SCREENING OF SOIL MICROORGANISMS ANTAGONISTIC TO *HELMINTHOSPORIUM SATIVUM* P., K. & B.

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CHAPTER I

INTRODUCTION

Root rot disease of cereal crops has long been a problem in the great plains of North America (11). The disease has been studied extensively since the principal causal agent, Helminthosporium sativum was discovered by Pammel, King, and Bakke (20). The disease has been of major importance in the economy of production of grass species and ranked as first importance on the grasses, particularly on wheat (24). The perfect stage of the parasite is Cochliobolus sativus (Ito and Kurib) Drechsl (27).

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 (13). The seedling blight is characteristically a dry rot type of tissue necrosis (11). Dark-brown to black lesions usually occur first on the coleoptile and progress inward. The seedling is killed before emergence or, more frequently, after emergence. The seedling leaves of infected plants are dark-green, erect with dark-brown lesions near the soil line that soon extend into the leaf balde. The seedling symptoms are similar in wheat, barley and some grasses. Cortical and leaf-sheath tissue are lesioned, tiller buds are blighted, and the crown-root system is invaded.

Winter or intercrop survival occurs on infested plant debris, and therefore inoculum tends to increase with continuous cropping, especially with wheat and barley (5, 7). Infected seed may also be a source of inoculum, however, as a result of head blight in the previous season (5). Disease development is usually more severe in late sown spring grain or early-sown winter grain; i.e. in warm soils. The fungus has been found to grow more rapidly at warmer temperatures (8), which has lead to recommendations for later fall planting or earlier spring planting ov cereal grains (23). The control of the disease is difficult and should be given more attention than in the past. Sanitation and crop rotation are important, but in the central and western plains grain area, where grains and grasses comprise such a large percentage of acreage, suitable rotations offer difficulties. Seed treatment with mercury compounds have been effective in increasing stands of vigorous seedlings (11). However, mercury compounds have not allowed to be used by EPA. Specific resistance in wheat or barley to H. sativum in the crown or root rot stage is unknow. However, breeders and pathologists seem to have developed cultivars with good field resistance or tolerance to the disease at current inoculum levels, since no major outbreaks of the disease have occured in the several decades preceding the introduction of minimum tillage systems and early fall planting of winter wheat for cattle pasture (2). More recent studies have indicated that the biological control of common root rot is effected in a basically different manner from that recognized at present (6).

It is well known that many microorganisms in soil are antibiotic and it has also been assumed that some of these microorganisms play

important roles in controlling soil-borne pathogens. Phytopathologists have neglected the role of the microbial population of the soil and considered only the parasite in disease studies. Alexopoulos and Herrick (1) studies the antagnostic property of 80 strains of Actinomycetes from the soil. They used Colletotrichum gleosporiodes as a test organism and found that 17 per cent of the Actinomycetes were strongly inhibitory and 44 per cent had no action. Cooper and Chilton (8, 9, 10) concluded that some Actinomycetes were antagonistic to Pythium spp. and Rhizoctonia solani on sugar cane roots. Meredith and Semeniuk (18) classified the organisms isolated from soil where the Panama disease occured into three groups; those which apparently increase the pathogen growth, those having no effect on its growth, and those which are antagonistic to it. Taubenhaus and Ezekiel (26) developed a system for the control of Texas root rot of cotton caused by Phymatotrichum omnivorum involving antibiotic activity in the soil. The increased emphasis placed on the use of antibiosis or other biological means of control of plant disease brought about this study. Elimination of the propagules of H. sativum, which usually exist in large numbers in the soils where wheats are grown, may be accomplished by a significant multiplication of the soil microorganisms which are antagonistic to it. Therefore, the objective of this study was to screen soil microorganisms isolated from soils where wheat was grown at different locations in Oklahoma for their antagonistic action on the saprophytic growth of H. sativum in the laboratory as well as their ability to significantly reduce seedling blight caused by this fungus under controlled conditions.

