effect seems to exist between the pathogens, as already reported in the literature (36, 68, 75). It is difficult, however, to say for sure how much yield reduction was caused by each of the pathogens separately in the combination study.

Effects on Root Yield

Results showed that H. sativum caused the lowest mean percent yield reduction of root in transplanted plants as compared to mean percent yield reduction caused by Fusarium alone and both pathogens combined (49.4% for H. sativum vs. 59.4 for Fusarium and 57.1 for both pathogens combined) (Table II). But in non-transplanted plants, H. sativum caused the highest mean percent yield reduced as compared to Fusarium alone and both pathogens combined (83.4 vs. 61.3 and 68.6), respectively (Table II). These results go further to suggest that continuous secondary infections by H. sativum help to increase damages it causes on host plant.

TABLE II

MEAN PERCENTAGE REDUCTION IN DRY ROOT YIELD CAUSED BY WHEAT ROOT ROT FUNGI AND SOIL MOISTURE STRESS IN TRANSPLANTED AND NON-TRANSPLANTED WHEAT SEEDLINGS CV 'DANNE'[a]

Treatment at the Rate of 250 Spores/g Soil	Transplanted Plants				Non-Transplanted Plants			
	Three Watering Regimes in Days			Mean of Watering Regimes	Three Watering Regimes in Days			Mean of Watering Regimes
	3	6	9		3	6	9	
Fusarium sp.	37.0*	62.5*	78.6**	59.4	29.6**	76.5**	83.3**	63.1
Fusarium sp. + *H. sativum*	44.4**	62.5*	64.3*	57.1	25.9*	88.2**	91.7**	68.6
H. sativum	14.8	62.5*	71.4*	49.4	70.4**	88.2**	91.7**	83.4
CV% (a)	6.0				CV% (a)		20.4	
CV% (b)	28.6				CV% (b)		41.5	

* = significantly different from control at P=0.05.

** = significantly different from control at P=0.01.

[a]Roots were harvested 84 days after planting.

Results also showed that root weight reduction was increased with increase in length of water stress in both transplanted and non-transplanted plants. The 9-day-watered plants gave the highest mean percent yield reduction and the 3-day watered plants gave the lowest mean percent yield reduction (Table II). In general, there were appreciable differences in yield reduction in plants in the same watering regimes in both transplanted and non-transplanted plants in all the treatments. For instance, the 9-day watered plants in transplanted and non-transplanted plants gave 78.6 vs. 83.3 mean percent yield in plants inoculated with Fusarium alone, 64.3 vs. 91.7 percent reduction in Fusarium + H. sativum inoculated plants, and 71.4 vs. 83.4 mean percent yield reduction in plants inoculated with H. sativum alone. No synergistic yield reduction was obtained in plants inoculated with both pathogens in transplanted and non-transplanted plants. In general, the amount of damage caused by both pathogens combined was smaller, or the same, than those caused by the pathogens alone (Table II).

Effects on Plant Height

In general, there were no significant differences in plant height as compared to control in all the treatments in the transplanted plants (Table III). But in non-transplanted plants, some significant differences were obtained with increase in period of water stress (Table III). Plant heights of 6 and 9-day watered plants in non-transplanted plants were significantly different as compared to their controls (Table III). These differences were not obvious in all the watering regimes in transplanted plants and in 3-day watered plants of non-transplanted plants (Table III).

TABLE III

PLANT HEIGHT (cm) BEFORE FORAGE HARVESTS AS INFLUENCED BY WHEAT ROOT ROT FUNGI AND SOIL MOISTURE STRESS IN TRANSPLANTED AND NON-TRANSPLANTED WHEAT SEEDLINGS CV 'DANNE'[a]

Treatment at the Rate of 250 Spores/g Soil	Transplanted Plants				Non-Transplanted Plants			
	Three Watering Regimes in Days			Mean of Watering Regimes	Three Watering Regimes in Days			Mean of Watering Regimes
	3	6	9		3	6	9	
Fusarium sp.	26.9	16.3	18.5	23.5	36.4	4.7**	7.6*	21.1
Fusarium sp. + *H. sativum*	24.2	16.1	12.0*	21.2	33.4	0.0**	0.0**	17.0
H. sativum	26.9	15.5	16.9	23.4	36.5	1.7**	1.7**	17.7
Control	30.0	18.6	21.9	24.4	39.7	8.5	13.5	23.5
CV% (a)	21.8				CV% (a)		14.1	
CV% (b)	15.0				CV% (b)		53.1	

* = significantly different from control at P=0.05.

** = significantly different from control at P=0.01.

[a]Plant height measured 84 days after planting, mean of three replicates.

Over-all results of this investigation showed that the pathogens are capable of causing significant reductions in wheat-plant yields especially when plants are under stresses (soil moisture and transplant stresses). The pathogens may be limiting root growth and root functions by incapacitating the xylem system which is responsible for water and nutrient uptake. Increase in disease severity by continuous reinfestation of host-plant by the pathogens cannot be overlooked in this study. This may be responsible for increase in wheat plant damage observed in the field as the growing season progresses, especially in years of scattered rainfall. Results of this investigation also noted the importance of water-stress-interval effects on yields irrespective of amount of water supplied later. Frequencies of water supply are very crucial to host-pathogen interaction leading to disease development and disease severity. This is an important consideration that has to be made in years of scattered rainfall versus years of uniform rainfall. Since both pathogens cause greatest damage on wheat plants in years of extreme (minimum and/or maximum) soil-moisture stress, then drought resistance should be considered as an important factor in any selective breeding program aimed at improving field resistance in wheat plants against these two pathogens.

