THE EFFECT OF STUBBLE MULCH TILLAGE SYSTEM ON THE INCIDENCE OF *HELMINTHOSPORIUM SATIVUM* IN HARD WINTER WHEAT

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CHAPTER I

INTRODUCTION

The root rot disease of cereal crops has long been a problem in the Great Plains area of North America. The disease has been studied extensively, as cited by Hayes (15), particularly since the discovery of the principle causal agent, Helminthosporium sativum P., K., & B., by Pammel, King, and Bakke in 1910. The literature concerning the disease and the fungus, the perfect stage of which is Cochliobolus sativum (Ito and Kurib) Dr. ex Dast (25), has been voluminous.

Several facts significant to the control of the disease have emerged from these studies. In the first place, the fungus has a wide host range, particularly among the Graminaceous plants and causes, in addition to root rot, leaf spot, seedling blight, crown or foot rot, stem lesions, spike and seed blight, and premature death particularly in wheat, barley, and rye (15).

Winter or intercrop survival occurs on infested plant debris, and therefore inoculum tends to increase with continuous cropping, especially with wheat and barley (3,7). Infected seed may also be a source of inoculum, however, as a result of head blight the previous season (24). Therefore, both rotation and seed treatment have been recommended as control measures.

The fungus has been found to grow rapidly at warmer temperatures (2) which has lead to recommendations for later fall plantings or

earlier spring planting of cereal grains (22). It is also known that H. sativum is a poor competitor in the soil under some conditions and antagonism and germination lysis have been suggested for control (6,8).

Specific resistance in wheat or barley to H. sativum in the crown or root rot stage is unknown. However, breeders and pathologists seem to have developed varieties with good field resistance or tolerance to the disease at current inoculum levels, since no major outbreaks of the disease have occurred in several decades.

The incidence of crown and root rot seems to be increasing in recent years, however, in conjunction with an increase in minimum tillage systems (unpublished data, Oklahoma Agricultural Extension Service). Craig (12), in 1962, stated that "Any person who advocates the destruction of crop residues beyond a protective amount in an area where they are needed for protection of the land against water or wind erosion hazards is doing a grave injustice to conservation of the land". While this is certainly true, little is now known of the effect of such tillage systems upon the incidence of root rot or upon the potential inoculum level of the organism or organisms causing root rot. Only one study has been found dealing with this problem. McCalla (19) found that there was an increase in the number and type of microorganisms in the surface one inch following stubble mulch tillage compared to a mouldboard plow or "clean tillage" system. Such an increase might lead to a more competitive situation in which H. sativum inoculum would tend to decrease; or, lacking rotation, the inoculum level of H. sativum might increase. It was the object of this study, then, to determine the effects of tillage systems on the incidence of propagules of the fungus H. sativum in the surface layer of soil.

CHAPTER II

LITERATURE REVIEW

Our knowledge of Helminthosporium sativum as the causal agent of spot blotch of barley dates back to 1909, when Pammel began investigations about the disease agent. Hayes (15) has reviewed the literature on the pathogenicity of H. sativum and points out that since 1910, when Pammel, King, and Bakke confirmed that spot blotch of barley was incited by Helminthosporium sativum further studies have shown this facultative parasite to be the agent that causes "leaf spot, root rot, foot-rot, stem lesions, basal stem rot, spike and seed blight, and premature death of wheat, barley, rye, and numerous grasses." Hayes and his co-workers (15) also made some crosses of barley varieties to find types resistant to H. sativum. They decided that resistance and susceptibility to attack by this fungus was an inherited characteristic, but that more than one gene was involved. The complicating factor of physiologic specialization was noted later by Christensen (10,11).

Since the work of Stakman (24), in 1920, many researchers have confirmed that inoculum in the soil is the primary source of the disease inoculum. Henry (16) confirmed that mycelium in plant refuse was capable of surviving the winter at St. Paul, Minnesota, but that H. sativum was the common cause of the black point disease of wheat kernels which could also serve as inocula the following season.

The ability of a fungus to survive through adverse environmental conditions and the ability to germinate when conditions are favorable

is an important factor for survival. In this connection Simmonds, Sallans, and Ledinghan (21) have found H. sativum to be extensive in soils of the wheat producing regions of Western Canada, and conidiospores of the fungus were found to over winter in those areas. Later Chinn, Sallans, and Ledingham (7) investigated conidial populations of H. sativum in field soils and studied the relationship of these populations to disease incidence on seedlings and mature plants. They also studied spore populations in field soils and in soils brought into the greenhouse. From 47 fields they found a range from less than 10 to over 250 viable conidiospores of H. sativum per gram of soil. In a similar study two years later they tested spore population in 200 soil samples from 100 cultivated fields in two areas. Using a flotation viability count method they developed for their previous work, and later modified for this study, they found conidiospores of H. sativum to number from less than ten to almost 900 per gram of field soil. Spore counts from summer-fallowed soil or from soils planted to wheat or barley did not differ significantly (9).