CHAPTER II

LITERATURE REVIEW

Helminthosporium sativum, the causal agent of spot blotch of barley, was described by Pammel, King, and Bakke (20). The mycelium is olivaceous to black when mature and develops abundantly. Conidia emerge from stomata or between epidermal cell walls, singly or two to three, rarely more, are 2-8 septate, and range in size from 110 to 150 X 6 to 8 u. The conidia are slightly to distinctly curved, thick-walled, reddish to dark-brown, 1-10 septate (mostly 7), and range in size from 60 to 120 X 5 to 20 u. H. sativum survives as conidia in soil (4, 7, 21). Mathre (16) studied the germination of conidiosphores of H. sativum. Among other things he observed that conidiosphores 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 conidiosphores of H. sativum were able to germinate at 100 per cent after a year of storage under all conditions tested. He found that the spores retained about 95 per cent viability for two years if stored dry at 4 C. He also noted that in many cases conidiosphores of H. sativum failed to germinate in the presence of other fungi. Meronuck and Pepper (19) revealed that when conidia of H. sativum are added to soil, the inner cells of some conidia are converted into chlamydospores.

Luttrell (15) examined the Saccardo-type specimen of H.

sorokinianum and those of H. sativum and reported their synonomy. Tinline (27) produced the perfect stage by mating compatible lines of the fungus on autoclaved barley kernels on nutrient media and studied the complete life cycle of the fungus in culture. He applied the binomial Cochliobolus sativus (Ito and Kurib.) Drechsl to the fungus. Hayes, et al. (13) has reviewed the literature on the pathogenicity of H. sativum.

Since the work of Stakman in 1920 (25), many researchers have confirmed that inoculum in the soil is the primary source of the disease. Dickson (11) reported that seedling and crown infection occured from seed-borne mycelium or from crop residues in the soil. This report supported the studies of Henry (14) who found that mycelia was capable of surviving in the winter in the soil at St. Paul, Minnesota. The abundant conidial inoculum results in severe infection of young tissues whenever environmental conditions are favorable. Dickson (11) also reported that plants retarted in development by injuries or unfavorable growing conditions are usually more susceptible to attack. Seed infection is frequently quite high. Surveys in Canada by Greaney and Machacek (12) indicated that high infection occured in years favorable for the disease. In this connection, Simmonds, Sallans and Ledingham (22) have found H. sativum to be extensive in soil of the wheat producing regions of Western Canada, and conidiospores of the fungus were found to over-winter in those areas. Later, Chinn, and Sallans, and Ledingham (7) investigated conidial populations of H. sativum in field soils and studied the relationship of these populations to disease incidence on seedling and mature plants. From 47 fields, they found a range from less than 10 to over 250 viable

conidiospores of H. sativum per gram of soil. Boosalis (4) 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 leterature concerning the survival of spores of H. sativum in the soil.

Chinn, Ledingham, Sallan and Simmonds (6) studied a mechanism for the control of common root rot of wheat. They empahsized the role that antibiosis plays in the control of plant diseases; however, the application of this hypothesis to control of common root rot of wheat caused by H. sativum and other fungi has given only limited success. Cooper and Chilton (8, 9, 10) reported the results of a survey made over a period of two years, and included an analysis of some of the factors responsible for the number and distribution of antibiotic Actinomycetes in the various soils. They concluded that some Actinomycetes were antagonistic to Pythium spp. and Rhizoctonia solani which caused a severe root rot of sugar cane. They found the number of Actinomycetes per gram of soil, determined by dilution cultures, varied from about 100,000-4,000,000 with an average of approximately 1,300,000. Based on 8,302 cultures isolated from 181 soil samples from five soil types, the percentage of isolates antibiotic to P. arrhenomanes ranged from 18.5 to 31.5 per cent for the different soils. The per cent of Actinomycete cultures antibiotic to P. arrhenomanes was comparable to that reported by Meredith and Semeniuk (18). These investigators reported that 21 per cent of the Actinomycetes cultures isolated from the Iowa soil were antibiotic to P. graminocolum Subra. Meredith and Semeniuk (17) also reported that the Actinomycetes were antagonistic to Fusarium oxysporum f. cubense. Of the soil organisms, mostly Actinomycetes, isolated from 66 soil samples in

Jamaica, 122 exhibited antagonism to F. oxysporum f. cubense. They were classified as 66 slightly antagonistic, 39 antagonistic, and 17 very antagonistic.

