STUDIES ON THE INFLUENCE OF SOIL COMPOSITION ON THE GROWTH AND NUTRITION OF CERTAIN FUNGI CAUSING FOOT- AND ROOT-ROT OF WHEAT

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

STUDIES ON THE INFLUENCE OF SOIL COMPOSITION ON THE GROWTH AND NUTRITION OF CERTAIN FUNGI CAUSING FOOT- AND ROOT-ROTS OF WHEAT

G. Semeniuk

INTRODUCTION

The ability of such cereal foot- and root-rotting fungi as *Helminthosporium sativum* P.K. and B., *Ophiobolus graminis* Sacc. and *Fusarium* spp. to exist either as plant parasites or as saprophytes is generally recognized. Under natural conditions a variety of substrata for saprophytic development is available to them, particularly the soil and various crop residues in or on it.

The effectiveness of crop rotations in reducing the severity of damage by these fungi is well known (4). Oats, sweet clover and corn for instance, tend to reduce the foot-rot damage to wheat following them. This reduction may be attributed to an effect on the development of the fungi in both parasitic and saprophytic stages. Immune or resistant crops reduce their parasitic development to a minimum and confine them mostly to their

saprophytic stages. Once in the soil medium their saprophytic development is probably conditioned by the nutritive materials present.

PRESENT STUDY

Purpose of the Investigation

Little is known on the nutritional value of soils and various crop residues which may be incorporated in them, as substrata for the saprophytic development of these fungi. The present studies were therefore undertaken to determine the relative value of the soil types, soils to which were added various plant residues and various chemical compounds as fertilizers, on the increase in amount of inoculum in the form of mycelium and in the form of spores.

Studies were also undertaken to determine the relative effect of soil types on the expression of seedling infection in wheat as affected by differences in the composition of soils.

Previous Work

The development of foot- and root-rotting fungi in the field as saprophytes has been mainly observed on plant residues. Dickson (9) considers that *Fusarium* spp. can develop as saprophytes on decaying crop residues near the surface of the soil. Henry (18) observed that the mycelia and spores of the *Helminthosporium* and *Fusarium* species tested could readily overwinter in plant debris in the soil or on its surface. Christensen (6) considers that *Helminthosporium sativum* develops saprophytically on plant residues in and on the surface of the soil. *Ophiobolus graminis* has been reported to be able to live saprophytically in the soil for a short time (22, 27). Russel (35) considers it capable of increasing in amount in the soil.

In Alberta, Foster (11) demonstrated that *H. sativum* and *Fusarium* spp. readily survived the severe winter of 1928-29 in barley seeds and wheat stubble. In addition, Davies (8) observed that when oat hulls bearing these fungi were placed in sterilized soil and exposed in the field to contamination for a period of three years, isolations of the fungi could be made from the inoculum.

Demonstration of the presence of some of these fungi in the soil medium is not readily done. Only very

rarely was Henry (18, 19) able to isolate H. sativum directly from the soil by the plate method and only in a few cases could spores of the fungus be found by examining the soil microscopically (19). Recently, Bisby (3) reports the isolation of H. sativum from the soil by the plate method. In no instance has Ophiobolus graminis been reported to be isolated by this method. The indirect or sterile seedling method, however, has resulted in isolation of these fungi from soil where present. Fusarium spp. are readily isolated by both methods.

Studies on the effect of cultural practices on the distribution and occurrence of these fungi have yielded interesting results. Out of 260 soil samples studied in each rotation, in no case did Davies (8) isolate H. sativum by the sterile wheat seedling method from soil taken from virgin sod, while that under alfalfa (grown for 5 successive years) gave 1 isolation, that in fallow following wheat gave 7, and that under continuous wheat for 4 years gave 14. These results are significant in view of the fact that all seedlings were subjected to similar conditions in the laboratory.

Results from field studies made on the prevalence of these organisms as detected by their presence on mature wheat plants are influenced by factors affecting the establishment of the pathological condition. Certain namely was [illegible] [illegible] which [illegible] isolated P. [illegible] directly from the soil by the [illegible] method and [illegible] of a few cases would [illegible] of the [illegible] [illegible] in examining the soil microorganisms (11). [illegible] [illegible] reports the isolation of P. [illegible] from the soil by the plate method. In [illegible] [illegible] [illegible] [illegible] was reported to be isolated [illegible] [illegible]. The [illegible] [illegible] sterile seedling method, however, the [illegible] [illegible] of these fungi from soil other methods. [illegible] [illegible] readily isolated by this method.

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Results from field studies made on the [illegible] of these organisms [illegible] [illegible] [illegible] [illegible] small plants are [illegible] [illegible] [illegible] establishment of the pyrethrum [illegible] [illegible] observations, however, have been made. Greaney and Bailey (14) concluded from their studies of the fungus flora of mature wheat roots that there was no tendency for root-rotting organisms to accumulate in the soil during six years' continuous cultivation of wheat. They observed that both Fusarium and Helminthosporium species were consistently present on the mature plants, with Fusarium tending to occur more frequently than Helminthosporium. Broadfoot (5) in a similar study observed that of 47,360 crown portions of mature plants, 43,305 plants yielded either H. sativum or Fusarium spp. on potato sucrose agar. Although H. sativum was isolated more often than F. culmorum, it was slightly less frequently isolated than all the Fusaria together. No significant effect of crop sequence or cultural practice on the relative prevalence of these fungi was observed.

Material Studied

1. Fungi.

The fungi used in the studies reported were Fusarium graminearum 2, Helminthosporium sativum 6 and Ophiobolus graminis 4. All are very pathogenic to wheat as determined by Davies (8). Since, however, Fusarium spp. do not cause severe damage to wheat in the field (18)

as compared with the other two fungi, most attention was given to H. sativum and O. graminis. H. sativum, in fact, was mainly used in most of the studies.

2. Soils.

The soils of the three major soil belts of Alberta were used; namely, brown, black, gray wooded or podsol. The brown soil, representing the silt loam type, was obtained from the C.P.R. irrigation experimental farm at Brooks; the black soil was a loam obtained from the University of Alberta experimental farm at Edmonton; and the podsol was a loam obtained from the University of Alberta experimental plots at Fallis. In all cases soils were taken from summerfallow land which had supported a wheat crop the previous year, and were selected as representative of their respective belts.

A brief description of the soils is as follows:

Brown soil - prairie vegetation, low in organic matter, exposed to conditions of scanty rainfall (11 inches) and excessive evaporation.

Black soil - grass vegetation, high in organic matter, exposed to conditions of moderate rainfall (17 inches) and decreased evaporation.

Podsol soil - wooded vegetation, low in organic matter, exposed to excessive leaching, moderate rainfall (17 inches) and decreased evaporation.

More detailed information on these soil types may be obtained from U. of A. bulletins (46, 47).

3. Crop residues.

Crop residue refers to the parts of the plants of a crop left in a field after harvesting operations and consists of the basal portions of the stems, together with as much of the roots as were dug up from the soil with the aid of a digging fork.

In all, residues of nine crops were used which included those of the grain crops, wheat, oats, barley, rye and flax; the legume crops, alfalfa and sweet clover; and the perennial grasses, brome grass (*Bromus inermis*) and western rye grass (*Agropyron tenerum*).

The alfalfa residue obtained represented plants from a field of a two-year old stand. As a cutting had been made, the above ground parts represented the older portions of the plants.

Brome grass residue was obtained from the growth bordering a fence. The above ground parts were cut down to a height of six inches and then diggings were made.

Western rye grass residue was obtained from a field in the grass for three years. The material was obtained after a cutting had been made in the fall.

Roots of certain of the crops were obtained from plant residues. The roots of individual plants were

clipped off with scissors as close to the stem as possible.

Corn meal used represented the commercial product sold in retail stores.

4. Chemical compounds.

The chemical compounds used were all of the c.p. quality. They represented sources of nitrogen, phosphorus and potassium. The nitrogen sources were ammonium carbonate, $(NH_4)_2CO_3.H_2O$, ammonium sulphate $(NH_4)_2SO_4$ and di-ammonium phosphate $(NH_4)_2HPO_4$. The sources of phosphorus were mono-calcium phosphate $CaH_4(PO_4)_2.H_2O$ and di-ammonium phosphate. The potassium sources were potassium nitrate, KNO_3, and di-hydrogen potassium phosphate KH_2PO_4. In addition sucrose and dextrose were used.

Carbon Dioxide as a Measure of Fungal Activity

In the study of the decomposition of organic materials in soils the indices of microbiological activity that have been used are the accumulation of ammonia (7, 13, 44) and nitrates (13), and the evolution of carbon dioxide. The disadvantages of using ammonia and nitrates as indices are due, 1. to differences between organisms in their ammonifying and nitrifying ability; 2. to the possibility of the nitrogen compounds being synthesized into body material of the organisms; and 3. to the influence

of organic material on the quantities that accumulate. Carbon dioxide as an index, on the other hand, does not have these disadvantages, but is sometimes objected to on the grounds that it may be evolved from chemical reactions in the soil and because in oxidation-reduction reactions micro-organisms may be active without its liberation. Despite these objections, however, its use has been generally accepted as a fair measure of microbiological activity.

Comparatively little work has been done in studying the relationship between the carbon dioxide evolved and the amount of mycelium produced by a fungus. Peterson, Fred and Schmidt (32) grew cultures of *Aspergillus niger* and *Penicillium glaucum* in a liquid medium containing xylose, and could account for nearly all (91 to 98%) of the carbon in the xylose reduced by the carbon in the mycelium and the carbon dioxide given off. More carbon was converted into mycelium by *P. glaucum* than by *A. niger*. In both, more was converted in the early stages of development than later. In the case of *A. niger*, 41% of the carbon of the xylose reduced was converted into carbon dioxide after 7 days' growth, 59% after 14 days and 55% after 28 days' growth. With *P. glaucum* slightly higher values were obtained. With seven different genera of fungi Harter and Weimer (15) reported that the coefficient of respiration (glucose in grams reduced per gram CO_2 formed) varied from 0.83 for *Botrytis cinerea* to 2.01 for *Mucor racemosus*. Three fungi out of seven gave values below 1.

The economical coefficient (glucose used in grams to produce 1 gram dry weight of mycelium) varied from 3.86 for Diplodia tubericola to 22.86 for M. racemosus. Five of the fungi ranged about a value of 4±1.

In an endeavour to relate the amount of carbon dioxide evolved with the quantity of mycelium produced, Harter and Weimer (15) concluded from their study of seven different fungi that there was no correlation between the amount of carbon dioxide evolved with the amount of dry matter formed or with the glucose reduced. The respiratory quotient for each fungus (dry weight in grams of mycelium produced per gram carbon dioxide given off) was found to vary considerably, although all were under the value of 1. However, Peterson et al (32) present a table which shows a fair correlation between the amount of carbon dioxide evolved and the mycelium produced in Aspergillus sp., A.niger and P. glaucum using xylose as a carbon source. When comparison is made in the results given by Harter and Weimer between the total amount of carbon dioxide given off and the dry weight of the mycelium produced, the data show a tendency for fungi that produce more mycelium by weight to evolve greater amounts of carbon dioxide. This was also brought out by Kunstmann who the authors cite.