CHAPTER VI

EFFECTS OF WHEAT ROOT ROT FUNGI ON SIX WINTER WHEAT CULTIVARS GROWN UNDER SOIL MOISTURE STRESS CONDITIONS

A growth chamber study was conducted to determine the effects of Fusarium and H. sativum, each alone and both combined, on six hard red winter wheat cultivars grown under soil moisture stress conditions. The cultivars studied were 'Payne,' 'Triumph 64,' 'Danne,' 'Vona,' 'TAM 101,' and 'Newton.'

Materials and Methods

Materials and methods used in this study were the same as previously described in Chapter III plus the following modifications. The study was conducted in a growth chamber at the Controlled Environmental Research Laboratory at Oklahoma State University, Stillwater, Oklahoma. Certified seeds of six hard red winter wheats: Payne, Triumph 64, Danne, Vona, TAM 101, and Newton, were used in the study. Lincoln fine sand (94.9% sand and 3.0% clay) treated with Methyl Bromide was thoroughly mixed with inoculum-suspension (macroconidia for Fusarium and conidia for H. sativum) of each pathogen alone and both combined for 30 mins at the rate of 250 spores/g soil in water sufficient to raise the water content of the soil to field

capacity (7.5%). Infested soil was put into plastic pots (11.0 x 14.5 cm). A total of 96 pots were used in the experiment. Five seeds of each cultivar were planted per pot to a depth of 5.1 cm. Plants were thinned to three plants per pot 5 days after emergence. A split-split-split-plot design with watering regimes as main plots, cultivars as subplots, and inoculated vs. uninoculated plants as sub-subplots, was used and each plot was completely randomized. Half of the plants were watered every 3 days with 50 ml of tap water and the other half with 100 ml of tap water every 7 days. Every pot ultimately received the same amount of water by end of the experiment. Each pot had a plastic container (11.0 x 8.0 cm) into which water was added and allowed to rise into pots by capillarity.

Plants were grown in a growth chamber. The growth chamber was maintained at 26 C day and 23 C night, and a 12 hour photoperiod from 0600 to 1800. Fluorescent and incandescent bulbs in the growth chamber supplied light at an intensity of 635 microeinsteins M^{-2} sec^{-1} as measured by Lambda (Lincoln, Nebraska) Instrument LI-185 Quantum, Radiometer, Photometer, in the region of exposed leaves. The relative humidity was between 50-70%. Plants were fertilized when they were four weeks old with water soluble fertilizer (15-30-15, 4 g/L). Plants were observed daily for 9 weeks when the experiment was terminated. The experiment was repeated three times.

The following measurements were taken to help determine the effects of the pathogens and soil moisture stress on the wheat cultivars.

Plant emergence was counted on a per pot basis when plants were 5 days old (before plants were thinned to 3 plants/pot).

Three forage yields were taken when plants were 21, 42, and 63

days old. Leaves were clipped on a per pot basis from the base of the first leaf and weighed on Mettler Analytical Balance (Mettler Instrument Corp., Highstown, New Jersey) to obtain fresh weights; oven-dried (24 hrs., 80 C) and weighed to obtain dry weights.

Plant height measurements were taken daily for 7 days when plants were in their third, sixth, and ninth week. Plant heights were determined by measuring distance from soil base to the tip of the tallest leaf of every plant.

Evapotranspiration rate was determined daily for 7 days when plants were also in their third, sixth, and ninth week. Measurements were taken by weighing each pot before watering, using a Sartorius 2351 scale, with maximum load of 7000 g, to the nearest 0.1 g.

Measurement of stomatal resistance helps to determine the water status of plants and in scheduling irrigations. Stomatal resistance was measured on alternate days of the week, when plants were in their third, sixth and ninth week with a calibrated stomatal diffusion porometer (33) (Model LI-65 and Sensor LI-20S, Lambda Instrument Corp., Lincoln, Nebraska). The upper leaf surface of the second leaf from the bottom of the plant was measured.

Soil samples were taken on a per pot basis with a sterile soil probe and put into small glass vials (14.5 x 45 mm, 1 Dram) sealed with plastic snap caps when plants were in their third, sixth and ninth week and taken to the laboratory to determine the soil water potential. A total of 96 samples were taken at each sampling period. Dew point readings were taken in microvolts, using the C-52 sample chamber attached to HR-33T, Dew Point Microvoltmeter by Wescor, Inc. (Longan, Utah). The soil water potential in negative bars (4) were calculated using

the dew point method. Mean soil water potential in the 3- and 7-day watered pots were -21 and -45 bars ±5 at 26 C, respectively. Very low soil water potential obtained indicates that the soil was quite dry.

Plants were rated for disease severity based on symptom expressions on leaves and stem and lesion sizes (area covered) on subcrown internodes on a scale of 1 to 5 where 1 = healthy (no visible symptoms), 2 = slight infection, 3 = moderate infection, 4 = severe infection and 5 = dead plants.

Number of tillers per plant were counted when plants were 21, 42, and 63 days old before forage harvests on same days.