Butler (3,4,5) studied the survival behavior of H. sativum on wheat straw buried in the soil. He determined the presence of the fungus within the tissue of the infected straws on acidified potato dextrose agar medium and found the fungus to remain viable in the soil for long periods of time.

Boosalis (1) was able to confirm the presence of conidiospores of H. sativum in the soil from Nebraska wheat fields and he made an extensive review of the literature concerning the survival of spores of H. sativum in the soil.

Meromick and Pepper (20) observed that the inner wall of a

conidium of H. sativum may be changed to the appearance of a chlamydospore if subjected to adverse environmental conditions. However, a detailed study of the mechanism of this change was not presented.

More recently, Mathre (18) studied the germination of conidiospores of H. sativum. Among other things he observed that conidiospores of H. sativum were able to germinate well at temperatures as high as 30 to 39°C and over a wide range of relative humidity. Interestingly, he found that conidiospores of H. sativum were able to germinate at 100% after a year of storage under all conditions tested. He found that the spores retained about 95% viability for two years if stored dry at 4°C. Significantly, he also noted that in many cases conidiospores of H. sativum failed to germinate in the presence of other fungi.

Tyner (26,27) studied three cereal straw composts and found that both wheat and barley straw-soil composts supported better growth of H. sativum than an oat straw compost. Wheat seedlings grown in the wheat and barley straw-soil composts also were more severely attacked by H. sativum than those grown in the oat compost. He then made a comparative study of fungus colonization on these different cereal crop residue composts. By means of a dilution plate technique he found a much higher number of fungus colonies (other than H. sativum) on oat straw compost than on the other two field plots having high infestations of root rot fungi including H. sativum. From his previous work and this one, he concluded that less infection of wheat seedlings occurred on the oat straw-soil compost because of the significantly higher number of colonies of fungi other than H. sativum in oat straw compost. It was confirmed, too, that there was very severe infection on the roots of wheat seedlings grown on wheat straw compost. Therefore, because of

the possibility of diseased wheat roots being a source of energy he also concluded that H. sativum would accumulate in a continuous wheat planting system and lead to a continuous danger of losses from root rot.

Planting dates have been altered in many cases to bring about increased crop yields, and the same practice has been used in controlling plant disease. Sprague (23), as early as 1948, suggested a late fall seeding practice as means of control of Helminthosporium wheat disease complexes. Slykhuis et al. (22) investigated the effects of planting dates upon the incidence of root rot and other disease of winter wheat. They conducted seeding date experiments at a weekly interval starting on August 2, and found higher severity of the disease among plants sown early and less disease among plants sown late. The fact that conidiospores of H. sativum will germinate well at high temperatures, as shown by Mathre (18), would be one explanation for the success of later planting of winter wheat.

Factors which predispose winter wheat plants to root rot disease were investigated by Fenster (14), in western Nebraska. He also found seeding date to be important in the incidence of the root rot disease, but he suggested that early planting favors rank growth in the fall if moisture is adequate and such growth may result in a severe water stress on the plants if moisture later becomes limiting. He believed that plants weakened due to water stress were more sensitive to attack by the root rotting fungus H. sativum. Although their findings were incomplete, these researchers also found that wheat plants weakened by the leaf rust disease were more vulnerable to the attack of root and crown rot of wheat than healthy plants.

CHAPTER III

MATERIALS AND METHODS

Field Experiments

The test cultivar selected for this work was a hard red winter wheat, "Triumph 64". Untreated seeds of this cultivar were obtained from the Department of Agronomy, Oklahoma State University. The cultivar has wide acceptance among Oklahoma farmers, is well-adapted in the State of Oklahoma, and has been grown commercially for several years. Unpublished records of several field surveys of stubble-mulched farms planted to this variety have shown an increase in the incidence of common root rot incited by Helminthosporium sativum P., K., and B.

A culture of Helminthosporium sativum P., K., and B., was obtained from Dr. H. C. Young, Jr. The original isolation was made from a single spore and the species confirmed by Dr. J. J. Christensen, University of Minnesota, St. Paul, Minn. The technique employed for the isolation work was that described by Davis (13). A stock culture was made from the original isolation on PDA medium in a Petri dish and kept in the refrigerator at $4^{o}C$. The stock culture remained effective throughout these experiments, although transfers were made every four or five months to maintain viability.