CHAPTER III

MATERIALS AND METHODS

A culture of Helminthosporium sativum P., K., and B. was obtained from an infected wheat seedling by surface sterilizing the seedling with a 1 to 10 dilution of sodium hypochlorite (Clorox) for a several minutes, cutting the infected tissue into small pieces and placing the pieces in a moist chamber for 48 hours. A single fresh conidium arising from the infected tissue was picked off by sterilized needle and transferred to Potato Dextrose Agar (PDA) medium in a 10 cm Petri dish and kept in the room temperature. A stock culture was made from this original single spore isolation and kept on PDA in Petri dishes in a refrigerator at 4 C. The stock culture remained pathogenic throughout these experiments, although transfers were made every month to maintain viability.

In the Fall of 1975, 19 soil samples were collected from seven different locations in wheat growing areas in Oklahoma (Stillwater, Guymon, Tipton, Medford, Fairview, Lahoma and Ponca City). These soil samples were dried in a hot air oven at 50 C for 24 hours. After passing through a 9- mesh screen, a 25 gram portion of each sample was added to an Erlenmeyer flask containing 250 ml of sterilized distilled water. Each flask was vigorously shaken periodically for approximately 3 hours. Following this agitation and dilution, 10 ml of soil suspension was withdrawn and added to 90 ml of sterilized distilled

water. This sample was further diluted to 1/1,000, 1.10,000 and 1/100,000. One ml of this final dilution was thoroughly mixed with 1 ml of propagules of H. sativum obtained from 1/day-old cultures or PDA which were blended into suspension with sterilized distilled water in a blender (Waring).

Approximately 25 ml of melted Glycerol Asparaginate Agar (28) was added into sterile plates already containing 2 ml a mixture of soil and disease propagule suspension. Twenty five dilution plates were poured from each soil sample and were incubated at 24 C for 10-14 days. Growth competition between H. sativum and the isolates of soil microorganisms was classified by the inhabition zones which occured at the interface between the mycelial growth of H. sativum and the colonies of soil microorganisms. The number of microorganism colonies showing some degree of inhibition zone in each plate was recorded. All individual colonies showing inhibition were transferred to Glycerol Asparaginate Agar slants and labelled serially according to origin of the soil sample; for example, T-111 was isolate number 111 from Tipton, Oklahoma. All colonies so isolated were held at 24 C until used to test for relative efficiency in the inhibition of H. sativum.

An in vitro test for antagonistic activity against a pathogenic isolate of H. sativum was made by a streak-plate method on the Czapek's Sucrose Nitrate Agar (28). Fifteen ml of melted Czapek's Sucrose Nitrate Agar was poured into each of 5 10-cm Petri dishes and kept overnight at room temperature. Each isolate to be tested was streaked along one edge of Petri dish as shown in Figure 1, and allowed to grow for 48 hours at 24 C. After incubation, an agar disc 2 mm in diameter, cut from an actively growing 10 day-old culture of H. sativum, was

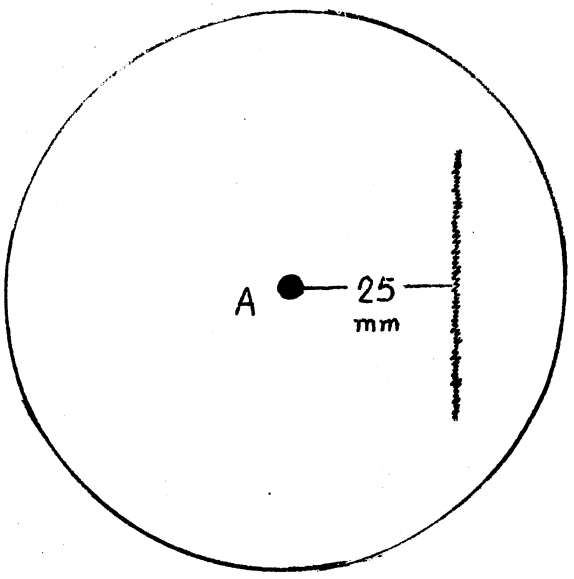

Figure 1. Diagram of the Technique used for testing the Antagonistic Efficiency of soil microorganisms to Helminthosporium sativum on Czapek's Sucrose Nitrate Agar in 10 cm Petri Dishes.
A- 2 mm disc of H. sativum culture.
B- soil microorganism culture.

placed in the center of the Petri dish. This procedure allowed 25 mm between each isolate to be tested and the inoculum. The distance from the foremost edge of H. sativum culture to the center of each soil isolate in each of the five Petri dishes was determined and recorded in millimeters after incubation for seven days at 24 C. A total of 413 soil samples obtained from all seven sample locations were tested by this procedure.