Roots were harvested when plants were 63 days old on a per pot basis. The roots were thoroughly washed with tap water on a 2.0 mesh sieve to get rid of soil particles and then blotted on Scottie tissues and weighted on Mettler Analytical Balance to obtain fresh weights. The roots were then oven-dried (24 hrs., 80 C) and weighed to obtain root dry weights.

Results and Discussion

Effects on Forage Yield

Results showed that the pathogens affected dry forage yield in 3 and 7-day watered plants. The difference in yield between the infected plants and the control was generally significant at P=0.01 level (Table IV). In 3-day watered plants, *Fusarium* alone caused the highest mean percentage yield reduction on Danne and Vona and the lowest percent yield reduction on Payne and TAM 101, as compared to

TABLE IV

MEAN PERCENTAGE YIELD REDUCTION OF DRY FORAGE OF SIX WHEAT CULTIVARS INOCULATED WITH *FUSARIUM* AND *H. SATIVUM*, EACH ALONE AND BOTH COMBINED, AND GROWN UNDER TWO WATERING REGIMES

	3-Day Watered Plants				7-Day Watered Plants			
Cultivar	F	H	F+H	Means	F	H	F+H	Mean
Payne	18.75	25.00*	25.00*	22.92	34.48**	37.93**	41.38**	37.93
Triumph 64	29.73**	32.43**	62.16**	41.44	61.29**	41.94**	58.06**	53.76
Danne	35.29**	44.12**	38.24**	39.22	44.83**	34.48**	34.48**	37.93
Vona	34.38**	37.50**	34.38**	35.42	60.71**	46.45**	64.29**	57.14
TAM 101	25.71*	48.57**	31.43**	35.24	53.57**	28.57*	21.43*	34.52
Newton	26.67*	50.00**	40.00**	38.89	43.33**	66.67**	57.14**	55.71
Mean	28.42	39.60	38.54		49.70	42.67	46.13	
CV% (a) 11.6					CV% (a) 11.6			
CV% (b) 17.5					CV% (b) 17.5			
CV% (c) 17.9					CV% (c) 17.9			

F = *Fusarium* inoculated plants (250 macroconidia/g soil), mean of three replicates.

H = *Helminthosporium sativum* inoculated plants (250 conidia/g soil), mean of three replicates.

* = significantly different from control at P=0.05.

** = significantly different from control at P=0.01.

other cultivars (Table IV). In 7-day watered plants, Fusarium alone caused the highest percent yield reduction on Triumph 64 and Vona, and the lowest percent yield reduction on Payne and Newton, as compared to other cultivars (Table IV). These results seem to suggest that Vona is the most susceptible cultivar and Payne the most resistant cultivar to Fusarium. Greater percentage yield reductions were caused by Fusarium alone on all the cultivars in 7-day watered plants than in 3-day watered plants. This indicates, as reported in the literature (13, 73), that Fusarium is very aggressive under extreme soil moisture stress.

In 3-day watered plants, H. sativum alone caused the highest mean percentage yield reduction on Newton and TAM 101 and the lowest percent yield reduction on Payne and Triumph 64, as compared to the other cultivars (Table IV). In 7-day watered plants, H. sativum alone caused the highest mean percent yield reduction on Newton and Vona, and the lowest percent yield reduction on Tam 101 and Danne and Payne, as compared to other cultivars (Table IV). These results seem to suggest that Newton is the most susceptible cultivar and Payne the most resistant cultivar to H. sativum alone. Mean percentage yield reductions caused by H. sativum alone on all the cultivars in 3- and 7-day watered plants did not differ appreciably, although yield reduction seemed to increase in 7-day watered plants (Table IV). This indicates that H. sativum alone causes greatest damage on host plants under extreme (maximum and minimum) soil moisture condition as reported in the literature (21).

In 3-day watered plants, Fusarium and H. sativum combined caused the highest mean percent yield reduction on Triumph 64, Newton, and Danne and the lowest percent yield reduction on Payne and Tam 101

Table IV). In 7-day watered plants, both pathogens combined caused the highest mean percent yield reduction on Vona, Triumph 64, and Newton and the lowest percent yield reduction on Tam 101 and Danne. These results seem to suggest that Triumph 64 and Newton are the most susceptible cultivars to both pathogens combined and Payne and Tam 101 the most resistant cultivars. Effects of both pathogens on the cultivars in 3 and 7-day watered plants did not differ appreciably (Table IV).

Overall means of cultivars over treatments showed that H. sativum alone caused higher percentage yield reduction in the 3-day watered plants than Fusarium (39.60% for H. sativum vs. 28.42% for Fusarium). But in 7-day watered plants, Fusarium alone caused higher percentage yield reduction than H. sativum (49.70% for Fusarium vs. 42.67% for H. sativum) (Table IV). These differences in disease severity between Fusarium and H. sativum on host plant under different soil moisture content support reports in the literature (21) that H. sativum's aggressiveness increases at relatively high soil moisture while Fusarium's aggressiveness increases under very dry soil moisture conditions (13, 73).

Results also showed that greater percentage yield reductions occurred in 7-day watered plants than 3-day watered plants in the presence of the pathogens (Table IV).

Overall results indicate that Payne is the most resistant cultivar to the pathogens while Newton is the most susceptible cultivar to the pathogens, under conditions of this study.