In the fall of 1970, an experimental field plot was designed on a Pond Creek silt loam soil at the North Central Agronomy Research Station, Oklahoma State University, Lahoma, Oklahoma. The entire plot was then

seeded with untreated seed of the cultivar "Triumph 64".

The experimental plot, measuring 60 m by 60 m, was then divided into two sub-plots, one of which was inoculated in a manner to be described later and one was not inoculated. Each of these sub-plots were further divided into two plots one of which was tilled with a mouldboard plow in the early summer of 1971, and designated here as "clean plowed" and the other was tilled with a "sweep" type implement, which disturbed only the top two inches or so of soil and is designated here as "stubble mulch".

Later, before planting time in 1971, an experiment designed to test the effectiveness of certain chemicals for the control of root rot and nematodes was incorporated with this study. For the purpose of this study, however, wheat plant samples were taken only from a treatment involving the fungicide, Terraclor and a check plot containing no chemical additive. The complete plot design is presented in Figure 1. There were two replications of each treatment in each of the two major sub-plots (inoculated and not inoculated).

The plot inoculated with H. sativum was inoculated twice, both times prior to the tillage operation following the 1970-1971 crop season. Two methods of inoculation were employed. In the first method, one hundred and thirty 100-ml Erlenmeyer flasks containing 20-ml of potato-dextrose broth were implanted with a 6 mm diameter plug from the stock culture of H. sativum. These cultures were allowed to grow for 10 days at room temperature. The fungal mycelial mats were then extracted from the spent media and placed in a blender with sterile distilled water at the rate of 50 ml of water per mycelial mat. This mixture was blended at a high speed for one minute, after which sterile distilled water was