The colonies that exhibited antagonism to H. sativum were classified into three classes: (1) not antagonistic (colonies that failed to show any inhibition to the growth of H. sativum) and the foremost edge of the test isolate merged with the H. sativum culture; (2) slightly antagonistic (organisms that slightly retard the growth of H. sativum) where the distance between the foremost edge of the test isolate culture and the H. sativum culture was between 1 to 5 mm and (3) very antagonistic (organisms that have ability to produce an area free from mycelial growth of H. sativum) where the distance between the test isolate culture and the H. sativum culture was between 6 and 17 mm. After testing for antagonistic activity, three of the most antagonistic microorganisms were used to test for seedling blight control of wheat caused by H. sativum in a growth chamber.

The three isolates chosen for this phase of the study were M-019, M-117 and M-010 (all from Medford, Oklahoma). These isolates were multiplied separately in liquid Glycerol Asparaginate Medium. Five millimeters discs of an agar culture of each organism were transferred to 500 ml Erlenmeyer flasks containing 250 ml of the liquid medium. These flasks were held at 24 C for one month before using in the experiment. During this time each flask was vigorously shaken

periodically. Each organism was used in three concentrations. Concentration A was the undiluted growth as it came from the flask. Concentration B was diluted with three parts of new Glycerol Asparaginate Medium to one part of month-old growth, and Concentration C was diluted with 15 parts of new medium to one part growth. Uninoculated, undiluted Glycerol Asparaginate Medium served as a control.

The inoculum of H. sativum was made by using a mixture of wheat straw and grain as a medium and infesting it with the fungus from the stock culture previously described. Infestation of the wheat straw-grain medium was accomplished by mixing a year-old wheat straw and grain which was then saoked with distilled water for 24 hours. The excess water was drained off, and the moist medium was autoclaved for 90 minutes at 121 C. After the sterile medium was cooled it was placed in wide mouth one 1 Mason jars. The jars were filled one-fourth full with this medium (approximately 250 ml). Each jar was then covered with a loose cap and sterilized for one hour at 121 C. This sterilization process was carried out twice with an interval of two days. The sterilized wheat medium was then inoculated with 1 ml of H. sativum spore and mycelium suspension grown in Potato Dextrose Broth Medium for 10 days. The fungus was allowed to grow on the wheat medium for 2 weeks at 24 C.

The soil used for planting, a one-two-one mixture of builders sand, sandy-loam soil and sphagnum peat, respectively, was put in 36 12 cm clay pots which had been steam heat sterilized twice at 115 C for 48 hours with an interval of 2 days.

The test cultivar selected for this study was the hard winter

wheat Danne CI 13876[1/]. This cultivar is widely grown in Oklahoma for commercial wheat production and is susceptible to H. sativum (29). Untreated seeds of this cultivar were obtained from the Department of Agronomy, Oklahoma State University. The experiment was made in split plot with randomized complete block design as diagrammed in Figure 2. The microorganisms to be tested for inhibition served as main plots and were randomized in three replications. The three concentrations and the control of each organism as sub-plots. The experiment was made in a growth chamber (Percival Model No. PGc 78c) at a light-time temperature of 24 C and a dark-time temperature of 10 C with approximately 5,382 lux of flourescent light at plant height with a 12 hour photoperiod.

The test microorganisms in liquid medium and the H. sativum propagules in straw-grain medium were placed in the soil in the following manner. Ten grams of inoculum of H. sativum grown in sterile wheat medium were placed at a 2.5 cm depth in all pots. At the same time, 30 seeds of the wheat cultivar "Danne" were soaked for 24 hours in 15 ml of each concentration of the test microorganism, after which the seeds were spread uniformly over the H. sativum inoculum, and the remainder of the test organism culture concentration was poured over the seeds. One hundred seeds were also used to test the germination of the seed lot in the same growth chamber.

The plants in each pot were examined 14 days after planting the number of dead plants and the number of alive but infected plants were recorded. At the end of the experiment, the root mat from each

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[1/] CI numbers are assigned by the Germplasm Resources Laboratory, A.R.S., U.S. Dept. of Agriculture, Beltsville, MD.