The results reported by these workers deal with differences between fungi. No reference has been found to

work done on the relationship between carbon dioxide evolved and mycelium produced when different sources of nutrient materials are used. Despite this lack of information it has been assumed in this investigation that there is a direct relationship between the nature of the substratum, as it affects the rate and amount of fungal growth and the carbon dioxide evolved for the particular fungus studied.

EXPERIMENTAL

PART I. GROWTH STUDIES OF *HELMINTHOSPORIUM SATIVUM*

Method

1. Respiration apparatus.

The amount of carbon dioxide liberated in respiration of the fungi was used to measure fungal activity. An apparatus was constructed similar in principle to that developed by Anderson (1) to collect the gas evolved. Since, however, the apparatus developed by him was used to measure the respiration of wheat seedlings at 0°C., numerous modifications were required in order to render it suitable for these investigations. Hence a more or less complete description of it seems necessary.

Reference to Plates 1 and 2 will show that the apparatus consisted of 9 large flasks and 14 glass towers connected to form a unified system, the glass towers being held in place by a wooden frame built on a table and against the wall. Each large erlenmeyer flask (1 l. capacity) comprised an individual unit, and when containing soil and the growing fungus, represented a treatment and was independent of the other eight. These erlenmeyer flasks are referred to as culture flasks.

The apparatus was so constructed that carbon dioxide-free air could be passed over the soil (17) in each flask, and the carbon dioxide evolved by the fungi drawn off. The parts to the right in Plate 1, represented by 5 glass towers, were concerned with producing carbon dioxide-free air. These are referred to as scrubbing towers. The other nine towers represented the part of the apparatus which had to do with collecting the carbon dioxide gas passing out from each erlenmeyer culture flask. These are referred to as absorbing towers. Each absorbing tower was connected to an erlenmeyer culture flask and was independent of the others.

The passage of air through each culture flask was effected by means of a water-pump just seen at the extreme left of Plate 1. By this means reduced pressure was created in the system and air was pulled in from the outside through the scrubbing towers, through each of the nine culture flasks and then through each of the nine

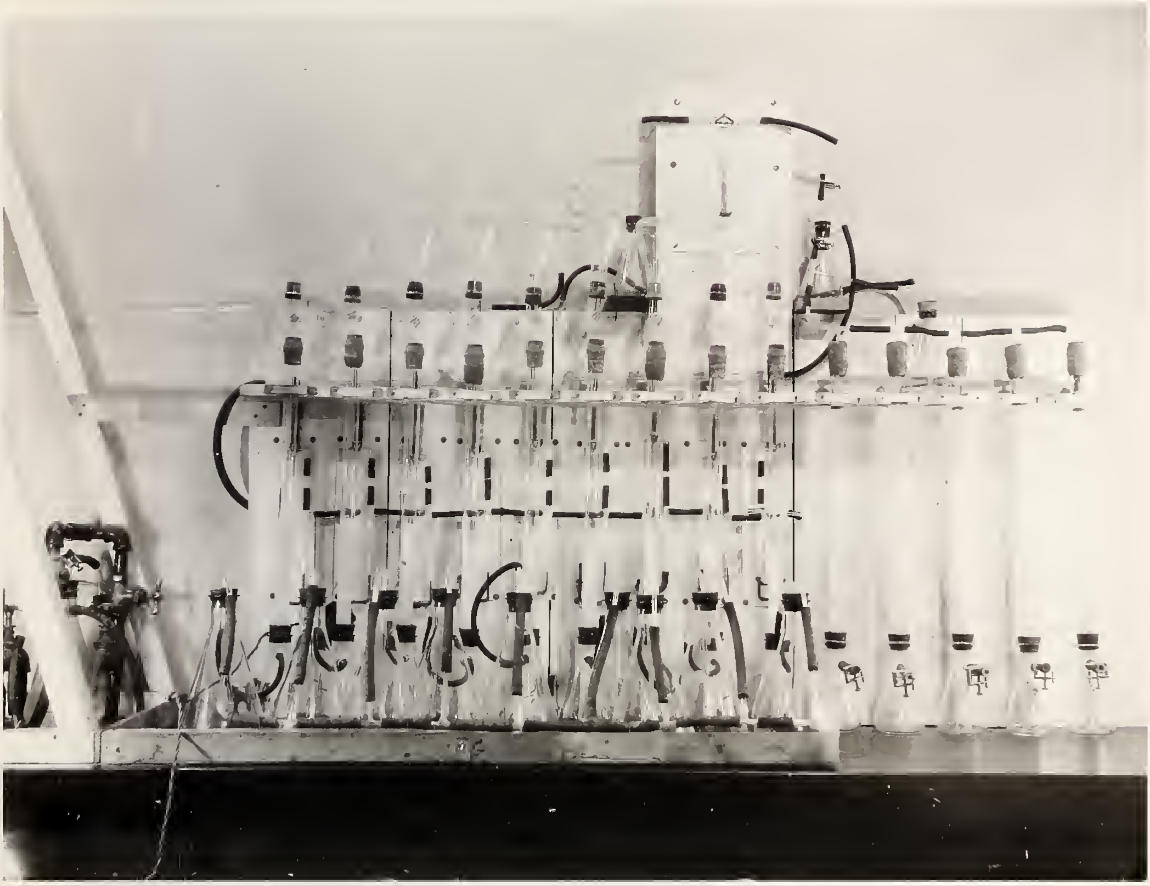

Plate 1. Respiration apparatus (Front view).

Plate 2. Respiration apparatus (Side view).

absorbing towers.

The towers were glass tubes 60 cm. long and 2.5 cm. outside diameter. The rim of the upper end of each tower was spread open to receive a rubber cork while the rim of the other end was bent inwards at four places to hold a perforated porcelain disc of a filter crucible. Each tube was inserted to the full extent into a 250 cc. side-neck erlenmeyer filter flask through a rubber stopper. Perforated glass beads stood in each tower to a height of 30 cm.

The lead from the scrubbing system went directly towards the connected line of T-tubes leading to each culture flask. The glass tube entering each culture flask extended to just below the rubber cork, while the lead-out tube extended from near the bottom. Connections were made to each of the absorbing towers by means of rubber tubes.

Through a rubber stopper at the top of each absorbing tower led a tube provided with a one-way stop-cock, which entered a 200 cc. erlenmeyer flask held in an inverted position. From each of these flasks, another tube led to a flow-meter fixed at a lower position. There were nine flow-meters arranged and fixed in a series to correspond to each of the nine absorbing towers. These were all joined together to a common lead by means of a connected system of T-tubes held in position behind the wooden frame. This common lead passed through a 200 cc. erlenmeyer flask,

through a flow-meter holding the highest position, through a number of flasks, and then to the water-pump.

Since air was pulled through the apparatus by virtue of reduced pressure, precautions had to be taken to avoid leakage inwards through connections. For this reason the rubber tubing used was all of the heavy type, and cork connections were made as tightly as possible with the exception of the top end of each tower. The difficulty encountered here was that pressing the rubber stoppers into the tubes resulted in a great deal of breakage. To reduce this, adhesive tape, 2.5 cm. wide, was wound on the outside upper end of each tube. However, even with this added protection, the joints could not be made air-tight. After trying vaseline, with no success, plastecine was tried and proved quite satisfactory for sealing purposes.

The flow-meters connected immediately to the absorbing towers were constructed of pyrex glass of 5 mm. outside diameter and with capillary tubes of 1 mm. bore. The rise of colored water in each indicated the passage of air, and by means of stop-cocks on each of the absorbing towers, the flow of air through the culture flasks was regulated. The single flow-meter located on the upper part of the wooden frame was placed between the water-pump and the single lead from the nine flow-meters. Mercury was used as the indicator, and the rise in its level in one tube indicated the total flow of air throughout the whole apparatus.

Prior to a determination, the towers were removed from the wooden frame and 50 cc. of sodium hydroxide solution were introduced into each side-neck erlenmeyer filtering flask. The scrubbing system received a 20% sodium hydroxide solution, while the absorbing system received approximately a 0.4 N solution from a standard burette fitted to a reservoir in a closed soda-lime system to exclude the carbon dioxide of the air. The towers were put back in place and cemented with plastecine.

Five towers in the scrubbing system were used to reduce the amount of bubbling that would take place if only one or two were used. This resulted in an increase in the efficiency of scrubbing and prolonged the length of time the sodium hydroxide solution could be used without changing. Screw clamps properly adjusted allowed for nearly uniform bubbling in the different towers. The reduced pressure created represented 3 cms. of mercury.

The level indicated for the rise of mercury was arrived at by trial, during which it was shown that a continuous flow of air could be had through each tower. This was obtained only when sufficient tension was produced that the flow could be regulated satisfactorily by stop-cocks. The level used represented a flow of 40 litres of air per hour.

The function of the inverted flasks above each of the absorbing towers was to act as cushions for the bursts of air that would be reflected in the unsteadiness of the fluid in each of the flow-meters. The erlenmeyer flasks on both sides of the single flow-meter and to the litre flask immediately attached to the pump were for a similar purpose.

The flask immediately adjacent to the connected leads from the scrubbing towers was concerned with regulating the humidity of the air. It contained 36% sulphuric acid and rendered the air approximately 70% saturated.

At the end of 24 hours' aspiration, the absorbing towers were removed and held in position in a series by burette clamps fixed to a stand. Distilled water, boiled and cooled, was added in 10 cc. quantities to each tower and the liquid allowed to drain. Ten such portions were added to each tower, the number representing complete washing as determined by phenolphthalein indicator.

<u>Carbon dioxide measurement</u>. The absorption of carbon dioxide by sodium hydroxide was according to the following reactions:

1. $NaOH + CO_2 \rightarrow NaHCO_3$
2. $NaHCO_3 + CO_2 \rightarrow Na_2CO_3 + H_2O$

The quantity and concentration (approximately 0.4 N) was in excess of the quantity actually involved in the

reactions. In no instance was more than 50% of the sodium hydroxide neutralized.

The method of determining the quantity of carbon dioxide absorbed depended on the reactions:

1. $Na_2CO_3 + HCl \rightarrow NaHCO_3 + NaCl$

2. $NaHCO_3 + HCl \rightarrow NaCl + H_2O + CO_2$

The acid required to complete the reaction represented in equation 2 was measured, it being the only known standard solution. This was made possible by using the double titration method, using as indicators phenolphthalein and B.D.H. Universal indicator.

To the solution in each side-neck erlenmeyer flask obtained from a tower was added three drops of phenolphthalein indicator and the solution titrated against approximately 0.4 N hydrochloric acid until the pink color began to fade. Further titration was carried out with a solution of standard (0.1 N approximately) hydrochloric acid until the pink color disappeared. Three drops of B.D.H. Universal indicator were then added and the volume of acid required to change the color from a greenish blue to the first appearance of a definite pink color was noted.

The accuracy of the titration method is indicated in Table I.