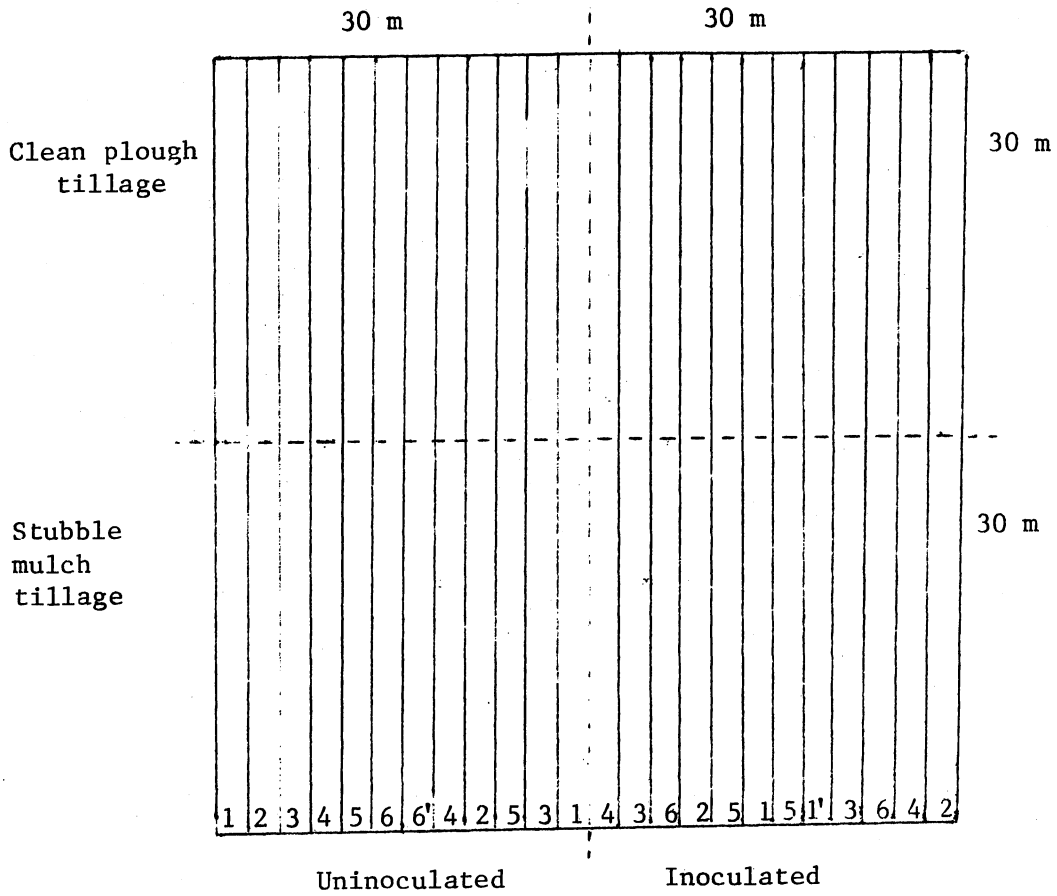

Figure 1. A Diagramatic Illustration of the Field Plot Used to Obtain Wheat Plant Samples for Examination for Seedling Infection. Samples were Obtained Only from Treatment I (Terraclor at 450g Per Acre AI Added at Planting Time) and Treatment Six (the Check). Other Treatments were Made for Another Study.

added to make a total volume of 20 ℓ. Just after anthesis, on May 13, 1971, the wheat on the "inoculated plots" was sprayed with the mycelium-spore suspension using a Hudson back-pack sprayer.

At harvest time, in June 1971, the entire experimental area was harvested with a normal combining operation except that care was taken to ensure that the straw, chaff, and other plant residues from each of the two major sub-plots were returned to their respective plots to avoid contamination of the non-inoculated plot.

A second inoculation was applied on July 16, 1971. This time the inoculum was spread over the stubble and straw residue remaining after the harvesting operation was completed. The inoculum was prepared by growing the fungus on a substrate containing a mixture of seven parts wheat and five parts oat kernels (10). Each of 50 one-quart glass jars were filled approximately one-half full with this mixture and 150 ml of distilled water was added to each jar. The jars containing this medium were steam sterilized twice with an interval of 48 hours between each sterilization. A 72 hour culture of H. sativum grown on potato-dextrose broth was added aseptically to each of the jars. The jars were then shaken to distribute the inoculum throughout the medium and the fungus was allowed to grow for a period of two weeks. Then each jar was emptied and the media macerated throughly. This inoculum, consisting of grain kernels throughly permeated by the fungus, was broadcast by hand on the plot to be inoculated. Ten days later the tillage operation, "clean plow" on one plot and "stubble mulch" on the other plot, was performed.

Untreated seed of the wheat cultivar, "Triumph 64" was planted on August 31, 1971, using a 10-row John Deere hoe drill which planted

the rows 10 inches apart. The soil was dry at the time of planting and for about 17 days thereafter. Poor germination resulted and the stand in some areas of the field remained poor until the later part of September when soil moisture became adequate for a more general germination and growth.

The chemical additive Terraclor was applied at planting time by metering the granular material into the soil covering the seed with a Gandy fertilizer spreader. This material was applied at the rate of 450g per acre of the active ingredient (Pentachloronitrobenzene, PCNB).

The level of inoculum in each of the plots was determined by a type of bio-assay that was accomplished in the following manner. On September 17, 1971 (17 days after planting), five plant samples from each of two random locations within each plot were dug with the roots intact. The 16 samples thus recovered were brought to the laboratory for examination. The soil was gently soaked and washed from the roots, which were then examined for signs of infection. A second sample was taken in the same manner on October 11, 1971 (42 days after planting), this time consisting of 10 plants per sample.

Each plant in the first sample was dissected into coleoptile, hypocotyl, and root. These tissues were then examined with a dissecting microscope for the presence of diseased tissue. Isolations from this apparently diseased tissue were made in a manner described later. The second sample was handled in the same manner except that the tissues were divided into primary leaf sheath and secondary roots in addition to the hypocotyl and primary root. The number of lesions and the relative amount of tissue damage to each plant part was recorded and each lesion on tissue that appeared to be diseased was then sectioned

from the healthy tissue, placed for one minute in a five percent solution of sodium hypochlorite, rinsed twice in sterile glass-distilled water and placed on acidified (17) potato-dextrose agar dishes. These dishes were held at room temperature for 48 hours and then examined for colonies of H. sativum. Since this examination had to be done with a microscope due to the presence of many fungi, and since there were a large number of dishes to examine (470 with the first sample and over 800 with the second sample) the period of examination extended over several days. Consequently, after the first growth period of 48 hours at room temperature, the Petri dishes were held in a cold room at approximately 4°C until each was examined.

Greenhouse Experiment

An experiment was designed to observe the effect of inoculum of H. sativum placed at different depths in the soil upon the incidence of seedling infection caused by this pathogen. The study was intended more or less to simulate the field conditions of clean plow and stubble mulch tillage practices.