Effect on Root Yield

Fusarium alone reduced the root yield of Danne and Vona significantly at P=0.05, compared to their controls, in 3-day watered plants,

TABLE V

MEAN PERCENTAGE YIELD REDUCTION OF DRY ROOT OF SIX WHEAT CULTIVARS INOCULATED WITH *FUSARIUM* AND *H. SATIVUM*, EACH ALONE AND BOTH COMBINED, AND GROWN UNDER TWO WATERING REGIMES

	3-Day Watered Plants				7-Day Watered Plants			
Cultivar	F	H	F+H	Mean	F	H	F+H	Mean
Payne	3.77	0.00	5.66	3.14	62.50	39.58	33.33	45.14
Triumph 64	31.82	22.73	63.64*	39.40	72.58*	30.65	66.13	56.45
Danne	58.11*	47.30	68.92*	58.11	44.23	32.69	51.92	42.95
Vona	68.00*	62.67*	21.33	50.67	87.10*	75.81*	72.58	78.45
TAM 101	19.51	82.93**	2.44	34.96	38.46	10.25	58.97	35.89
Newton	23.08	92.31**	51.28*	55.56	68.42*	92.98*	84.21*	81.87
Mean	34.05	51.32	35.55		62.22	46.99	61.19	
CV% (a) 40.6 CV% (b) 70.8 CV% (c) 56.0					CV% (a) 40.6 CV% (b) 70.8 CV% (c) 56.0			

F = *Fusarium* inoculated plants (250 macroconidia/g soil) means of three replicates.

H = *Helminthosporium sativum* inoculated plants (250 conidia/g soil) mean of three replicates.

* = significantly different from control at P=0.05.

** = significantly different from control at P=0.01.

while for the other cultivars, root yield was not significantly reduced (Table V). Fusarium alone caused the lowest mean percent yield reduction on Payne as compared to other cultivars (Table V). In 7-day watered plants, Fusarium alone significantly reduced root yields of Vona, Triumph 64, and Newton, compared to their controls, and TAM 101 was the least affected (Table V).

Helminthosporium sativum alone significantly reduced root yield of Newton and TAM 101 (P=0.01) and Vona (P=0.05) in the 3-day watered plants, as compared to their controls. H. sativum alone did not affect root yield of Payne (Table V). In the 7-day watered plants, H. sativum alone significantly reduced root yield of Newton and Vona (P=0.05), as compared to their controls. The cultivar, TAM 101, was the least affected, as compared to its control. The other cultivars were not significantly reduced.

Fusarium and H. sativum combined significantly reduced root yield to Triumph 64, Danne, and Newton (P=0.05) in the 3-day watered plants, and TAM 101 and Payne were the least affected by both pathogens combined. In the 7-day watered plants, both pathogens combined significantly reduced root yield of Vona and Newton (P=0.05), as compared to control, and Payne was the least destroyed by the pathogens (Table V).

Days of watering did not significantly reduce root yield of the cultivars, except that greater reduction seemed to occur in the 7-day watered plants than in the 3-day watered plants.

Results obtained on root yield agreed with results obtained on forage yield, in that Payne is the most resistant cultivar to the pathogens and Newton and Vona are the most susceptible cultivars to the pathogens. The pathogens' ability to destroy the cultivars' root

systems obviously accounted for reduced plant vigor and low forage production, as compared to their controls. The pathogens limited the plants' ability to absorb water and nutrients for normal growth. In addition, moisture stress probably contributed to predispose the plants to secondary infections by the pathogens. Thus, a wheat cultivar with a root system resistant to the pathogens and tolerant to drought, like Payne, is desirable for wheat improvement programs in Oklahoma.

Effects on Plant Height and Tiller Production

In general, plant heights of treated plants did not differ significantly from controls in all the cultivars (Table VI). However, in 3-day watered plants, both pathogens combined significantly reduced plant height of Triumph 64 (P=0.05), as compared to control (Table VI). Also in 7-day watered plants, Fusarium alone, and Fusarium and H. sativum combined, significantly reduced plant height of Triumph 64 (P=0.05) and H. sativum alone significantly reduced plant height of Newton (P=0.05), as compared to their controls (Table VI).

It is reported in the literature (73) that wheat root rot pathogens cause infected wheat plants to produce few tillers. But results of this study showed, in general, no significant tiller reduction in all the cultivars, as compared to their controls, in the two watering regimes (Table VI). However, although not shown clearly on Table VI, mean number of tillers produced by the cultivars decreased significantly in the watering regimes (P=0.01 level) (i.e., 2.8 tillers in 3-day watered plants and 1.8 tillers in 7-day watered plants).