Table I

Results of tests to determine the accuracy of the titration method

Na_2CO_3 used to obtain approximate mgms. CO_2	Mgms. CO_2* as given by acid required to titrate to end point of		Difference in end points
	Phenolphthalein	B.D.H. Universal	
5	4.7	4.9	0.2
10	9.2	10.3	1.1
25	24.0	24.1	0.1
50	49.5	48.3	1.2
100	93.9	87.1	6.8
200	183.9	175.8	8.1

* Average of 6 replicates.

It will be noticed that the carbon dioxide as revealed by the phenolphthalein indicator is approximately equal to that given by the B.D.H. indicator.

Scrubbing system. The efficiency of the scrubbing system in removing carbon dioxide from the air entering the apparatus is indicated by the data given in Table II.

The table reveals a number of important points:

1. The variation (barring tower 8 where the values are very high) between towers in any run was quite small.

2. The average value for a run varied from day to day, and even on two different times on the same day.

Table II

CO_2 values obtained after different periods of aspiration when culture flasks were empty

Run No.	Date	Time of aspi-ration hrs.	Mgms. CO_2 in each absorption tower									Mgms. CO_2 in 50 cc. NaOH solu-tion used
			1	2	3	4	5	6	7	8	9	
1	28/4/33	2	6.2	6.2	6.8	6.2	6.6	6.2	*			
2	31/5/33	2	7.9	8.5	8.0	6.2	8.5	7.3	*			1.7
3	5/6/33	2	4.4	3.6	3.9	4.6	4.6	4.1	*			
1	7/7/33	4	5.4	5.0	5.4	5.4	5.1	5.6	5.6	5.8	6.0	
2	7/7/33	4	4.4	4.4	4.4	4.4	4.4	4.6	4.4	18.4	4.4	1.7
3	18/7/33	4	4.6	5.2	5.1	4.8	4.7	5.5	5.2	5.4	5.7	
1	7-8/11/33	24	5.4	5.4	4.9	7.5	9.1	5.7	5.6	5.6	5.4	
2	4-5/12/33	24	8.5	10.6	9.7	8.5	9.3	8.5	7.6	37.8	8.3	2.2
3	12-13/2/34	24	4.3	4.7	5.4	4.4	4.6	4.8	4.3	4.6	4.4	

* Apparatus consisted of 6 absorbing towers only.

3. The values obtained apparently were independent of the length of time of aspiration up to 24 hours. This tends to show that the efficiency of the scrubbing system was quite high.

4. The values obtained were not entirely due to the presence of carbonates in the stock solution of sodium hydroxide.

To determine whether those values were comparatively low, the apparatus was allowed to operate and air drawn in which was not freed of its carbon dioxide content. The data are recorded in Table III.

Table III

CO_2 values obtained after different periods of aspiration when culture flasks were empty and scrubbing towers disconnected

Run No.	Time of aspiration hrs.	Mgms. CO_2 in each absorbing tower 1	2	3	4	5	6	7	8	9
1	4	18.3	19.5	15.4	20.4	20.1	17.2	*		
2	24	38.7	48.8	63.1	31.5	43.2	34.4	42.7	57.0	60.2

* Apparatus consisted of 6 absorbing towers only.

They show that when atmospheric air was allowed to enter without passing through the scrubbing system the values are much higher.

The main source of the carbon dioxide in the checks may, therefore, be attributed to the procedure of setting the apparatus in preparation for aspiration. The time necessary to introduce sodium hydroxide into the flasks of each of the absorbing units and to fix the plastecine on each tower involved 30 to 45 minutes. During this time the solution was in contact with atmospheric air and carbon dioxide was absorbed. In addition a certain amount of ordinary air was present in the apparatus.

The length of time 20% sodium hydroxide solution could be used in the scrubbing system lasted only a short

while. Anderson (1) pointed out that with each succeeding day that such a solution was used, the efficiency was reduced quite rapidly. The procedure used with the method reported in this paper was to change the solution once after six periods of 24 hours' aspiration. Table IV gives data to show that the efficiency remained relatively constant for that period.

Table IV

Efficiency of 20% NaOH solution in scrubbing towers

Exp. No.	Contents of culture flask	Mgms. CO_2 after each 24-hour aspiration period					
		1	2	3	4	5	6
1	sterilized soil	9.1	7.8	7.6	7.0	6.7	8.9
2	sterilized soil	11.2	16.2	10.6	11.4	14.9	11.4

<u>Absorbing system</u>. To determine whether in the process of aspiration the carbon dioxide evolved in one culture flask would diffuse into an adjacent one, an experiment was conducted with the results given in Table V.

Towers 2, 4 and 7 were connected to empty culture flasks while towers 1, 3, 5, 6, 8, 9 were connected to culture flasks containing an actively growing fungus. It was concluded that diffusion of carbon dioxide, if any, was negligible.

Table V

Mgms. CO_2 evolved and the diffusion of the gas between adjacent culture flasks

Absorption tower numbers								
1	2	3	4	5	6	7	8	9
58.9	4.0	71.3	3.7	51.1	57.3	4.4	64.0	54.8

Leakage. The presence of carbon dioxide values as shown in Table II by tower number 8 that deviated so greatly from others, indicated that leakage occurred in the particular unit to which the absorbing tower was connected. Although precaution was taken to note any leakage in any part of the apparatus, especial attention was paid to the rubber corks of the culture flasks and of the absorbing units which held the glass towers in the side-necked erlenmeyer flasks. To ensure non-leakage and that corks were kept tightly in their places, distilled water was sprinkled on the connections from time to time. Values obtained which deviated widely from its duplicate were viewed with suspicion and considered due to leakage. In addition, if in subsequent determinations the particular duplicate treatments in which a leak occurred previously, gave approximately equal carbon dioxide values, the value obtained thought due to leakage was considered confirmed.

2. Preparation and quantity of material used.

a. Soil. The soils studied were brought to the laboratory in the summer of 1933 in gunny sacks and stored away from the sun in a dry cool place in a wooden shed. When required, a quantity was brought to the laboratory, passed through a fine mesh sieve, air dried in the greenhouse and stored in tin containers kept under room conditions.

Two hundred grams was the quantity used for each test throughout the experiments. This quantity when introduced into a 1 litre culture flask, formed a layer approximately one inch in depth.

b. Plant residues. In the fall of the years of 1932 and 1933 the residues of different crop plants were dug up in the field. Care was exercised to include as much of the roots as possible. In addition, fields were selected in which the stubble was of the length generally left after harvesting operations. The residues were washed with water to remove adhering dirt and spread out to dry rapidly. After passing the materials through a straw cutter, the residues were ground in a Wiley mill. They were stored in tin containers and kept in the laboratory.

On introducing the residues as types of organic matter to the soil, quantities were added on the water-free basis which corresponded to one percent by weight of the air-dry soil. The mixture was prepared in a large container

by thoroughly mixing the two together by means of a tin scoop. Two hundred gram portions were then removed and one added to each culture flask.

c. Chemical compounds. In all cases chemical compounds were used in the form of water solutions. Quantities were added to soil to correspond to 200 lbs. of each of the elements, nitrogen, potassium and phosphorus per acre. The acre value was taken as two million pounds of soil. This is equivalent to 0.01 grams of an element to 100 grams of soil. After the chemicals were added, the flasks were sterilized.

3. Treatment of substratum for fungal development.

a. Moisture. In all cases, the moisture content of the substratum was brought up to 70% of the water-holding capacity. This value was taken since it was thought that it represented optimum field conditions.

b. Sterilization. Heating at 15 lbs. steam pressure for three hours at 120°C. was adopted as sufficient for the conditions of the experiments. That this time was sufficient is indicated in Table VI, the results given representing mgms. of carbon dioxide evolved in 24 hours from black soil heated for different lengths of time and allowed to incubate for four days to allow for the development of any living micro-organisms present.

Table VI

Effect of method of heating black soil on the CO_2 evolved after 4 days of incubation

Exp. No.	Method of heating	Mgms. CO_2 evolved in 24 hrs.		Check* - empty culture flask
		1	2	
1	1 hr. only	44.0	41.2	
	2 hrs. only	6.1	6.6	6.4
	3 hrs. only	7.6	6.9	
2	3 hrs. on 1 day	6.6	9.6	
	3 hrs. on 2 days	6.9	8.0	7.0
	3 hrs. on 3 days	11.8	8.7	

* Average of 3 replicates.

The data show that heating for two hours reduced the activity of the soil organisms sufficiently that development was not detected after four days of incubation. However, three hours' heating on one day only was chosen. That this time of heating was sufficient was further brought out by experiments which involved determinations after each day of incubation for eight days. The amount of carbon dioxide obtained was relatively constant over the whole period.

4. Initiation of soil cultures of the fungus.

In every case spores of the fungus were obtained from a two week old test-tube culture. Sterilized distilled water was added to a test tube culture and by

means of a heated platinum wire the spores on the agar surface were loosened. The spore suspension obtained was then transferred to a 200 cc. erlenmeyer flask containing sterilized distilled water, the final suspension consisting of spores in 30 cc. of water.

With the aid of a sterilized 1 cc. pipette, a 1 cc. portion was distributed by drops over the surface of the soil in each flask. Care was exercised that the distribution was uniform and covered as much of the soil surface as the number of drops would permit.

The effect of using different spore concentrations in this method was determined by adding to soils 1 cc. suspensions containing different numbers of spores. Black soil was used and from 3-5 days were allowed for incubation. The data on carbon dioxide evolved in 24 hours after these periods of incubation are given in Table VII.

Table VII

CO_2 evolved from black soil which received different spore concentrations of *H. sativum*

Spores in 1 cc. suspension	Relative spore conc.	Av. mgms. CO_2 evolved in 24 hrs. after incubation for		
		3 days	4 days	5 days
2,300	1	37.1	39.8	30.7
600	1/4	36.8	35.9	42.0
260	1/10	33.1	40.2	43.8
20	1/100	26.4	38.2	43.3
0	0	7.2	7.9	6.5

The data bring out two interesting points:

1. The slight variations that may exist in the number of spores applied in an experiment to various substrata, cannot be attributed entirely, if at all, to differences in spore concentration.

2. The time for maximum evolution of carbon dioxide depends on the quantity of spores added to each soil. Soils which received a great number of spores reached the peak of maximum carbon dioxide evolution sooner than soils to which a smaller number of spores were added.

Similar results were obtained with _Fusarium graminearum_ when introduced into black soil containing 1% cornmeal. Kopeloff (24) observed with a number of fungi that when their spores were introduced into soil containing dried blood or cotton seed meal equivalent to 155 mgms. N per 100 gm. of soil the quantity of ammonia accumulated increased with concentrations of spores up to a certain value beyond which greater concentrations of spores produced no marked effect.

5. Method of studying fungal activity.

The experimental method followed early in the study consisted in attaching the culture flasks to the apparatus and subjecting them to continuous aspiration for one week, air being drawn over the soil. The difficulties of soil contamination and insufficient replication, however, were so

great that this procedure was discarded in favor of discontinuous aspiration. The advantages of this method, together with its ease of operation, were such as to recommend its use; but a knowledge of the behavior of the organism was first necessary.