The inoculum of H. sativum was made using wheat straw as a medium and infesting it with the fungus from the stock culture previously described. Infestation of the wheat straw was accomplished in the following manner. Two year old baled wheat straw was obtained from the Department of Animal Husbandry, Oklahoma State University. Approximately five kg of straw were placed in a 20 liter metal barrel and then throughly soaked with distilled water. The excess water was then drained off, and the straw was autoclaved for 90 minutes at 121 C and 15 psi. After it was cooled the straw was cut into pieces (1-2 cm)

and placed in 20 one-liter glass jars. The jars were filled one half full with straw and 100 ml distilled water was added. Each jar was then plugged with cotton and sterilized for one hour at 121 C and 15 psi. The sterilization process was carried out twice with an interval of two days. The sterilized wheat straw medium was implanted with about 10 ml of H. sativum spore and mycelium suspensions grown in potato-dextrose broth medium for 10 days. The fungus was allowed to grow on the straw for two weeks at room temperature.

The soil used for planting, a one-two-one mixture of builders sand, sandy-loam soil and sphagnum peat, respectively, was placed in several wooden flats and together with 35 four-liter glazed crocks, were steam heat sterilized at 115 C for 48 hours.

The experiment consisted of five replications of seven treatments. One four-liter glazed crock composed one replication of one treatment. Each crock was planted with 40 hand-picked, sterilized (one minute in five percent sodium hypochlorite) kernels of the cultivar "Triumph 64". The seven treatments consisted of inoculum of Helminthosporium sativum grown on sterile wheat straw placed at: (1) 2.5 cm (2) 5 cm; (3) 10 cm; and sterile wheat straw placed at: (4) 2.5 cm; (5) 5 cm; (6) 10 cm; (7) sterile soil check. The crocks were then placed in a greenhouse at a temperature of 22±3 C in a randomized block design. Approximately 500 ft-c of supplemental fluorecent light was used. Each crock was watered as needed to provide optimum moisture. At the end of the experiment the root mat from each crock was soaked and washed gently in water over a fine-mesh screen to remove soil. The volume of each root mass was measured by water displacement. Small samples of roots,

hypocotyls and coleoptiles were selected for planning on potato-dextrose agar after which each root mass was oven dried and weighed.

CHAPTER IV

RESULTS

Field Experiment

Insufficient rainfall was a limiting factor in the experiment. This was especially true during the first 17 days after planting and again a few months before harvest. This resulted in poor germination, stand and seeding growth, and consequently, plant specimens suitable for making isolations were difficult to obtain during the first sampling. In one plot no sample was obtained at all.

Examination of the fungal colonies obtained from the first sampling revealed that colonies of H. sativum were found in the stubble mulch area more frequently than from the clean plowed area (Table I). In the clean plowed area there were more isolates of H. sativum from the portion treated with Terraclor 30G than from the untreated portions. This also occurred with isolates from the stubble mulch area. Also, there were more isolates recovered from the inoculated portions than from the uninoculated portions.

A second sampling was made 42 days after planting and on this occasion a record was made of the number of tillers present as well as the number of hypocotyls and sheaths of the basal tiller leaves showing lesions or evidence of infection. Also, the relative percent of damaged tissue on each hypocotyl or each sheath of the basal leaf of each tiller was estimated. These results are presented in Tables II

TABLE I

NUMBER OF COLONIES OF *HELMINTHOSPORIUM SATIVUM* RECOVERED FROM SEEDLING PLANTS OF THE WHEAT CULTIVAR TRIUMPH 64 17 DAYS AFTER PLANTING. LAHOMA RESEARCH STATION, LAHOMA, OKLAHOMA

Treatments	Coleoptiles	Hypocotyl	Primary Root
Clean plowed			
Uninoculated, check	2[1/]	0	0
Uninoculated, treated[2/]	0[3/]	0	0
Inoculated, check[4/]	5	2	6
Inoculated, treated	2	1	4
Stubble mulched			
Uninoculated, check	6	1	1
Uninoculated, treated	14	5	6
Inoculated, check	10	2	4
Inoculated, treated	17	10	5

[1/] Each figure represents the number of colonies derived from two 5-plant samples from each of 2 replications.

[2/] Each "treated" plot received Terraclor 30G at 1500 g per acre at the time of planting.

[3/] Samples from only one replication obtained due to drought.

[4/] Each "inoculated" plot was inoculated the previous season by spraying the plants with a spore-mycelial suspension of *Helminthosporium sativum*, and infested after harvest and prior to tillage by spreading grain infested with the same fungus.

through IV.