TABLE VI

MEAN HEIGHT AND NUMBER OF TILLERS BEFORE HARVESTS OF SIX WHEAT CULTIVARS INOCULATED WITH *FUSARIUM* AND *H. SATIVUM*, EACH ALONE AND BOTH COMBINED, AND GROWN UNDER TWO WATERING REGIMES

	3-Day Watered Plants								7-Day Watered Plants							
	Plant Height (cm)				Tillers (no/plant)				Plant Height (cm)				Tillers (no/plant)			
Cultivars	C	F	H	F+H	C	F	H	F+H	C	F	H	F+H	C	F	H	F+H
Payne	17.3	21.2	18.3	18.5	3.8	3.7	2.3	2.7	19.2	19.0	17.8	18.0	3.5	1.9	2.3	1.5
Triumph 64	21.7	17.0	19.7	14.9*	3.8	4.1	2.5	1.4*	22.2	16.5*	19.2	14.6*	2.3	0.4*	2.2	0.9
Danne	17.9	18.0	15.5	17.4	4.6	4.0	2.6*	3.6	18.2	15.7	14.9	17.0	3.8	2.4	2.8	2.1*
Vona	17.6	17.0	16.9	16.9	3.2	3.5	2.5	2.5	17.0	15.8	1.67	14.3	2.9	0.8*	1.7	1.3
TAM 101	17.0	17.2	14.9	18.3	2.6	2.3	1.6	1.9	17.7	16.4	14.5	17.9	2.3	1.1	1.8	1.3
Newton	16.4	16.5	17.1	16.2	3.1	2.9	0.9*	1.6	19.2	15.9	15.0*	15.7	2.2	1.5	0.7	0.8
LSD (0.05) for plant height				3.7					CV% (a) for plant height			35.8				
									(b) for plant height			11.4				
									(c) for plant height			10.4				
LSD (0.05) for tillers				1.6					CV% (a) for tillers			5.8				
									(b) for tillers			35.4	(c) for tillers			33.3

C = Control, mean of three replicates.

F = *Fusarium* inoculated plants (250 macroconidia/g soil), mean of three replicates.

H = *H. sativum* inoculated plants (250 conidia/g soil), mean of three replicates.

* = significantly different from control at P=0.05.

Seedling Emergence and Plant Disease Rating

Seedling emergence was recorded 5 days after planting to compare difference between treated and control. No significant differences were found between treated and control seedlings. However, more seedlings emerged in controls than in treated plants in all the cultivars, although not significant. Seedlings inoculated with Fusarium gave the lowest number for emergence in all the cultivars, as compared to seedling emergence in plants inoculated with H. sativum alone and both pathogens combined.

Plants were rated on disease severity based on lesion size (total area covered) on the leaves and subcrown internodes throughout the entire experiment. Results showed that the pathogens caused severe infections on treated plants as compared to controls (Table VII). In the 3-day watered plants, Triumph 64 and Danne cultivars were significantly damaged by Fusarium alone, as compared to their controls. The rest of the cultivars were not significantly damaged, as compared to the other cultivars (Table VII). In the 7-day watered plants, Fusarium alone significantly damaged all the cultivars, as compared to their controls (Table VII). Triumph 64 also appeared to be the most susceptible cultivar to Fusarium alone, while Danne under this soil moisture stress condition appeared to be the most resistant cultivar. However, differences among cultivars were not significantly different in plants inoculated with Fusarium alone. Fusarium alone caused greater damage on all the cultivars in 7-day watered plants than in 3-day watered plants. The reason for this is obvious, because Fusarium alone has been noted to be more aggressive and destructive on plants

TABLE VII

MEAN DISEASE SEVERITY RATING BASED ON LESION SIZE (TOTAL AREA COVERED) ON THE LEAVES AND SUBCROWN INTERNODES OF SIX WHEAT CULTIVARS INOCULATED WITH *FUSARIUM* AND *H. SATIVUM*, EACH ALONE AND BOTH COMBINED, AND GROWN UNDER TWO WATERING REGIMES

	3-Day Watered Plants				7-Day Watered Plants			
Cultivar	C	F	H	F+H	C	F	H	F+H
Payne	1.6	2.3	3.7**	4.1**	1.1	3.4**	4.1	4.2**
Triumph 64	1.2	2.7**	2.8**	3.6**	1.3	3.6**	3.2**	3.7**
Danne	1.0	2.2*	3.7**	3.7**	1.1	2.7**	4.2**	3.4**
Vona	1.6	1.5	3.1**	3.3**	1.3	3.4**	3.0**	3.4**
TAM 101	1.6	1.5	3.7**	3.6**	1.3	2.9**	3.1**	3.4**
Newton	1.9	2.1	4.6**	4.9**	1.8	3.2*	4.6**	4.7**
LSD (0.05)	1.1			CV% (a)	19.8			
LSD (0.01)	1.5			CV% (b)	20.6			
				CV% (c)	19.8			

C = Control, mean of three replicates.

F = *Fusarium* inoculated plants (250 macroconidia/g soil), mean of three replicates.

H = *H. sativum* inoculated plants (250 conidia/g soil), mean of three replicates.

Disease rating scale: 1 = healthy (no visible symptoms), 2 = slight infection, 3 = moderate infection, 4 = severe infection and 5 = dead plant.

* = significantly different from control at P=0.01.

** = significantly different from control at P=0.05.

under extremely dry soil conditions (13, 73).

There were no significant differences in damage caused by H. sativum alone on all the cultivars in both the 3- and 7-day watered plants (Table VII). The reason for this is because H. sativum has been noted to cause severe damage on plants under minimum and maximum soil moisture conditions (21). Newton appeared to be the most susceptible cultivar to H. sativum alone, while Triumph 64 was the most resistant cultivar in 3-day watered plants. In the 7-day watered plants, Newton also appeared as the most susceptible cultivar to H. sativum alone while Vona, Tam 101, and Triumph 64 were most resistant cultivars (Table VII).