Spores of the fungus were introduced into culture flasks containing sterilized soils and the fungus given time to develop. Brown, black and podsol soils were used since they represented low quantities of available energy material and possible differences that were desirable to detect. After seven days of incubation the development in each soil was studied by the carbon dioxide evolved in a given time of aspiration. Aspiration periods of 2, 4, 8 and 24 hours were tried to determine which would give sufficient differences between the different soils. The results obtained are shown in Table VIII.

Table VIII

Length of aspiration period as affecting the appearance of differences in carbon dioxide evolved from soil types supporting a culture of H. sativum after 7 days' incubation

Exp. No.	Time of aspiration	Av. mgms. CO_2 evolved in soil types		
		Black	Brown	Podsol
1	4	0.4	1.2	0.2
2	6	2.3	4.2	0.1
3	8	1.2	0.2	0.1
4	24	16.3	13.0	8.4

The data show that 24 hours of aspiration gave differences which, though not great, were measurable. The values obtained after 4, 6 and 8 hours of aspiration were so greatly affected by the values obtained from the check flasks, which were always subtracted, that even a negative value became a possibility. An added advantage in the 24 hours'aspiration period was the possibility of obtaining a total carbon dioxide yield from an experiment over a period of days.

Since in the discontinuous aspiration method the carbon dioxide evolved was determined after a period of development, it was necessary to know at what period maximum carbon dioxide was evolved to obtain greatest differences between treatments. Sixty-three culture flasks were prepared to represent different substrata and different incubation periods. Spores were introduced in all of the flasks at the same time, except in the check flasks. At the proper day, a set of nine flasks was attached to the apparatus and aspirated for 24 hours. The results obtained from the seven sets are given in Table IX and are represented in Figure 1.

Table IX

Period of maximum evolution of CO_2 by H. sativum growing on different substrata

Days of incubation prior to aspiration	Av. mgms. CO_2 evolved in 24 hrs. Soil alone	Soil + wheat	Soil + cornmeal
1	6.7	13.4	28.3
2	19.0	26.4	178.0
3	30.8	48.6	304.4
4	36.5	52.0	340.3
5	24.5	40.3	246.1
6	12.7	37.3	127.4
7	8.1	32.9	84.0

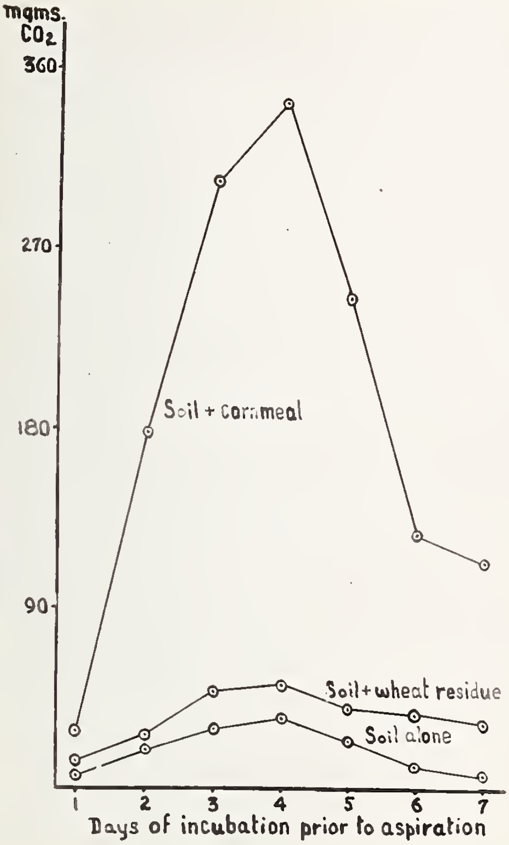

Figure 1. Respiration rate of H. sativum on substrata of different energy content after different periods of incubation.

The data show that the incubation period for maximum carbon dioxide evolution in the development of H. sativum was the same for the three substrata tried even though they differed widely in their available energy content. However, it was thought that in certain cases the period of 24 hours of aspiration might be too long to reveal any differences in time of maximum carbon dioxide evolution. In addition, some forms of organic materials which might be added to soil might differ in their immediate availability. In view of this, therefore, experiments were planned in which three sets of nine flasks representing periods of incubation of 3, 4 and 5 days were used. Not only did this take care of possible variations in the period of maximum carbon dioxide evolution as influenced by the substratum, but also it afforded a means of obtaining a number of replications, since any difference that was consistently present in each of the three sets could be considered as significant.

Treatment of cultures prior to aspiration. The incubation period allowed for fungal development made it possible for carbon dioxide to accumulate in each culture flask. To remove this, air was passed over the soil for one hour before the aspiration period for determining the carbon dioxide evolved was begun. The carbon dioxide content of the soil and air was considered then to be equal in all the culture flasks.

To determine whether, in the process of incubation, the formation and possible accumulation of carbon dioxide reduced the activity of Helminthosporium sativum, experiments were conducted, the results of which showed no appreciable difference. The variations between duplicate flasks that were aerated continuously for four days and those not aerated were as great as between the treatments. Gainey (13) reported that discontinuous aeration resulted in reducing the rate of carbon dioxide evolution. However, it was assumed in the present studies that, if any effect at all was present, it was small and the cultures would be comparable since they were subjected to similar conditions.

Results

1. Effect of Alberta soil types on the activity of Helminthosporium sativum.

a. Soil alone. Alberta soil types differ in chemical as well as in biological properties. Wyatt et al (46, 47) have reported differences in the average chemical compositions, while Bedford (2) has reported biological differences. An analysis of the three soils used in these studies is given in Table X and represent comparisons of the organic matter and nitrogen contents.

Table X

Results of a chemical analysis of Alberta soil types

Soil type	O.M. %	N %	O.M./N.
Black	13.6	0.39	34.9
Brown	5.6	0.19	29.5
Podsol	3.2	0.12	26.7

O.M. = organic matter,   N = nitrogen.

Organic matter was determined by the heat combustion method, while nitrogen was determined by Gunning's Kjeldahl method. The data show that the organic matter is highest in black soil and least in podsol soil, and that nitrogen holds a similar relationship. The ratio of O.M./N apparently is greatest in black and least in podsol soils.

To determine the relative value of the different soil types as substrata for the growth of H. sativum, the fungus was grown on them following sterilization, and its activity measured after six periods of incubation by determining the CO_2 evolved in respiration. The results are shown in Table XI and graphed in Figure 2.

The results obtained indicate a greater activity in both black and brown soils over the period studied than in the podsol soil. Other experiments, similarly conducted, showed essentially the same relationship.

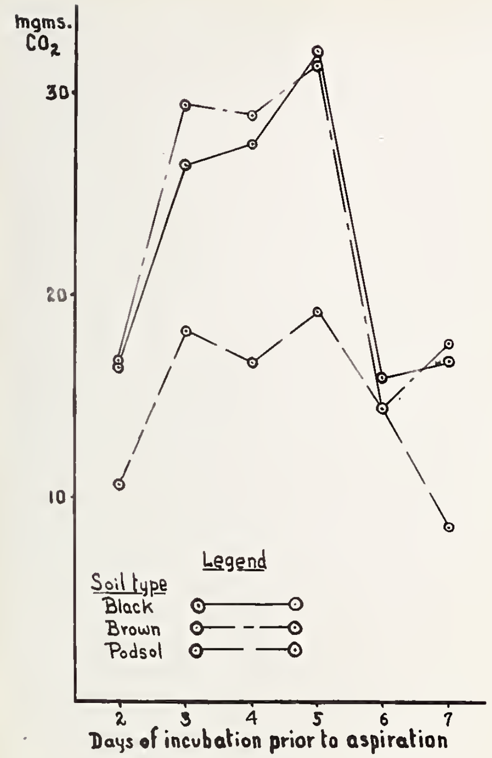

Figure 2. Respiration rate of H. sativum on Alberta soil types after different periods of incubation.

Table XI

Activity of H. sativum on three soil types as measured by the amounts of CO_2 evolved

Days of incubation prior to aspiration	Mgms. CO_2 in 24 hrs. aspiration Soil types Black	Brown	Podsol
2	16.4	16.8	10.7
3	26.5	29.4	18.3
4	27.5	28.9	16.8
5	32.1	31.4	19.3
6	16.0	14.5	14.5
7	16.9	17.8	8.7
Total	135.4	138.8	88.3

Apparently there is no definite relation between the O.M. - nitrogen ratio and the carbon dioxide evolved, although black soil with a higher ratio gave rise to greater activity than did podsol soil with a much lower ratio. In an endeavor to find a reason for this behavior, Table XII was prepared in which the total carbon dioxide evolved from each soil was reduced to a unit basis of the organic matter - nitrogen ratio.

Table XII

Relation of CO_2 evolved by H. sativum to the organic matter-nitrogen ratio of the soil types

	Soil types Black	Brown	Podsol
Total CO_2 (Table XI)	135.4	138.8	88.3
O.M./ N (Table X)	34.9	29.5	26.7
CO_2/O.M./N	3.88	4.71	3.31

The data indicate that the organic material in podsol soil in less suited to the development of the organism than is that in either the brown or black soils, and since its quantity in the soil is much less, the development of the organism is, therefore, considerably reduced. Comparing the brown and black soils, the relative activity of H. sativum is greater in the brown soil per unit of organic matter-nitrogen ratio. However, since the quantity of organic matter is much higher in black soils, the actual amount of available energy apparently is as great as that in the brown soil.

Bedford (2) observed a somewhat similar relationship to nitrification in both prairie and park belt soils and concluded that to compare the relative fertility of soils, it was necessary to use the soil organic matter as a unit and not the soil as a whole.

In view of these results, therefore, it becomes evident that the development of H. sativum is at least dependent on two factors in these soils:

1. The relative availability of the organic material in the soil.

2. The quantity of such material in the soil.

b. Soil water extract. To determine whether the soil solution of each of these soil types influences the activity of H. sativum an experiment was made using Ophiobolus graminis for comparison.

To each of three soils was added tap water in the proportion by weight of 5 parts water : 1 part air dry soil (20). The liquid was then filtered through filter paper till clear. Soil extract agar was prepared by using 12.5 grams of washed agar agar to 1000 cc. of extract (12). The two were heated in the autoclave for 10 minutes, filtered and tubed (15 cc. to each test tube) and sterilized for 20 minutes at 15 lbs. pressure. The agar from the tubes was poured into petri dishes into which on hardening was introduced a piece of water agar bearing young mycelia of either fungus. After a period of incubation at room temperature, measurements were made of the diameter of the colonies. The average values for ten replicates are given in Table XIII.

Table XIII

Effect of soil extracts of three soil types on the growth of *H. sativum* and *Ophiobolus graminis*

Fungus	Time of incubation days	Diameter of colonies in mm. on extracts of			
		Black soil	Brown soil	Podsol soil	H_2O check
Helminthosporium sativum	5	69	71	74	71
Ophiobolus graminis	12	81	77	78	66

In the case of *H. sativum*, the diameters of the colonies on the different soil extract agars show no definite increase or decrease in growth over those of the check on water agar. This would indicate that substances in the soil solution do not materially affect the development of this fungus. Slight differences, however, in response existed amongst the soils, podsol soil extract apparently being a more favorable medium for growth than either the black or the brown soil extracts.