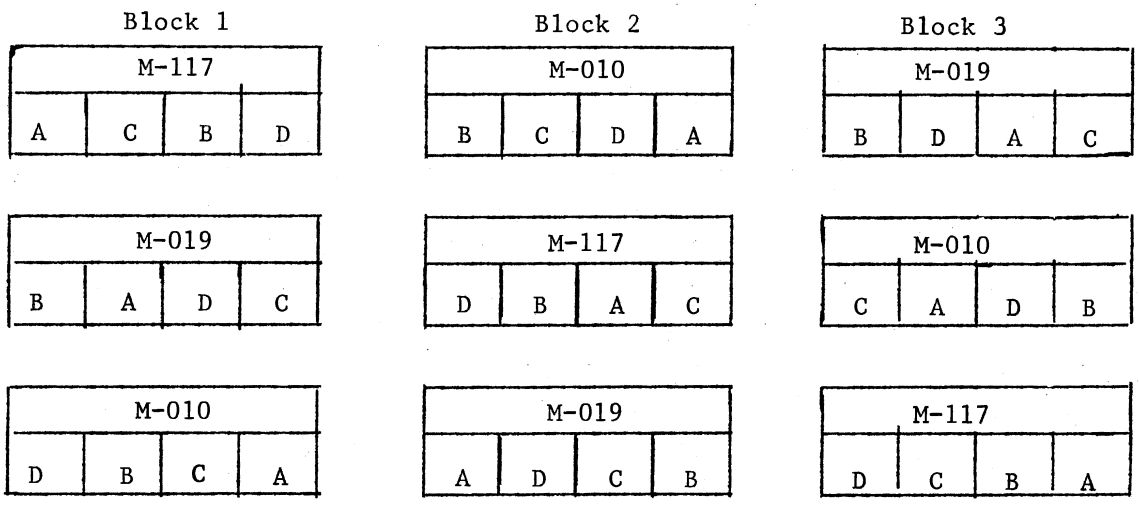

Figure 2. Diagram of the experiment design used for the relative Ability of Certain Soil Microorganisms to inhibit Helminthosporium sativum infection of the Wheat Cultivar "Danne" in a Growth Chamber. The test organisms were from Medford, Oklahoma and labelled M-010, M-019, and M-119. The concentration of each test organism were lettered as follows: (A) Concentrated Organisms (not diluted) and H. sativum Culture; (B) A 1/4 Dilution with Glycerol Asparaginate Medium; (C) a 1/16 Dilution with Glycerol Asparaginate Medium and (D) Glycerol Asparaginate Medium control.

pot was soaked and washed gently in water over a fine-mesh screen to remove soil. The volume of roots in each pot was measured by water displacement in a 10 ml graduated cylinder. After the volumes of the roots were recorded, they were oven dried at 50 C for 96 hours and weighed.

CHAPTER IV

RESULTS

As expected, the number of organisms in the soil samples antagonistic to H. sativum varied considerably since the soil samples were collected from different types of soils and at different locations. From 25 dilution plates of each soil sample, only the colonies of organisms growing in plates of 1/1,000, 1/10,000 and 1/100,000 dilutions were recorded for antagonistic activity and isolated for further testing. A total of 413 colonies of soil microorganisms were obtained from 19 soil samples made in Oklahoma and isolated for use in the "streak-method" test for antagonism. These colonies showed different degrees of antagonistic activity based on the zone of inhibition between the colony and the culture of H. sativum. The inhibition zones of some colonies are shown in Figure 3. The soil microorganisms that appeared in agar dilution plates were not identified other than is note that most of them were bacterial and Actinomycetes of different color and colony characteristics. About 60 per cent of all soil microorganisms isolated were not antagonistic at all, about 30 per cent were slightly antagonistic and about 10 per cent were very antagonistic (Table I). Isolates from Stillwater, Fariview, Lahoma and Ponca City had 0 to 5 per cent that were very antagonistic, while Guymon, Medford and Tipton had 10 to 17 per cent that were very antagonistic.