Fusarium and H. sativum combined were destructive on the plants. Both pathogens combined significantly damaged all the cultivars in 3- and 7-day watered plants (Table VII). Newton and Payne were the most severely damaged of all the cultivars in both the 3- and 7-day watered plants (Table VII).

Payne, which consistently out-yielded all the other cultivars in dry forage and root yields, had higher severity rating by Fusarium and H. sativum, each alone and both combined (Table VII). This seems to suggest that Payne is a more tolerant cultivar than a resistant cultivar to these pathogens under wet and dry soil conditions. Newton, however, consistently appeared the most susceptible cultivar. None of the cultivars clearly qualified to be called a resistant cultivar to the pathogens. Each of them has some degree of tolerance to the pathogens. The six cultivars could serve as standard cultivars for resistance screening trials.

Effects on Evapotranspiration Rate

Evaporation and transpiration from plant communities is frequently termed evapotranspiration (35). The relative amount of water removed from soil by evaporation and transpiration is of great interest, particularly in regions of limited rainfall because it can be used as a guide for irrigation (35). As water potential decreases (water stress increases) the stomata close and the rate of transpiration decreases. In general, water stress and root rot diseases cause decrease in transpiration (34). A decrease in supply of water to plant tissues (diseased, water-stressed tissue) or an increase in water loss from the tissues is usually attributed to changes in permeability in the tissues (34). The decrease in water supply may result from failure of root to take up water or of the vascular system to conduct water (34). Blockages of the vascular system have been reported to be common among vascular wilt diseases caused by species of Fusarium (22) and Verticillum (44).

Results of this study showed that the pathogens and soil moisture stress seemed to cause a decrease in evapotranspiration rate of the cultivars but the decrease was not significantly different from controls with the exception of Newton, which differed significantly from its control ($P=0.05$) (Table VIII). Evapotranspiration seemed to decrease with increase in soil-moisture stress (evapotranspiration rates in 3-day watered plants vs. those of 7-day watered plants) (Table VIII).

Considering the six cultivars, the rate of evapotranspiration did not differ significantly with age of the plants (Table VIII). The reason for this is because evapotranspiration rates of the cultivars were measured when plants were in their third, sixth and ninth weeks.

TABLE VIII

MEAN EVAPOTRANSPIRATION RATE (g/day) OF SIX WHEAT CULTIVARS INOCULATED WITH *FUSARIUM* AND *H. SATIVUM*, EACH ALONE AND BOTH COMBINED, AND GROWN UNDER TWO WATERING REGIMES

	3-Day Watered Plants				7-Day Watered Plants			
Cultivar	C	F	H	F+H	C	F	H	F+H
Payne	9.8	8.0	9.2	8.0	6.6	5.6	5.4	5.7
Triumph 64	8.5	8.5	7.6	8.4	6.5	6.4	5.4	5.4
Danne	8.5	8.5	8.4	8.1	6.3	6.2	5.4	5.6
Vona	8.9	8.8	8.0	8.2	6.4	6.3	5.7	5.7
TAM 101	9.4	8.1	9.2	7.8	6.2	5.2	5.3	5.3
Newton	9.8	8.0	8.2	7.6*	6.4	6.0	5.1	5.0
LSD (0.05)	2.1		CV% (a)	10.6				
LSD (0.01)	2.8		CV% (b)	17.7				
			CV% (c)	14.4)				

C = Control, mean of three replicates.

F = *Fusarium* inoculated plants (250 macroconidia/g soil), mean of three replicates.

H = *H. sativum* inoculated plants (250 conidia/g soil) mean of three replicates.

Plants grown in temperature controlled growth chamber, temperature 26 C day and 23 C night.

* = significantly different from control at P = 0.05.

Forage was harvested when plants were 3, 6, and 9 weeks old and leaves allowed to regrow. Therefore, transpiration rates of the plants measured were from leaves of the same age (35).

The measuring system used may have been a limiting factor in detecting real differences in evapotranspiration between treated and check plants, because the measuring instrument used can only measure to the nearest 0.1 g.

Effects on Stomatal Resistance

Stomatal closure is a plant response mechanism that allows leaf tissues to conserve water and maintain turgor when water becomes limiting (44). Water stress and plant pathogens generally cause reduction in cell turgor, which is important in relation to the opening and closing of stomata. Vascular wilt diseases such as Verticillium and Fusarium species, which are confined to the vascular system of host plants, generally cause decrease in transpiration rate and increase in stomatal resistance (22, 44). Some root pathogens such as Helminthosporium species, which produce toxins that interfere with metabolism of host-plants, generally cause stomatal opening and decrease in stomatal resistance (64, 72).

In healthy plants, stomatal resistances are commonly measured using a diffusion porometer with a humidity-sensitive element, but in diseased plants stomatal resistances are sometimes difficult to measure because of many variable effects of pathogens on host-plant water relations. However, in this study, an attempt was made to determine the effects of wheat-root rot pathogens and soil-moisture stress on stomatal resistance using a diffusion porometer (33).

In general, the pathogens had no significant effects on stomatal resistance as compared to controls (Table IX). But Fusarium alone seemed to cause an increase in stomatal resistance in 7-day watered plants as compared to controls, while H. sativum alone seemed to cause decrease in stomatal resistance in both 3- and 7-day watered plants, as compared to controls (Table IX). Both pathogens combined seemed to cause a decrease in stomatal resistance, as compared to controls (Table IX).