The data for *O. graminis*, on the other hand, show a marked beneficial effect of the soil extracts as compared with water. This is in sharp contrast to that of *H. sativum*, indicating possibly, that the beneficial effects of the soil extracts depend on the nutritional requirements of the organism. This is further indicated by the differences in the soil extracts as to which produces the greatest beneficial effect. Podsol soil extract agar is more suitable to the growth of *H. sativum* than black soil extract agar, while the reverse is true for *O. graminis*.

2. Effect of plant residues on the development of *Helminthosporium sativum*.

Foot- and root-rotting fungi of cereals as pathogenes are at present known to be confined mainly to the grass family. Of the cereals, oats appear to be the least affected, while wheat is most severely attacked. Simmonds (40) in a survey made in Saskatchewan during the

In the case of *P. [illegible]*, the differences in the colonies on the different soil extract agars were in terms of increase or decrease in growth over those on the check or water agar. This would indicate that substances in the soil solution do not radically affect the development of this fungus. Slight differences, however, do appear which suggest that some of the soils [illegible] more favorable media for growth than others [illegible] brown soil extracts.

The data for *P. [illegible]*, on the other hand, show a marked beneficial effect of the soil extracts as compared with water. This is in marked contrast to that of *P. [illegible]*, [illegible] possibly, that the beneficial effects of the soil extracts depend on the nutritional requirements of the organism. This is further indicated by the differences in the soil extracts as to which produces the greatest beneficial effect. [illegible] soil extract seems to be more suitable to the growth of *P. [illegible]* than those with extract agar, while the reverse is true for *P. [illegible]*.

1. Effect of [illegible] on the development of [illegible] colonies.

Soil and non-sterile [illegible] of [illegible] [illegible] and [illegible] of [illegible] [illegible] [illegible] the [illegible]. [illegible] the [illegible], [illegible] [illegible] [illegible] least affected, while [illegible] [illegible] [illegible] extract [illegible].

years 1925, 1926 and 1927, reported no instance of oat plants being affected by *Ophiobolus graminis*. Pot experiments showed that wheat, oats and barley could be readily attacked by *Fusarium* spp., but oats were the least affected, if at all, by various strains of *H. sativum*, and in no instance did it appear affected by *O. graminis*. Experiments with the latter organism showed that the order beginning with the most resistant was oats, rye, barley and wheat. Oats in crop rotations have generally been observed to reduce the foot- and root-rot damage to wheat, while wheat, barley and rye tend to increase it.

The relation of certain grasses to these diseases has recently been discussed by Padwick and Henry (31). It is pointed out that the kind of grass preceding wheat may determine in large measure the severity with which the wheat crop is affected with foot- and root-rot diseases. Brome grass (*Bromus inermis*) and western rye grass (*Agropyron tenerum*) are reported as readily attacked by *H. sativum* and *O. graminis* and to a lesser extent by *F. graminearum*. Western rye grass in particular and also brome grass to an important extent, were found to result in marked increase in severity of "take-all" caused by *O. graminis* in the following wheat crop. It would be especially interesting to know if the residues of these crops favor the saprophytic development of the foot- and root-rotting fungi.

Of the non-grasses, flax, alfalfa and sweet clover, no report has been made of their relative resistance or susceptibility to cereal foot- and root-rotting fungi, though it is probably safe to assume that they are immune. In crop rotation studies, sweet clover has been observed (4) to reduce foot-rot infection in wheat, and flax has been noted to act in a similar manner.

a. Plant residues added to black soil. In order to study the relative effect of residues of these plants added to soil on the growth of *H. sativum* as measured by its respiration rate, the procedure was divided into a number of experiments. This was necessary since the respiration apparatus had only nine culture flasks. In a single experiment, therefore, three substrata each with two replicates could be tested, the remaining three flasks one for each substratum serving as checks. The fungus was of course not added to the latter.

The results shown in Table XIV represent carbon dioxide evolved, the amount produced by the check flask being subtracted. It will be seen that the results of the experiments are summarized in the table. The results are strictly comparable only within a single experiment and for the particular incubation period.

Table XIV

Activity of H. sativum on soil containing various plant residues as measured by CO_2 evolved

Exp. No.	One percent plant residue added to black soil	Mgms. CO_2 in 24 hrs.' aspiration Incubation period in days prior to aspiration						Total of averages	Relative CO_2 values	CO_2 index	Total difference between replicates	Total difference %	Variation index
		3		4		5							
		1	2	1	2	1	2						
1	None - check	29.3	32.3	37.8	35.2	25.6	23.3	92.8	65.1	1.00	7.9	8.52	0.13
	Wheat	48.4	48.8	51.4	52.6	38.1	42.7	141.0	100.0	1.54	6.2	4.40	0.07
	Cornmeal	305.6	303.3	343.3	337.3	248.0	244.3	891.0	631.8	9.71	12.1	1.36	0.02
2	Wheat	13.1	17.3	10.3	13.6	8.5	12.2	37.5	100.0	1.54	11.2	29.87	0.46
	Oats	13.0	12.2	14.2	11.0	9.5	8.7	34.3	91.5	1.49	4.8	13.99	0.26
	Barley	14.6	15.5	12.4	11.5	11.2	13.5	40.0	103.9	1.60	4.1	10.27	0.16
3	Western rye	25.4	23.6	21.6	19.1	16.2	18.3	62.1	208.2	3.20	6.4	10.30	0.16
	Brome	25.4	22.3	20.6	17.4	20.0	10.3	57.8	194.2	2.98	15.7	27.16	0.42
	Rye	9.8	14.6	8.2	10.7	7.9	11.0	31.1	104.3	1.60	10.4	33.44	0.51
4	Flax	49.7	36.1	23.4	33.9	48.1	53.6	122.4	81.5	1.25	29.6	24.18	0.37
	Alfalfa	96.0	93.6	95.4	96.6	103.2	97.5	291.2	193.9	2.98	9.3	3.19	0.05
	Sweet clover	43.3	47.4	46.1	55.7	58.0	64.0	157.2	104.8	1.60	19.7	12.53	0.19
5	Wheat	38.1	39.4	50.4	48.3	42.7	50.5	134.7	100.0	1.54	11.2	8.31	0.13
	Rye	44.4	35.1	48.9	47.8	53.1	51.6	140.4	104.3	1.60	12.0	8.55	0.13
	Sweet clover	37.7	37.3	47.7	46.7	56.1	56.7	141.1	104.8	1.60	2.0	1.42	0.02

In comparing these plant residues by their effect on the activity of H. sativum, it is necessary to assume that the relationship existing between plant residues in any experiment is the same even though the quantity of carbon dioxide evolved is different. With this in mind, experiment 5 was conducted wherein there was established the relation between wheat, rye and sweet clover. The relationships of the other residues were calculated using the ratio in experiment 5 as a basis.

To facilitate comparisons, the average figures for each incubation period were totalled. The value for wheat in each case was taken as 100, and those for the other residues were changed in proportion to the total carbon dioxide evolved. By dividing these figures by 65.1, carbon dioxide figures were obtained which showed the ratio of the carbon dioxide produced by plant residues in soil to that of soil alone taken as 1.00.

To obtain some value indicating the reliability of the carbon dioxide index, a method of computation was used which did not conform to any known method of statistical analysis. The differences between replicates of any treatment were totalled and expressed as a percentage of the total amount of carbon dioxide produced. The values obtained were then divided by 65.1 to be used as an indication of the variation existing between the replicates as reflected in the carbon dioxide index.

It will be seen from the data that the addition of the various plant residues including cornmeal to black soil greatly increased the activity of H. sativum over that in soil alone. Although the amount of plant residue added represented only 1% of the dry weight of the soil, sufficient energy was supplied to increase the activity of the fungus in all cases. However, the increase in activity is apparently determined by the kind of plant material incorporated in the soil, being greatest in the case of cornmeal and least in the case of flax. It is worthy of note that the grasses, western rye grass (Agropyron tenerum) and brome grass (Bromus inermis) resulted in a very marked increase in the activity of H. sativum and were only approached very closely by alfalfa.

No difference between the residues of the grain crops, if they existed, could be detected with the apparatus used. Although in some instances slight differences were obtained, definite conclusions could not be made in view of the variation index, although it might be pointed out that flax tended to be a less favorable substratum for the activity of H. sativum than did the other residues.

Apparently there exists no definite relationship between susceptibility of a plant to H. sativum and the suitability of its residue to the saprophytic development of the fungus. Of the legume crops, sweet clover held about

the same relative position as the grain crops. Alfalfa, however, is to be questioned since it represented material taken from a two year stand. When ground it had a tinge of green color, indicating that it possibly represented an immature crop. However, it is to be noted that it did occasion greater activity of the fungus.

Cornmeal caused a marked increase in activity, in fact 9.71 times greater than that in ordinary soil. This is important in view of the smaller increase in activity produced by plant residues. The latter possibly provide a poorer supply of readily available energy material.

b. Plant residue extracts. Since fungi in their nutrition require materials in solution (43) the presence of readily available energy material will result in immediate activity. The greater the concentration of such materials in solution, within limits, the greater will the response be to its presence. In contradistinction to this, the presence of toxic materials in solution acts in the opposite manner, the effect is one of retardation in activity. It is therefore easy to conceive that when both are present in the same solution, the response of a fungus will be conditioned by the relative importance of the two. In a medium rich in nutrients, the toxic substance may readily express itself, while in a medium poor in nutrients, the importance of the water soluble energy material may be overshadowed by the presence of the toxic substance or it may definitely express

itself. It is necessary, therefore, to postulate that both are operative, but that the expression of either is dependent upon the available energy supply to the fungus.

The method of studying the effect of plant residue extracts on the growth of H. sativum was similar to that used by Padwick (30) for Ophiobolus graminis, except that 200 cc. of distilled water was added to 20 grams of air dry plant material. The two media, potato dextrose agar and soil extract agar, and the method of adding extracts to media were also similar. The inoculum consisted of pieces of water agar (12.5 gms. agar + 1 l. water) of approximately equal size bearing mycelia of the fungus. In all, 10 replications were used. After five days of incubation at room temperature the diameters of the colonies were measured and are recorded in Table XV. Since it was thought that differences if any might be partly due to differences in the reaction, pH readings were made of the water extracts with a Quinhydrone calomel electrode and are recorded in the same table.

The main points brought out by the table are:

1. Water extracts of all the plant materials studied when added to potato dextrose agar, a medium rich in nutrient supply, resulted in a retardation in the growth of H. sativum.