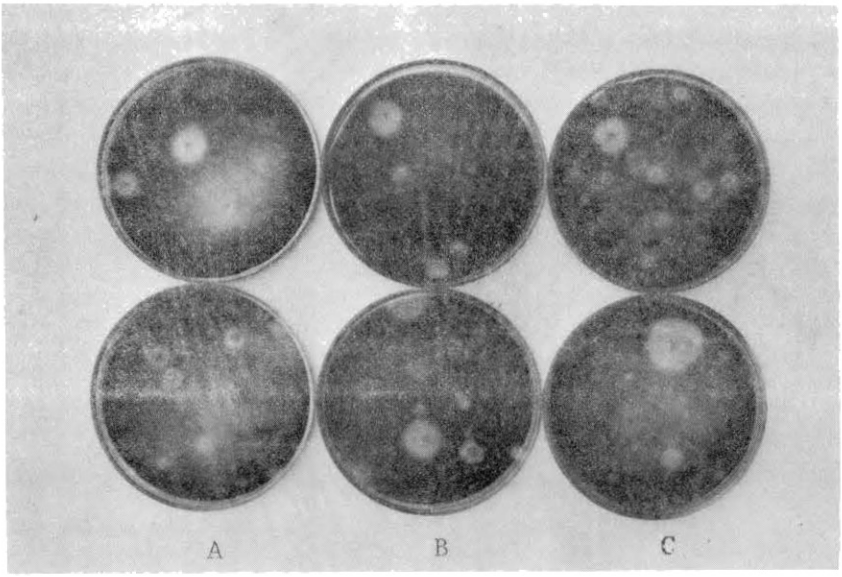

Figure 3. Inhibition Zones Between Certain Soil Microorganisms and Helminthosporium sativum on 1/1,000 Dilution Plates of Soil Samples from (A) Guymon, (B) Medford and (C) Tipton.

TABLE I

RELATIVE ANTAGONISTIC ACTIVITY OF SOME SOIL MICROORGANISMS ON *HELMINTHOSPORIUM SATIVUM* GROWING ON CZAPEK'S SUCROSE NITRATE AGAR

Soil Samples From	Number of Organisms Examined	Number of Organisms: [1/] Not Antagonistic	Slightly Antagonistic	Very Antagonistic
Stillwater	48	36	12	0
Guymon	35	21	8	6
Tipton	47	23	16	8
Medford	123	69	41	13
Fairview	58	43	12	3
Lahoma	62	43	17	2
Ponca City	40	27	12	1
	413	262	118	33

[1/] Number of colonies from all soil samples from a location.

From the 33 colonies in the very antagonistic class, the best 10 isolates were tested again for their antagonistic properties by the streak plate method. Each isolate was tested in each of five plates and the results are given in Table II. All of these organisms, with the possible exception of G-013 would again be classified as very antagonistic, and the zone of inhibition for each isolate was almost identical with the zone measured in the first test. The highest degree of inhibition was obtained from M-117, M-019 and M-010, all of which had inhibition zones of over 14 mm. It is interesting that all of these isolates have different colony colors and characteristics, but all are from Medford, Oklahoma, soil samples. These isolates were multiplied in the laboratory and used in the growth chamber test for control of seedling infections of wheat by H. sativum.

Measurements of the growth of these organisms in the liquid Glycerol Asparaginate Medium were not made but these cultures were allowed to grow for one month. The inoculum of H. sativum on the mixture of wheat straw and grain produced a high concentration of conidia in as little as two weeks.

It was evident that the strain of H. sativum used in the experiment was very pathogenic. Death of seedling plants occurred right after emergence in all pots, even the ones in which the concentrated soil organisms were applied (Table III). The average number of dead plants in the control pots of all of the soil isolates exceeded 26 out of 30. The culture of the soil isolates used did effect the development of the disease, and in proportion to the concentration of the culture. For example when the undiluted culture of isolate M-019 was applied only an average 11.3 plants were dead after 14 days, but when

TABLE II

ANTAGONISTIC EFFECT ON 10 SOIL ISOLATES ON HELMINTHOSPORIUM SATIVUM GROWN ON CZAPEK'S SUCROSE NITRATE AGAR

Soil Isolate Number	Zone of Inhibition in mm [1/] Petri Dish or Replicate Number 1	2	3	4	5	Mean
M-117	16	17	18	17	17	17.0
M-019	15	15	15	14	17	15.2
M-010	11	16	16	12	15	14.0
T-021	15	13	13	13	12	13.2
G-028	13	13	14	12	13	13.0
G-014	13	13	12	11	12	12.2
T-007	12	13	12	10	12	11.8
M-049	13	10	12	12	12	11.8
G-018	12	9	10	11	12	10.8
G-013	8	11	10	8	9	9.2

[1/] The distance between the foremost edge of the colony of H. sativum and the colony of the soil isolate culture in millimeters.