The data, therefore, obtained suggest that Fusarium alone caused the stomata to close and prevent water loss, while H. sativum alone, and both pathogens combined, caused the stomata to open and much water was lost. Increase in stomatal resistance by Fusarium alone seemed to indicate that the pathogen may have blocked the vascular system of the host plant, thereby causing decrease in transpiration rate and increase in stomatal resistance. Duniway (22) found that the diffusive resistance of leaves to water-vapor loss in tomato plants infected with Fusarium oxysporum f. sp. lycopersici was as high, or higher, than the resistance in healthy plants at a given leaf water potential.

The decrease in stomatal resistance of the plants by H. sativum alone seemed to suggest that H. sativum may have released a phytotoxin which was carried up the shoot where it induced stomatal opening and, thereby, decreased stomatal resistance. Helminthosporium sacchari, the cause of "eyespot" of sugarcane, is reported to release a diffusible toxin that causes appearance of a "runner" (64). This toxin altered the membrane permeability causing increase in transpiration and decrease in stomatal resistance (64). Turner (66) and Turner and Graniti (67) have shown that fusicoccin, victorin, and phytotoxins lower stomatal

TABLE IX

MEAN STOMATAL RESISTANCE (sec cm^{-1}) OF SIX WHEAT CULTIVARS INOCULATED WITH FUSARIUM, AND H. SATIVUM, EACH ALONE AND BOTH COMBINED, AND GROWN UNDER TWO WATERING REGIMES

	3-Day Watered Plants				7-Day Watered Plants			
Cultivar	C	F	H	F+H	C	F	H	F+H
Payne	31.5	18.8	9.7*	11.9	22.0	26.9	32.9	23.6
Triumph 64	16.8	16.3	19.9	39.1*	28.0	52.4	27.0	25.6
Danne	22.9	36.1	17.4	23.1	27.6	19.4	28.6	29.3
Vona	19.8	28.4	10.4	15.2	32.0	36.8	17.5	28.5
TAM 101	20.3	21.1	42.7*	23.2	33.5	34.4	29.8	32.6
Newton	44.8	29.1	30.0	30.7	33.3	33.8	32.3	32.4
Mean	26.0	25.0	21.7	23.9	29.4	34.0	28.0	28.7
LSD (0.05)	19.2			CV% (a)	60.5			
LSD (0.01)	25.8			CV% (b)	32.8			
				CV% (c)	35.2			

C = Control, mean of three replicates.

F = Fusarium inoculated plants (250 macroconidia/g soil), mean of three replicates.

H = Helminthosporium sativum inoculated plants (250 conidia/g soil) mean of three replicates.

* = significantly different from control at P=0.05.

resistance of Avena sativa.

The decrease in stomatal resistance in plants inoculated with both pathogens combined may be more of the effects of H. sativum alone than that of Fusarium at this plant stage, because H. sativum is normally the first fungus to enter roots before it was outcompeted by Fusarium, as a growing season progresses (73).

Results also showed that stomatal resistance tends to increase with increase in water stress (Table IX). Mean stomatal resistance of 3- and 7-day watered plants combined showed that Fusarium-inoculated plants had higher stomatal resistances and H. sativum and both pathogens combined had lower stomatal resistances as comapred to controls (Table X).

Although not included in Table IX, Newton had the highest stomatal resistance in all the treatments while Payne had the lowest stomatal resistance (33 sec cm^{-1} for Newton vs. 22 sec cm^{-1} for Payne; highly significant) (P=0.01). This means that Payne has a better stomatal closure mechanism, which can prevent tissue desiccation under conditions of high internal water stress, than Newton. Newton's stomatal resistance was significantly different from those of Danne and Vona (P=0.05) and not significant from those of TAM 101 and Triumph 64.

Overall results of this study consistently showed that Payne is the most resistant cultivar to the pathogens under soil-moisture-stress conditions, while Newton is the most susceptible cultivar. Adjei (1) reported that the wheat cultivars, Centurk and Concho, have desirable adaptive characteristics for dryland areas in his study of the effects of soil-moisture-stress on some wheat cultivars. Comparative studies of the effects of the pathogens on Payne, Centurk, and Concho under soil-moisture-stress conditions may be a worthwhile screening program

TABLE X

MEAN STOMATAL RESISTANCE (sec cm^{-1}) OF 3 AND 7-DAY WATERED PLANTS COMBINED OF SIX WHEAT CULTIVARS INOCULATED WITH *FUSARIUM* AND *H. SATIVUM* EACH ALONE AND BOTH COMBINED[a]

Treatment at the Rate of 250 Spores/g Soil	Mean of 3-Day and 7-Day Watered Plants Combined Stomatal Resistance (sec/cm^{-1})			
	Week 3	Week 6	Week 9	Mean
Fusarium	18.9	18.6	50.9	29.5
Fusarium + *H. sativum*	20.8	14.3*	43.6	26.2
H. sativum	25.0	13.4*	36.1	24.8
Control	20.8	21.2	41.1	27.7
LSD (0.05)	6.2	6.6	14.1	
LSD (0.01)	9.4	10.0	21.14	
CV% (a)	2.7	7.4	8.2	
CV% (b)	41.2	55.5	46.6	
CV% (c)	58.6	83.4	47.9	

* = significantly different from control at P=0.05.