Table XV

Effect of plant residue water extracts added to two types of media on the growth of <u>H</u>. <u>sativum</u> after 5 days' incubation

Plant residue extract	pH of extract	Potato dextrose agar		Soil extract agar	
		Diameter of colony in mm.	Increased diameter over check in mm.	Diameter of colony in mm.	Increased diameter over check in mm.
Wheat	7.1	57	-23	63	+2
Oats	7.1	61	-19	67	+6
Barley	7.4	72	- 8	65	+4
Rye	6.4	62	-18	56	-5
Flax	6.9	61	-19	55	-6
Brome grass	6.4	72	- 8	69	+8
Western rye grass	6.0	70	-10	68	+7
Sweet clover	7.0	59	-21	65	+4
Alfalfa	5.9	65	-15	66	+5
Wheat roots	6.4	72	- 8	62	+1
Oat roots	6.5	58	-22	62	+1
Western rye grass roots	6.8	69	-11	66	+5
Cornmeal	6.9	79	- 1	64	+3
Water - check	5.8	80	-	61	--

2. When, however, such extracts were added to soil extract agar, a medium poor in nutrient supply, increased growth resulted in all cases with the exceptions of flax and rye.

3. The development of <u>H</u>. <u>sativum</u> as conditioned by the water extracts was apparently dependent upon the presence of both a toxic substance, or substances, and soluble energy material.

4. That toxicity was not due entirely, if at all, to the pH of the extracts is indicated by the variations in values as compared amongst themselves and with water, and the diameter of the colonies.

5. The retarding effect of all plant material extracts does not apparently conform rigidly to the relative susceptibility of the various crops to attack by *H. sativum*. Oats and flax are considered to be relatively resistant, yet their residue extracts retarded growth to nearly the same degree as that of wheat.

Of the plants studied, however, there is a tendency for the water extracts of susceptible plants such as barley residue, brome grass residue, western rye grass residue and roots and wheat roots, to be less toxic to *H. sativum* than plants which are resistant. With root extracts of various plants, Padwick (31) did not obtain a similar tendency with *Ophiobolus graminis*.

6. Of the grain crops, least retardation to growth was exhibited by barley. The amount of retardation by wheat, oats, rye and flax was quite similar and approximately equal to that of sweet clover.

7. The nutritive value of the water extracts as determined on soil extract agar does not conform to the relative susceptibility of the plants to disease. Rye is known to be more readily attacked by the pathogene than oats, and yet there is quite a difference in the nutritive

values of their water extracts; that of rye being quite low, while that of oats is much higher.

8. There appears to be no definite relationship between the toxicity of the water extract and its nutritional value to H. sativum.

9. It is important to note the behavior of brome grass and western rye grass. Their water extracts showed low toxicity and high nutritive value. They can therefore serve as excellent substrata for the initial development of the fungus.

10. In general, the nutritive value of the plant extracts is not very great.

c. Relationships between plant residues in soil, extracts of plant residues and nitrogen content of plant residues, on the activity of H. sativum. Table XVI was prepared from data obtained from tables already given.

On comparing the activity of H. sativum on soil containing various plant residues with the nitrogen content of such residues, the data show a tendency for the two to coincide. Brome grass, western rye grass and alfalfa are all relatively rich in nitrogen and all resulted in increased activity of H. sativum. The other crop residues being comparatively low in nitrogen did not produce as great an increase in activity. The effect of cornmeal is extraordinary in that it contains a high content of starch which appears to be readily utilized as an energy source.

Table XVI

Development of H. sativum as influenced by the composition of plant residues

Plant residue	Nitrogen content in %	CO_2 produced in soil + residue/CO_2 produced in soil alone	Increase of growth in mm. in extract of residues over soil check
Check - black soil	0.39	1.00	--
Wheat	1.06	1.54	+2
Oats	0.59	1.49	+6
Barley	0.95	1.60	+4
Rye	0.78	1.60	-5
Flax	0.67	1.25	-6
Brome	1.28	2.98	+8
Western rye	1.62	3.20	+7
Sweet clover	0.68	1.60	+4
Alfalfa	1.84	2.98	+5
Cornmeal	1.41	9.71	+3

No relationship apparently exists between the effect of plant extracts on activity and nitrogen content of plant residues, since almost as great a stimulation was obtained with oats of a low nitrogen value as with western rye grass of a high nitrogen value. This is to be expected since the nitrogen in the plant materials is mostly of a protein nature and sparingly soluble in water.

The relationship between activity in soil plus plant materials and on the water extracts of such materials added to a solid medium poor in nutrients, is in most cases approximate and borders on being non-significant. Alfalfa and rye differ greatly in their nutritive value when applied

to soil and their water extracts hold a similar relationship. However, rye and sweet clover behaved differently in that wide differences were obtained only with the water extracts.

3. Effect of inorganic nitrogen compounds on the development of *Helminthosporium sativum*.

Field practices of applying commercial fertilizers to soils result in changes in the composition of the soil. The relation of such changes to the foot- and root-rot problem of wheat has received some attention, but has led to no definite conclusions. The possible effect of these compounds in altering the soil medium for the saprophytic development of the foot- and root-rotting fungi is a problem arising out of such practices.

Soils poor in fertility are generally deficient in nitrogen, though also phosphorus and potassium may be slightly reduced in quantity. The addition of nitrogen alone or in combination with the other two elements results in increased fertility as determined by crop yields. The effect of these when added to soil on the activity of *H. sativum* was studied.

A preliminary experiment was made to determine the relative ability of *H. sativum* to utilize nitrogen from various inorganic compounds.

a. *Availability of nitrogen from inorganic compounds to H. sativum*. Duggar's Solution for fungi was used for inorganic nitrogen comparisons as reported by Neal, Wester and Gunn (28). A stock solution devoid of nitrogen was prepared by mixing solutions of $\frac{M}{2}$ dextrose, $\frac{M}{4}$ KH_2PO_4, $\frac{M}{10}$ $MgSO_4.7H_2O$, $\frac{M}{1000}$ $FeCl_3.6H_2O$ in the proportion 12:5:2.5:0.05. Portions of 20 cc. each were then pipetted into 200 cc. erlenmeyer flasks and followed by 5 cc. of a nitrogen compound solution. The quantities of the various nitrogen compounds added were equivalent to 2.4 gms. of elemental nitrogen per litre. This was obtained by introducing 5 cc. from solutions of the following concentrations: $\frac{M}{2}(NH_4)_2CO_3.H_2O$, $\frac{M}{2}(NH_4)_2SO_4$, $\frac{M}{2}$ NH_4NO_3, $\frac{M}{2}(NH_4)_2HPO_4$, $\frac{M}{2}$ $Ca(NO_3)_2.4H_2O$, $\frac{M}{1}$ KNO_3, $\frac{M}{1}$ $NaNO_3$, $\frac{M}{1}$ $NaNO_2$. In the case of checks, 5 cc. of water were added. The flasks were plugged with cotton, covered with tin foil and sterilized in the Arnold steamer for one-half hour on three consecutive days. One cubic centimeter quantities of a suspension of *H. sativum* spores were introduced and allowed to incubate at room temperature for three weeks. Three cultures of each nitrogen source for each incubation period were prepared. At the end of each week of incubation, the fungal growth of the three replicates of each nitrogen source was filtered on tarred filter paper and thoroughly washed down with distilled water. The mycelial mats were brought to constant weight by heating at 100°C.

Table XVII

Effect of the addition of inorganic nitrogen compounds to Duggar's solution to the development of *H. sativum* and pH of filtrates after 1, 2 and 3 weeks of incubation

Inorganic nitrogen source added to Duggar's solution	Total dry weights of fungal mats in mgms. after			pH value of uninoculated solution after sterilization	pH value of filtrates after		
	1 week	2 weeks	3 weeks		1 week	2 weeks	3 weeks
$(NH_4)_2CO_3 \cdot H_2O$	23	96	93	7.2	7.5	7.8	6.5
$(NH_4)_2SO_4$	60	219	239	4.9	3.2	3.0	2.8
$(NH_4)_2HPO_4$	615	1015	1210	7.0	6.0	5.9	5.5
NH_4NO_3	81	223	148*	4.2	3.6	3.0	2.9
$Ca(NO_3)_2 \cdot 4H_2O$	16	58	39	3.7	3.4	-+	4.0
KNO_3	78	141	286	4.2	5.1	5.5	5.7
$NaNO_3$	58	103	245	4.4	4.8	5.2	5.6
$NaNO_2$	3	6	1	5.3	5.6	5.7	6.0
H_2O - check	11	10	10	-+	4.6	4.4	4.0

* One flask did not support as good growth as the other two.

\+ Filtrate lost.

Hydrogen-ion determinations by the Quinhydrone calomel electrode were made of the filtrates after each incubation period, and of the liquid prior to inoculation but after sterilization. The results obtained are shown in Table XVII and graphed in Figure 3.

The data show that $(NH_4)_2HPO_4$ caused the greatest development over the whole period of three weeks (not graphed in Figure 3), while $NaNO_2$ supported the least growth. Since the growth with $NaNO_2$ was slightly less than that for the water check, it appears that the nitrite ion is unavailable or possibly toxic. In regard to the solution of $(NH_4)_2HPO_4$ it was noticed that sterilization changed the color of the solution from a colorless to a brown. This effect may have been due to carmelization of the sugars, the carbon source in the solution. It is also possible that the $(NH_4)_2HPO_4$ itself may have been changed.

By tracing the trends of the mycelial weights and the pH values of the filtrates, an indication is obtained as to the relative availability of the nitrogen sources under the conditions of the experiment. Apparently the nitrate radicals in KNO_3 and $NaNO_3$ are less ready sources than are the ammonium groups in $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$ and NH_4NO_3 in the early stages of fungal development. As the acid radicals accumulated from the ammonium compounds, the acidity apparently increased to a sufficient extent to

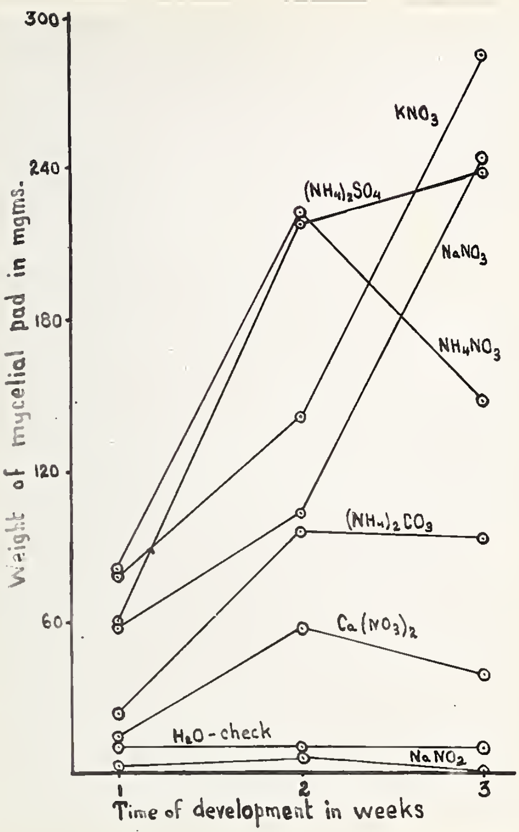

Figure 3. Effect of inorganic nitrogen compounds on the development of *H. sativum*.