TABLE III

MEAN VALUES OF DEAD PLANTS, INFECTED PLANTS, ROOT VOLUMES, AND ROOT DRY WEIGHTS OF WHEAT CULTIVAR "DANNE" GROWN IN CLAY POTS IN THE GROWTH CHAMBER AND INOCULATED WITH HELMINTHOSPORIUM SATIVUM AND CERTAIN SOIL MICROORGANISMS

Soil Isolate	Concentration[1]	Dead Plants	Infected Plants[2]	Root Volume (ml)	Root Dry Weight (g)
M-010	A	15.7[3]	16.3	0.4	0.07
	B	19.0	22.7	0.5	0.06
	C	22.7	25.0	0.2	0.03
	D	26.3	27.7	0.1	0.01
M-019	A	11.3	12.0	0.9	0.09
	B	18.7	19.3	0.5	0.06
	C	27.3	27.7	0.1	0.01
	D	28.0	29.7	0.1	0.01
M-117	A	12.3	14.3	0.7	0.08
	B	15.7	21.0	0.4	0.05
	C	22.0	27.0	0.1	0.02
	D	28.0	29.3	0.1	0.01

[1] A-concentrated isolate (not diluted)
B-a 1/4 dilution of each isolate with liquid Glycerol Asparaginate medium.
C-a 1/16 dilution of each isolate with liquid Glycerol Asparaginate medium.
D-sterile liquid Glycerol Asparaginate medium (control)

[2] Each figure includes the number of dead plants in addition to live plants showing symptoms of seedling blight or root rot.

[3] Each figure is an average of 3 replications of 30 plants.

M-019 was not applied at all, 28.0 plants were dead. When isolate M-019 was diluted one to four with sterile liquid medium 18.7 plants were killed by the end of 14 days, and when this same isolate was diluted 1 to 16 with sterile medium, 27.3 plants were dead. When the remainder of the plants were examined 14 days after planting, several additional plants were found to be diseased in all pots.

After the notes on the number of infected plants were recorded, the volume and dry weight of the root mass of each pot were measured and recorded. These values are also given in Table III. In almost all cases, the root volume and dry weight were inversely correlated with both the number of dead plants and the total number of diseased plants at the end of 14 days. The higher the number of dead or diseased plants, the smaller was the root volume and root weight. In most cases the number of roots remaining in the pot was almost too small to measure. A statistical analysis of each of these characteristics was made. When the data for each soil isolate were pooled it was found that there were no significant differences between the three isloates used in their effect upon the development of H. sativum on the seedlings of Danne wheat (Table IV).

However, when the three soil isolates were pooled at each of the three concentrations and the control there were highly significant differences (Table V). There were significant differences between the controls and even the weakest concentration of the soil isolates, in some cases at the 0.01 level of probability.

TABLE IV

A COMPARISON OF MEANS OF THREE CONCENTRATIONS AND THREE REPLICATIONS OF THREE SOIL ORGANISMS AND THEIR EFFECT ON INFECTED PLANTS, ROOT VOLUMES, AND ROOT DRY WEIGHTS PRODUCED BY THE WHEAT CULTIVAR "DANNE", GROWN IN CLAY POTS IN THE GROWTH CHAMBER, INOCULATED WITH HELMINTHOSPORIUM SATIVUM AND THESE ORGANISMS

Soil Isolate	Dead Plants	Infected[1/] Plants	Root Volumes (ml)	Root Dry Weight (g)
M-010	20.9[2/]	22.9	0.3	0.04
M-019	21.3	22.2	0.4	0.04
M-117	19.5	22.9	0.3	0.04
LSD .05	8.78	5.58	0.8	0.07

1/ (See note 2/, Table III).

2/ Each figure is a mean of three replications and three concentrations plus a control of 30 plants.