As mentioned before the uneven pattern of drought or moisture stress seriously influenced the experiment. Evidence of this can be seen in the number of tillers per plant (Table II). The stubble mulch area of the experimental design was almost uniformily affected by moisture stress, but only the uninoculated section of the clean plowed area was seriously affected. The number of tillers per plant in the one area (inoculated, clean plowed) not subjected to moisture stress was about double the number of tillers per plant in the area affected.

Examination of the hypocotyls (Table III) indicated that the number of plants infected was much higher in the stubble mulch area than in the clean plowed area when no inoculation or infestation was made. When inoculation or infestation of the soil with Helminthosporium sativum, was made, the number of plants infected was much higher in the infested portion than the uninfested portion of the clean plowed area, but about the same as the uninfested portion in the stubble mulch area.

When the percent of the total hypocotyl tissue damaged is considered, essentially the same conclusion may be reached; that is, infestation of clean plowed soil with Helminthosporium sativum greatly increased the amount of tissue damage, but infestation of stubble mulched soils increased the amount of hypocotyl tissue damage little, if any.

Treating the clean plowed uninoculated soil with Terraclor 30G had little effect on either the number of plants infected or the amount of tissue damaged (Table III). Actually, the check area showed a little less damage than the treated areas and perhaps would indicate some

TABLE II

NUMBER OF TILLERS PER PLANT ON WHEAT CULTIVAR "TRIUMPH 64"
LAHOMA RESEARCH STATION, LAHOMA, OKLAHOMA, 1971

Treatment	Average Number of Tillers per plant[1]
Clean plowed	
Uninoculated, check	3.7
Uninoculated, treated[2]	3.2
Inoculated, check[3]	6.2
Inoculated, treated	5.9
Stubble mulch	
Uninoculated, check	2.1
Uninoculated, treated	3.3
Inoculated, check	3.2
Inoculated, treated	3.9

[1] Each figure is an average of two ten-plant samples from each of two replications.

[2] Each "treated" plot received Terraclor 30G at 1500g per acre at the time of planting.

[3] Samples from only one replication obtained due to drought.

TABLE III

THE PERCENT OF HYPOCOTYLS WITH VISIBLE LESIONS, AND THE PERCENT OF HYPOCOTYL TISSUE DAMAGED ON 42 DAY OLD SEEDLINGS OF THE WHEAT CULTIVAR "TRIUMPH 64", LAHOMA RESEARCH STATION, LAHOMA, OKLAHOMA, 1971

Treatment	Percent of hypocotyls with visible lesions[1]	Percent of hypocotyl tissue damaged
Clean Plowed		
Uninoculated, check	15.0	1.5
Uninoculated, treated[2]	16.6	3.1
Inoculated, check[3]	65.0	29.0
Inoculated, treated	75.0	58.8
Stubble mulch		
Uninoculated, check	50.0	16.0
Uninoculated, treated	35.0	7.0
Inoculated, check	60.0	22.8
Inoculated, treated	60.0	15.8

[1] Each figure is an average of two ten-plant samples from each of two replications.

[2] Each "treated" plot received Terraclor 30G at 1500g per acre at the time of planting.

[3] Samples from only one replication obtained due to drought.

TABLE IV

THE PERCENT OF SHEATHS OF BASAL LEAVES OF EACH TILLER OF THE WHEAT CULTIVAR "TRIUMPH 64" SHOWING EVIDENCE OF INFECTION AND THE PERCENT OF THE TOTAL BASAL LEAF SHEATH TISSUE DAMAGED. LAHOMA RESEARCH STATION, LAHOMA, OKLAHOMA, 1971

Treatment	Percent of basal leaf sheaths with visible lesions	Percent of total basal leaf sheath tissue damaged
Clean plowed		
Uninoculated, check	7.3[1]	10.3
Uninoculated, treated[2]	81.3	11.9
Inoculated, check[3]	46.7	27.3
Inoculated, treated	50.8	31.3
Stubble mulch		
Uninoculated, check	76.2	15.0
Uninoculated, treated	57.5	26.3
Inoculated, check	75.0	14.3
Inoculated, treated	51.3	32.8

[1] Each figure is an average of two ten-plant samples from each of two replications.

[2] Each "treated" plot received Terraclor 30G at 1500g per acre at the time of planting.

[3] Samples from only one replication obtained due to drought.

control of non-pathogenic competitors. In the stubble mulch areas, treatment with Terraclor 30G did seem to reduce the amount and severity of seedling attack, particularly in the uninoculated or uninfested portions.