$(NH_4)_2HPO_4$ curve not shown)

result in reduced development. However, in the cases of the nitrate compounds, KNO_3 and $NaNO_3$, the acidity decreased towards a probably more favorable value. The continued increase in acid reaction in the presence of NH_4NO_3 probably indicates a preferential selection of the ammonium cation over the nitrate anion. The reaction of the medium containing $(NH_4)_2HPO_4$ being almost neutral, provided not only a ready source of nitrogen, but also a favorable pH for development.

Ammonium carbonate in solution decomposes at approximately 85°C. The reaction being alkaline, ammonia is liberated which according to Klotz (23) can either remain as NH_4OH or be precipitated as $MgNH_4PO_4.6H_2O$. Observations of the cultures inoculated with <u>H. sativum</u> revealed white particles on the bottom of the flasks, presumably the precipitate. Since no fungal growth was visible it is concluded that ammonia is toxic to <u>H. sativum</u> at the concentration present. The results of experiments with ammonia gas on growing fungal cultures tend to support this conclusion. Somewhat similar toxicity has been observed by others to hold for <u>Phymatotrichum omnivorum</u> (28).

The failure of rapid development in the presence of $Ca(NO_3)_2$ cannot entirely be explained since to <u>Phymatotrichum omnivorum</u> (28) it proved a ready source of nitrogen. Apparently nutritional differences exist between these two fungi.

b. Chemical compounds added to soil. A number of chemical compounds as fertilizers were added singly or in combination to black soil at the rate of 200 lb. per acre of an element. The effect on the development of *H. sativum* was determined by the CO_2 evolved in 24 hours. The results obtained are given in Table XVIII.

Table XVIII

Effect of the addition of various chemical compounds as sources of nitrogen on the activity of *H. sativum* as determined by the CO_2 evolved

Exp. No.	Chemical compounds added to black soil	CO_2 evolved in mgms. in 24 hrs. aspiration						Total of average CO_2 produced in 24 hrs.	Total deviation between replicates in %
		Incubation period in days prior to aspiration							
		3		4		5			
		1	2	1	2	1	2		
1	H_2O - check	34.2	33.3	36.5	36.0	13.8	35.8	189.6	12.3
	$(NH_4)_2SO_4$	32.8	34.6	35.5	32.8	37.6	35.8	209.1	3.0
	$CaH_4(PO_4)_2$	31.5	32.0	28.9	35.8	19.8	34.0	182.0	11.9
	K_2SO_4	17.7	36.0	37.8	33.1	42.2	31.4	198.2	17.0
2	$(NH_4)_2SO_4$	73.7	62.8	25.4	38.2	19.4	29.3	248.8	13.3
	$(NH_4)_2SO_4$ + $CaH_4(PO_4)_2$	28.4	34.8	13.7	56.8	43.0	54.8	241.5	25.4
	$CaH_4(PO_4)_2$ + K_2SO_4	23.6	24.1	47.9	27.7	21.4	31.7	176.4	17.6
3	H_2O - check	14.7	14.5	10.9	33.2	17.4	23.3	114.0	24.9
	$(NH_4)_2SO_4$	62.1	15.1	11.7	18.7	36.1	18.1	161.8	44.5
	$(NH_4)_2HPO_4$	36.5	33.5	8.7	13.0	19.2	18.4	129.3	6.7
	KNO_3	13.4	27.1	29.2	18.3	16.5	20.3	124.8	22.8

The data show that in no case did the addition of chemicals at the rates used materially affect the development

of H. sativum. It is worthy of note that whereas $(NH_4)_2HPO_4$ produced much greater growth in the liquid culture experiment already described, the effect in soil was not shown when compared with the water check or with $(NH_4)_2SO_4$ or KNO_3. To determine whether the lack of any effect in these experiments was due to an insufficient amount of nitrogen added, a similar experiment was conducted with $(NH_4)_2SO_4$. Different quantities of inorganic nitrogen were added to black soil. The results obtained are shown in Table XIX.

Table XIX.

The effect of adding $(NH_4)_2SO_4$ to black soil in quantities to represent varying amounts of nitrogen on the activity of H. sativum as measured by the CO_2 evolved

Amount nitrogen added to black soil in %	CO_2 evolved in mgms. in 24 hrs. aspiration						Total of average CO_2	Total difference between replicates %
	Incubation period in days prior to aspriation							
	3		4		5			
	1	2	1	2	1	2		
0.01	27.8	25.8	49.4	26.8	27.2	25.8	91.4	28.4
0.02	30.8	27.6	25.0	26.1	14.8	17.4	70.9	9.7
0.05	28.6	26.7	28.6	26.3	10.5	19.1	69.9	18.3
0.20	29.3	31.8	30.5	34.8	28.9	21.0	88.1	16.7

The data show no significant difference with inorganic nitrogen applications. These results therefore tend to indicate that the quantities of elemental nitrogen

at least are present in sufficient quantities in black soil for the growth of H. sativum in bare soil. Additions of phosphorus and potassium did not result in any significant increase in growth whether used alone or in combinations with each other or with nitrogen.

Since soil analysis (Table X) showed reduced nitrogen content in black, brown and podsol soils in the order named, it was thought that by adding nitrogen in the form of $(NH_4)_2SO_4$, the differences in growth of Helminthosporium sativum in the three soil types might be overcome. Quantities of $(NH_4)_2SO_4$ were therefore added to the three soil types equivalent to the ordinary fertilizing practices; that is, quantities equivalent to 200 lb. of nitrogen per acre. Since in previous experiments the addition of $(NH_4)_2SO_4$ did not affect the growth of H. sativum in black soil, it was thought that a similar practice applied to other soils might result in a noticeable effect. The results of an experiment to test this are given in Table XX.

Table XX

Effect of addition of $(NH_4)_2SO_4$ to soil types on the activity of H. sativum as measured by the CO_2 evolved

Soil type	CO_2 evolved in 24 hrs. aspiration Incubation period in days prior to aspiration						Total of average CO_2	Total difference between replicates
	3		4		5			
	1	2	1	2	1	2		%
Black	39.0	34.6	33.9	23.4	30.9	26.6	94.2	31.0
Brown	58.2	33.0	35.0	34.3	29.3	34.4	112.1	27.7
Podsol	40.2	12.5	24.3	26.3	21.9	25.6	75.4	44.3

The addition of $(NH_4)_2SO_4$ did not affect the relationship existing in the soil types as to their suitability for the growth of H. sativum.

In general the results obtained from the studies on the addition of chemical compounds to soil tend to point to the conclusion that the ordinary practices of adding chemical fertilizers to the field do not result in the increased activity of H. sativum as a saprophyte in the soil. In fact, the stimulation in numbers of other micro-organisms and in their activity by the addition of fertilizers would probably result in a tendency to reduce the activity of H. sativum by virtue of the suppressive action of the soil microflora and fauna.

4. Available energy source as a factor in the activity of Helminthosporium sativum.

From the experiments already discussed it becomes evident that a much greater effect is produced on the activity of H. sativum by the addition of organic matter to the soil than by the addition of inorganic chemical compounds as fertilizers. The organic matter of the natural soil is in a reduced condition and consists of resistant materials (26, 41) not readily utilized as a nutrient source by fungi. The addition of inorganic chemical fertilizers apparently does not increase the availability of this energy source, whereas the addition of organic matter in the form of crop residues provides a ready source.

The differences existing in the three soil types on the activity of H. sativum would suggest differences in the immediately available carbohydrates. This is supported in part by the lack of any effect produced by the addition of $(NH_4)_2SO_4$ as fertilizer. To test further the possibility of the important part played by the available energy source in the development of the organism, the following experiments were conducted:

(a) Relation between decomposed and undecomposed plant residues on the activity of H. sativum. The effect of decomposed plant materials was tested as to their ability to affect the growth of the fungus. To black soil was added 1% quantities of a number of plant residues and the mixtures were placed in glass fruit jars, in 400 gram quantities. Moisture was brought up to 70% of the water holding capacity of the mixture and they were then allowed to stand at room temperature. From time to time the mixtures were stirred and water was added to the 70% value. After a period of three months the contents of each jar was spread out and allowed to dry. Each lot was divided in two and placed in separate culture flasks. At the same time soil mixtures were prepared containing undecomposed plant residue. After the mixtures were sterilized, H. sativum was grown on them as in previous experiments and its activity measured by the CO_2 evolved.

The results obtained are given in Table XXI and are comparable only within an experiment.

Table XXI

Effect of decomposed organic matter in black soil on the activity of *H. sativum*

Exp. No.	Plant residue added to black soil	Mgms. CO_2 produced after 4 days' incubation			
		Treatment of plant residue			
		Decomposed		Undecomposed	
		1*	2*	1	2
1	None - check	27.0	36.5	29.2	30.0
	Western rye	21.4	32.2	100.7	95.6
2	Sweet clover	48.0	45.4	69.0	64.8
	Oats	45.7	36.8	64.2	58.7
3	Wheat	47.0	41.9	53.5	54.9
	Alfalfa	38.8	35.4	74.6	77.4

* Replicates

In every case the data show that *H. sativum* was more active in soils containing undecomposed plant residues than in soils containing plant residues that had been acted upon by soil micro-organisms for a period of three months. This is more evident when comparison is made with the results obtained from the checks of ordinary soil with no organic matter added. The effect apparently is due to a reduction in quantity of available food material essential to the rapid development of the fungus. However,

the rapidity with which such materials are reduced apparently varies with kind of plant (26, 41). Western rye grass residue shows a comparatively rapid reduction to a level equal to that of untreated soil. Sweet clover residue, however, appears to be slightly more resistant to decomposition than oat residue, and in the same way wheat appears to be more resistant than alfalfa. It appears, therefore, that available food material is essential to rapid development of _H. sativum_; and the quantity supplied by a soil will depend on the particular organic matter it contains and the stage of decomposition in which it is.

(b) _Relation between addition of carbohydrate source and nitrogen source to soil on the activity of H. sativum_. To demonstrate that the addition of a carbohydrate source in a soil is more important to development than is a nitrogen source, an experiment was conducted in which nitrogen and carbohydrates were added. $(NH_4)_2SO_4$ was used as a nitrogen source and was applied to black soil at the rate of 0.01% N. Dextrose and sucrose were applied as carbohydrate sources in quantities equimolar to $(NH_4)_2SO_4$. The results obtained are given in Table XXII.

Table XXII

Effect of equimolar concentrations of chemical compounds applied to black soil at the rate equivalent to 0.01% N on the activity of _H. sativum_

Chemical compounds added to black soil in equimolar concentrations	Mgms. CO_2 evolved Incubation period in days prior to aspiration						Total of average CO_2	Total difference between replicates %
	3		4		5			
	1	2	1	2	1	2		
H_2O - check	38.6	-*	38.7	40.0	22.2	30.0	104.0	8.7
$(NH_4)_2SO_4$	33.3	35.9	50.3	19.1	26.5	30.9	98.0	40.0
Dextrose	41.2	45.1	54.3	46.3	30.3	33.0	125.1	11.7
Sucrose	54.9	50.4	50.1	48.8	41.5	39.9	142.8	5.0

* Lost.