[a]Mean of three replicates.

leading to development of resistant cultivars.

General Conclusion

Information obtained in the studies such as pathogens variabilities in pathogenicity and virulence; inoculum density levels of pathogens that can cause economic damage on host plants; effects of pathogens continuous re-infection by pathogens of host plants; and effects of moisture stress and pathogens on host physiology (transpiration, stomatal resistance, growth, yield) will be helpful in developing effective resistance screening trials, which will provide a basis for integrated control of wheat root rot disease.

Wheat-root-rot research program in Oklahoma has reached a stage where changes in population structure, density, and distribution of pathogens need to be monitored occasionally before and after the growing season. Data collected will be helpful in developing a sound predictive model of disease epidemics and for breeding drought and pathogen-resistant cultivars.

CHAPTER VII

SUMMARY

1. Pathogencity study of five isolates of H. sativum and six isolates of Fusarium collected from diseased wheat plants from various areas of Oklahoma showed that isolates of both fungi were pathogenic.

2. There were no significant differences in the ability of H. sativum isolates to attack host plants. But the isolates differed significantly in relation to their degrees of virulence.

3. Based on mean-percentage-disease-severity rating of subcrown internodes, it appeared that isolate 47 of H. sativum from Custer City is the most virulent of H. sativum isolates.

4. Results also showed variability in virulence among isolates 47, 53, and 58 of H. sativum collected from same field location. This seems to account for the variability in disease severity always observed in same wheat field.

5. Fusarium isolates varied with respect to their degree of pathogenicity and virulence.

6. Based also on mean-percentage-disease-severity rating of subcrown internodes, it appeared that isolate 34 of Fusarium from Geary City is the most virulent of Fusarium isolates.

7. Over-all results of the pathogenicity study showed that Fusarium isolates exhibited less aggressiveness than H. sativum

isolates, based on percentage-recovery data of the pathogens and disease-severity rating of subcrown internodes, as caused by the pathogens.

8. Inoculum density level study of isolate 47 of H. sativum and isolate 34 of Fusarium showed that both pathogens were different in their inoculum/infection number and inoculum/disease severity.

9. Results of mean percentage recovery of H. sativum from subcrown internodes ranged from 48-97%, as the inoculum levels increased from 1-1000 conidia/g soil, respectively. The percentage recovery plateaued between 250 and 1000 conidia/g soil. Disease-severity rating of the subcrown internodes followed similar trend.

10. Percentage recovery of Fusarium from subcrown internodes ranged from 41-57% as inoculum levels increased from 1-100 macroconidia/g soil, plateaued between 100 and 500 macroconidia/g soil, and finally declined to 48% at 1000 macroconidia/g soil. Disease severity rating of the subcrown internodes followed similar trend. This suggests competition among the spores as inoculum density reached 1000 macroconidia/g soil.

11. The penetration study of wheat seedlings by H. sativum, Fusarium and R. solani showed that H. sativum and Fusarium can penetrate the first subcrown internode and the first seminal root within 24 hours period following inoculation, but R. solani never did penetrate within that time.

12. The subcrown internodes and the region of differentiation of the first seminal root were found to be the most susceptible regions to the pathogens, and the root cap region was the least susceptible part. This indicates that the pathogens penetrated older plant tissues

rather than younger tissues.

13. Effects of H. sativum and Fusarium on transplanted and non-transplanted seedlings, grown under three watering regimes, showed that the pathogens under soil-moisture-stress conditions significantly decrease plant growth and yield. Also results showed that once the pathogens are established in host plant, transferring plants into non-infected soil does not prevent increase in disease severity and yield losses.

14. Transplanted plants produced higher yield than non-transplanted plants in all three watering regimes and pathogen treatments. The reason for this is because continuous re-infection of host plants increased disease severity.

15. Results of six cultivars of winter wheat inoculated with H. sativum and Fusarium, and both pathogens combined, and grown under two watering regimes in the growth chamber, showed that Payne exhibited most desirable adaptive characters for dryland areas infected with Fusarium and H. sativum.

16. All the six cultivars were susceptible to the pathogens, but Payne was most tolerant of all of the cultivars.

17. The pathogens had no significant effect on evapotranspiration rate, but evapotranspiration rate tended to decrease with increase in soil-water-stress. There were no significant differences in evapo-transpiration rate among the six cultivars, since the leaves were of the same age.

18. Evapotranspiration rate did not differ significantly in any of the cultivars as compared to controls.

19. Results also showed that the Fusarium alone seemed to cause

the stomata to close rather than to open, while H. sativum alone, and both pathogens combined, seemed to cause the stomata to open under soil moisture stress conditions. Why the stomata reacted in these manners to the pathogens is not known, but there may be possibilities that Fusarium blocks the xylem system of the plants, thereby inhibiting water uptake, decrease in transpiration and increase in stomatal resistance, while H. sativum seemed to produce a toxin that was carried up the shoot and altered the membrane permeability, leading to increase in transpiration rate and decrease in stomatal resistance.

20. The cultivar, Newton, had the highest stomatal resistance, while Payne had the lowest stomatal resistance. This suggests that Payne might be better adapted for dryland areas. The stomatal resistances of the other cultivars fell between that of Payne and Newton.

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