The results of the examination of the basal leaf sheath of each tiller are given in Table IV. In the clean plowed area more tiller leaf sheaths were infected in the uninfested or uninoculated areas than in the infested area. The total damage in the uninfested area was only about half as much as in the infested area, however, indicating that in the uninfested area the lesions were more numerous, but smaller.

In the stubble mulch area, the infested and uninfested portions had about the same number and size of lesions, and treatment of the soil with Terraclor 30G reduced the number but increased the size of the lesions. If it can be assumed that the larger lesions were due to infection by Helminthosporium sativum, which is supported by the results indicated in the clean plowed area where inoculations or infestation with this organism produced fewer, larger lesions, then it seems likely again that the Terraclor 30G treatment was exerting some control of less pathogenic competitors.

Portions of the tissue examined were placed on acidified potato dextrose agar to test for the presence of H. sativum. Table V contains the results of the isolations made from the second sampling.

By far the most colonies of H. sativum were obtained from the stubble mulched area. Inoculation of infestation of the plots with this fungus increased the isolates obtained from infected seedlings in both the clean plowed and the stubble mulched areas. Also, further evidence was obtained that treating with Terraclor 30G increased the

TABLE V

NUMBER OF COLONIES OF *HELMINTHOSPORIUM SATIVUM* RECOVERED FROM SEEDLING PLANTS OF THE WHEAT CULTIVAR "TRIUMPH 64" 42 DAYS AFTER PLANTING. LAHOMA RESEARCH STATION, LAHOMA, OKLAHOMA, 1971

	Coleoptile	Hypocotyl	Primary Roots	Secondary Roots	Basal leaf of each tiller
Clean plowed					
Uninoculated, check	2[1]	0	4	0	7
Uninoculated, treated[2]	1	2	4	2	14
Inoculated, check[3]	3	7	2	5	4
Inoculated, treated	2	8	14	22	28
Stubble mulched					
Uninoculated, check	8	1	10	3	15
Uninoculated, treated	16	12	15	10	24
Inoculated, check	30	29	25	56	46
Inoculated, treated	32	26	22	39	59

[1] Each figure represents the number of colonies derived from two 10-plant samples from each of two replications.

[2] Each "treated" plot received Terraclor 30G at 1500g per acre at the time of planting.

[3] Samples from only one replication obtained due to drought.

incidence of H. sativum.

Greenhouse Experiment

Isolations were made from a greenhouse experiment designed to determine the relationship of the depth at which the inoculum was placed to the degree of infection of wheat seedlings. It is evident from the results (Table VI) that the depth at which the inoculum was applied affected the degree of infection, based on counts of colonies of H. sativum derived from various parts of the seedling plants. When the inoculum was applied in the surface 2.5 cm of the soil the number of colonies isolated was greater than when the inoculum was applied at 5-10 cm below the soil surface. When inoculum was placed at 2.5 cm, the coleoptiles and hypocotyls produced many more colonies than the roots, probably because the coleoptiles and hypocotyls came in more close contact with the inoculum. Observations on the characteristics of the roots in relation to depth placement of the inoculum showed that the main roots about 5-7 cm from the crown were severely diseased. Portions of the roots beyond 7 cm from the crown showed no lesions. The volume and dry weight of the roots indicated little or no difference between the various treatments (Table VII). If any trend is indicated it is that the greater root volumes and weights were recorded for treatments where the inoculum or sterile media were placed in the surface layers of the soil.

The pathogen did not appear to have any effect on the germination of the wheat seeds. An average of 93% germination was recorded.

TABLE VI

THE NUMBER OF ISOLATES OF HELMINTHOSPORIUM SATIVUM DERIVED FROM SEEDLINGS OF THE WHEAT CULTIVAR "TRIUMPH 64" GROWN IN THE GREENHOUSE IN SOIL INFESTED AT DIFFERENT LEVELS

Treatments		Number of isolates from Coleoptiles	Hypocotyles	Roots
Inoculum[1] placed at:	2.5 cm	94[2]	95	69
	5 cm	71	52	55
	10 cm	70	49	46
Sterile media[3] placed at	2.5 cm	70	56	71
	5 cm	59	37	31
	10 cm	41	53	39
Sterile soil, check		33	27	35

[1] Helminthosporium sativum grown on sterile wheat straw for 14 days.

[2] Each figure represents the isolates obtained from five selections of each plant part selected at random in each of five replications.

[3] Sterile wheat straw.