TABLE V

A COMPARISON OF THE MEAN OF THREE SOIL ISOLATES AT THREE CONCENTRATIONS AND A CONTROL OF THE NUMBER OF DEAD PLANTS, NUMBER OF INFECTED PLANTS, ROOT VOLUME, ROOT DRY WEIGHT PRODUCED BY THE WHEAT CULTIVAR "DANNE" GROWN IN CLAY POTS IN THE GROWTH CHAMBER AND INOCULATED WITH HELMINTHOSPORIUM SATIVUM AND THREE SOIL ORGANISMS

Concentration	Dead Plants	Infected Plants	Root Volume (ml)	Root Dry Weight (g)
A (undiluted)	13.1[1/]	14.2	0.7	0.08
B (diluted 1 to 4)	17.8	21.0	0.5	0.05
C (duluted 1 to 16)	24.0	26.6	0.1	0.02
D (control)	27.4	28.9	0.1	0.01
LSD .05	2.5	2.2	0.4	0.04
.01	3.8	3.3	0.6	0.05

[1/] Each figure is a mean of three replications of 30 plants and three soil isolates.

CHAPTER V

DISCUSSION

Isolates of the soil organisms concerned here were collected only from plates of 1/1,000, 1/10,000 and 1/100,000 dilutions. The spread of the organisms and the appearance of the inhibition zones in these plates were convenient for recording and isolation. Plates of 1/10 and 1/100 dilutions were not used because of the population of the organisms produced in each plate was too dense to be screened. The best organisms, in terms of antagonistic ability, may have been lost in this manner; however, a fair number of active inhibitor organisms were obtained. The organisms collected in this study are mostly bacteria and Actinomycetes and it seems likely that most of the organisms which pronounced significant inhibition are Actinomycetes.

The screening technique used indicated that soils from Stillwater, Ponca City, Lahoma and Fairview yielded fewer antagonistic organisms than soils from Medford, Tipton and Guymon. However, the sampling was probably too small to draw conclusions in this regard.

Of the 413 colonies of organisms isolated from the initial dilution plates, less than 10 per cent were classified as very antagonistic by the streak-method. This may indicate that the population of antagonistic soil organisms in nature is not sufficiently high to play any significant role in the control of H. sativum in these soils. The identification of the collected organisms has not been done in this

study, but if all the isolates were Actinomycetes and less than 10 per cent were (7.99 per cent) antagonistic to H. sativum, this does not compare favorably to that reported by Meredith and Semeiuk (17) in Iowa where they found 21 per cent of the Actinomycetes were antagonistic to Pythium graminicolum, nor to the work of Cooper and Chilton (9) who found that 23.4 per cent of Actinomycetes cultures isolated from the Louisiana soils were antagonistic to P. arrhenomanes.

The results of testing for control of root rot infection on the wheat cultivar "Danne" by H. sativum in the growth chamber probably could have been expected. The high percentage of infection occured in all treatments including the ones that concentrated organisms were added, probably because the level of inoculum of H. sativum culture (10 grams per pot) was too high. The three organisms used in this study showed almost the same efficiency of control. Statistical analysis indicated the heavier the concentration of the test organism, the more control of root rot infection by H. sativum was obtained. Probably a more realistic view could have been obtained if the concentration of inoculum of the pathogen had also been varied as well as the concentrations of the test organisms.

CHAPTER VI

SUMMARY

1. Nineteen soil samples collected from Stillwater, Guymon, Tipton, Medford, Fairview, Lahoma and Ponca City, Oklahoma were used to screen microorganisms which could inhibit the growth of Helminthosporium sativum P., K., and B., a cause of root rot infection on wheat and other grasses.

2. Four hundred and thirteen colonies of soil microorganisms were obtained from different locations by a screening technique in dilution plates containing Glycerol Asparaginate Agar.

3. The test for antagonistic activity against a pathogenic isolate of H. sativum was made by a streaking-plate technique on Czapek's Sucrose Nitrate Agar.

4. The 413 colonies that were screened for antagonism to H. sativum were classified into three classes: (1) not antagonistic (organisms that have failed to inhibit the growth of H. sativum), 63.4 per cent; (2) slightly antagonistic (organisms that slightly retard the growth of H. sativum), 28.6 per cent and (3) very antagonistic (organisms that have the ability to produce an area free from mycelial growth of H. sativum around the colony), 8.0 per cent.

5. Three organisms from Medford soils were considered to be the most antagonistic to H. sativum.

6. Three organisms from Medford soils gave some control of seedling blight and root rot caused by H. sativum in growth chamber tests.

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