TABLE VII

THE VOLUME AND DRY WEIGHT OF ROOTS PRODUCED ON WHEAT PLANTS OF THE CULTIVAR "TRIUMPH 64" GROWN IN GLAZED CROCKS IN THE GREENHOUSE AND INOCULATED WITH *HELMINTHOSPORIUM SATIVUM*

Treatment		Average Root Volume in ml.	Average Root Dry wt. in gms.
Inoculum[1] placed at	2.5 cm	0.237[2]	0.020
	5 cm	0.199	0.018
	10 cm	0.182	0.018
Sterile media[3] placed at	2.5 cm	0.252	0.018
	5 cm	0.188	0.018
	10 cm	0.170	0.014
Sterile soil, check		0.158	0.011

[1] *Helminthosporium sativum* grown on sterile wheat straw for 14 days.

[2] Each figure is for an average single plant obtained by measuring displacement and weighing the root mass from a single container and dividing by the number of plants.

[3] Sterile wheat straw.

CHAPTER V

DISCUSSION

Little has been published pertaining to the behavior of root rotting fungi in relation to the stubble mulch or minimal tillage farming practices. In this experiment stubble mulch tillage did increase the incidence of Helminthosporium sativum more than where clean cultivation was used. Crop residue, wheat straw particularly, left on or in the surface layer of soil may provide an energy source for fungus growth. Greater numbers of H. sativum colonies were recovered from the plot that was stubble-mulched and inoculated with H. sativum than from the plot that was clean-plowed and inoculated. The increased severity of the disease on the stubble-mulched, inoculated plot demonstrated that wheat straw refuse in the surface layers of the soil is a very favorable substrate for H. sativum as has been pointed out by others (3,26,27). These results were confirmed when this medium was used in the greenhouse experiment.

Infestation of the previous wheat crop and infestation of the soil with H. sativum prior to tillage raised the level of infection in all plots, but more in the clean tilled plots than in the stubble mulch plots. Perhaps there was inoculum already present on the stubble refuse from natural infection, so that additional inoculum had little noticeable effects. It is also possible that other fungi antibiotic to H. sativum on the stubble refuse restricted the growth of the additional inoculum.

In almost all cases where Terraclor 30G was added to the soil at seeding time the incidence of seedling infection by H. sativum was increased. It is possible that some phytotoxicity predisposed the plants to infection, and it is also possible that this chemical controlled the growth of some natural competitor or competitors to H. sativum in the soil.

The experimental design for a field study such as this where different methods of tillage are involved is difficult. In the studies reported here, the effective randomization and replication of treatments was almost negated by uneven moisture stress. The studies are being continued and a new design is being used to attempt to correct such errors. However, since the practice of stubble mulch tillage is a moisture conservation measure, as opposed to clean tillage, the effect of moisture stress may still not be successfully compensated.

The greenhouse experiment was affected by still another problem. The abundant fruitification of H. sativum on wheat straw near the surface of the soil can provide such abundant inoculum for distribution by air currents or splashing water that inoculated pots can not be randomly distributed throughout uninoculated pots without some provision for stopping this contaminating inoculum. Either that must be done, or another experimental design must be used.

CHAPTER VI

SUMMARY

1. Development of Helminthosporium sativum on hard winter wheat cultivar "Triumph 64" in both stubble mulch and clean plowed tillage systems was investigated in field and greenhouse experiments.

2. In the field experiment 17 days after planting there were more isolates of H. sativum found in the stubble mulched area than in the clean plowed area and a still greater number of isolates of H. sativum in the inoculated area than in the uninoculated area of each tillage system.

3. After 42 days the number of tillers per plant in an area of the experiment affected by drought was only about one half of the number found in an unaffected area.

4. After 42 days the percent of hypocotyls with visible lesions averaged about 16 for the uninoculated clean plowed treatment and about 50 for inoculated clean plowed and both the inoculated and the uninoculated stubble mulch treatments. The percent of hypocotyl tissue damage was much higher for the inoculated plots of both systems than the uninoculated plots.

5. Treatment of the soil with Terraclor 30G increased the percent of infection of the sheath of the basal leaves in both the clean plowed area and in the stubble mulch area. The percent of sheath tissue damage was increased in all Terraclor treated plots.

6. Isolates of H. sativum recovered from various seedling plant parts was higher in the stubble mulch area than in the clean plowed areas. Inoculation or infestation with H. sativum the previous season increased the incidence of isolations of H. sativum in both tillage systems, but more in the clean plowed system than in the stubble mulch system.

7. In the greenhouse there was more tissue damage from inoculum applied in the surface 2-3 cm of the soil than from inoculum applied at two or three times this depth below the soil surface.

8. There was no difference in root volume or root dry weight between the different treatments in the greenhouse experiment.

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