The data indicate that the addition of sugar either dextrose or sucrose to the soil provides a very valuable nutrient source for the development of _H. sativum_. Ammonium sulphate as a nitrogen source did not produce any definite effect on fungal activity. It is quite probable that the ordinary amount of nitrogen present in a soil is sufficient for the amount of carbohydrates available for fungal metabolism. The addition of nitrogen, therefore, will not increase the nutritive value of the soil, whereas a definite increase is had with the addition of available carbohydrates.

(c) _Addition of wheat residue to soil types on the activity of H. sativum._ Since the available food supply

in the form of carbohydrates plays such an important part in the development of H. sativum, the differences found in the nutritive value of the various soil types are probably not due to differences in nitrogen (Table XX) but more to differences in the organic matter content as to quantity and stage of decomposition. That this difference is overcome when undecomposed organic matter is added is shown quite clearly in Table XXIII.

Table XXIII

Effect of 1% wheat residue added to soil types on the activity of H. sativum

Soil type	Mgms. CO_2 evolved				Total of average CO_2
	Incubation period in days prior to aspiration				
	3		4		
	1	2	1	2	
Black	39.1	36.4	47.4	47.4	85.1
Brown	45.0	44.6	53.7	59.4	101.4
Podsol	46.6	42.4	49.0	51.3	94.6

The data show that growth in podsol soil is as great, if not greater, than in other soil types. The differences shown between soils might probably be due to development mainly taking place on the organic material and to a greater extent in the soils poor in nutritional value than in that richer in this respect.

In view of these results, therefore, the differences existing between the soils in their nutritive value for H. sativum are apparently due to the weathering conditions under which the soils were formed. It is believed that, if plant material was added to the three soil types and decomposition was allowed to proceed under similar conditions, the differences existing between the soils in their nutritive value to H. sativum would disappear. The low nutritive value of podsol soil is believed to be due to the excessive leaching to which it has been subjected resulting in a marked reduction in the surface layers quantities of available nutrient. This effect does not take place to as great an extent under black and brown soil conditions.

In addition, the type of vegetation which becomes incorporated in the soil would be expected also to have a marked influence on its value as an energy source and the rate at which it is decomposed (26, 41). Podsol soil organic matter is mostly from dead tree leaves and decaying wood, while that in the brown soils is of grass origin. Black soil organic matter is somewhat intermediate in that the vegetation is of a park belt character.

PART II. INFLUENCE OF SOIL COMPOSITION ON THE SPORULATION OF HELMINTHOSPORIUM SATIVUM

Spores of pathogenic fungi are of importance in a number of ways, e.g. in reproduction, dissemination, inoculation and survival. Henry (19) has observed the ability of H. sativum as a saprophyte to sporulate in sterilized soil and has pointed out differences that soils induced in the number of spores formed. In view of this, experiments were made on the effect of soils, organic matter and chemical compounds added to soil, on the sporulation of the fungus under sterilized conditions.

The method employed was essentially similar to that of Henry except that water was added to 70% of the water-holding capacity of the soil. Plant residues were added at the rate of 1% of the dry weight of the soil, and chemical compounds at the rate of 200 lbs. of each element to the acre. The results obtained after allowing four weeks for the organism to develop at 23°C. are shown in Table XXIV and represent the relative number of spores obtained from a water suspension of soil taken at a distance one inch away from the piece of water agar bearing the organism. The spore numbers represent the number of spores counted in 0.1 cc. of the suspension and multiplied by 10.

Table XXIV

Effect of different soils and soil treatments on the relative sporulation of <u>H. sativum</u> after 4 weeks' development

Soil type	Treatment of soil	Average spore number in each replicate		Average spore number of both replicates
		1	2	
Black	None	70	100	85
Brown	"	30	43	37
Podsol	"	30	32	31
Black	Wheat residue	431	537	484
Brown	" "	222	423	323
Podsol	" "	435	383	409
Black	Oat "	456	590	523
"	Barley "	590	517	554
"	Rye "	470	517	494
"	Flax "	953	*	953
"	Brome grass residue	445	336	391
"	W. rye grass "	290	460	375
"	Sweet clover residue	657	490	574
"	Alfalfa residue	490	658	574
"	Wheat roots	670	334	502
"	$(NH_4)_2CO_3$	0	*	0
Brown	"	5	0	3
Podsol	"	0	0	0
Black	$(NH_4)_2HPO_4$	0	0	0
"	$(NH_4)_2CO_3 + K_2HPO_4$	0	1	1
"	$(NH_4)_2SO_4$	3	18	11
"	$(NH_4)_2SO_4 + CaH_4(PO_4)_2$	21	3	12
"	$(NH_4)_2SO_4 + CaH_4(PO_4)_2 + K_2SO_4$	0	2	1

* Contaminated.

The table shows a number of important points.

1. The sporulation on soil alone is much lower than when organic matter is added in the form of undecomposed plant residues.

2. The addition of chemical compounds to soil did not result in an increase in sporulation.

3. Sporulation induced by the soil types is apparently greater on black soil than on either brown or podsol soils. Henry (19) pointed out a somewhat similar behavior.

4. Differences in sporulation induced by different plant residues added to soil could not be detected; the variations in counts of spores in one treatment were as great as between treatments.

5. The addition of wheat plant residues to the three soil types resulted in marked increase in sporulation in all, with no apparent difference between soil types.

PART III. INFLUENCE OF ALBERTA SOIL TYPES ON WHEAT SEEDLING INFECTION CAUSED BY *HELMINTHOSPORIUM SATIVUM* AND *FUSARIUM GRAMINEARUM*

The composition of the soil influences the saprophytic behavior of the foot- and root-rotting fungi, it influences the development and composition of the higher plants, and it might therefore be expected to influence the expression of the disease condition occasioned by their interaction. Sanford (38) reported that the severity of the take-all disease of wheat was much greater on the black soil

than on the brown. Christensen (6) observed that the extent to which wheat and barley were affected by H. sativum depended upon the amount of inoculum and the host reaction as influenced by soil environment such as soil type, amount of available plant food. He considered that plants grown on peat and sandy soils were more likely to be attacked because they are weaker than those growing on better soils. However, not all plant diseases are affected in this manner (25).

In an endeavor to determine whether the differences in the composition of Alberta soil types would affect wheat seedling infection by H. sativum and F. graminearum, two experiments were made. In one approximately equal quantities of water were added to the soil types, and in the other water was added to 70% of the moisture holding capacity of each.

Method

The soils were used just as they came from the field except for screening which removed the large lumps. Moisture was added prior to three hours of steam sterilization to facilitate subsequent easy imbibition of water.

Marquis wheat, grown the previous year, was used as seed. The method of seed inoculation with a spore suspension obtained from two week old cultures as recommended

by Sallans (37) was used. The seed was dried in a desiccator over $CaCl_2$ prior to planting.

The disease ratings given to plants were divided into two parts, root infection and foot infection. The infections on both were divided into four classes, namely, no infection, slight, medium and heavy infection, and designated as 0, 1, 2, 3 respectively.

1. Approximately equal moisture added to soils.

The soils were placed in five inch flower pots and one-half the number were sterilized. After planting, sand was used to cover the surface of soil to reduce hardening of the surface from baking. Moisture was added from day to day to pots of soil that appeared dry on the surface. After three weeks, the plants were removed and rated for disease infection. The results obtained are given in Table XXV.

The data indicate that with *H. sativum* and *F. graminearum* on sterilized and unsterilized soil, greater infection was present on both foot and roots of wheat seedlings grown in podsol soil than in black soil. The apparent effect of brown and black soil on the seedlings was the same.

Table XXV

Influence of soil types on wheat seedling infection on sterilized and unsterilized soils given approximately equal quantities of water

Soil type	Soil treatment	Fungus	Percent emergence	Av. height of plants in cms.	Av. per-cent degree infection Foot	Root
Black	Sterilized	Check - none	91.2	40.7	5.0	0.3
"	Unsterilized	"	70.4	29.4	2.7	0.7
Brown	Sterilized	"	88.0	35.2	5.7	0.0
"	Unsterilized	"	83.2	30.0	11.5	0.0
Podsol	Sterilized	"	78.4	33.7	3.0	1.2
"	Unsterilized	"	90.4	26.7	2.4	0.0
Black	Sterilized	<u>H</u>. <u>sativum</u>	78.4	38.4	46.1	32.6
"	Unsterilized	"	56.8	31.8	22.0	1.0
Brown	Sterilized	"	74.4	36.4	51.7	53.0
"	Unsterilized	"	88.8	28.6	25.8	4.2
Podsol	Sterilized	"	47.2	28.5	56.7	55.8
"	Unsterilized	"	76.8	30.6	47.7	17.8
Black	Sterilized	<u>F</u>. <u>graminearum</u>	45.6	28.9	49.7	39.1
"	Unsterilized	"	40.0	32.7	10.9	1.2
Brown	Sterilized	"	64.8	29.6	47.1	35.8
"	Unsterilized	"	82.4	30.9	19.5	2.8
Podsol	Sterilized	"	28.0	25.1	76.4	65.9
"	Unsterilized	"	73.6	31.5	35.4	33.4

2. <u>Moisture added to 70% water-holding capacity of soil</u>.

The soils were introduced into gallon crocks and sterilized. Subsequent treatment was as in the previous experiment except that water was added to the 70% water-holding capacity of each soil. The results obtained are given in Table XXVI.

Table XXVI

Influence of soil types on wheat seedling infection on sterilized soils given water to 70% of water-holding capacity

Soil type	Soil treatment	Fungus	Percent emergence	Av. height of plants in cms.	Av. percent degree infection Foot	Av. percent degree infection Root
Black	Sterilized	Check - none	82.0	33.5	1.6	1.1
Brown	"	"	90.7	37.9	0.2	0.5
Podsol	"	"	88.7	35.7	1.7	0.5
Black	"	<u>H. sativum</u>	52.0	31.7	50.5	40.0
Brown	"	"	80.6	34.7	49.4	49.3
Podsol	"	"	76.0	34.0	49.6	48.0
Black	"	<u>F. graminearum</u>	64.0	32.4	28.2	32.8
Brown	"	"	71.4	35.3	26.7	26.9
Podsol	"	"	62.7	41.3	20.4	22.0

The data indicate that under these conditions the different soils induced no marked differences in the infection of the seedlings.

Discussion of Results

Differences in the severity of infection in the first experiment can be explained partly by the differences in the moisture present in the soils. Sallans (36) could obtain no differences in infection on wheat by <u>H. sativum</u> at soil moistures ranging from 40 to 80% of the water-holding capacity. Dosdall (10), however, obtained greatest infection

at both maximum and minimum amounts of moisture in the soil and considered this as influencing the general health of the plants and predisposing them to disease. She attributed the differences in infection obtained in different soils to differences in moisture content.

The soils used in these experiments varied in their moisture-holding capacity, podsol soil having the least, and black soil having the greatest water-holding capacity. The addition of approximately equal amounts of water resulted in an increased water condition with the decrease in the water-holding capacity of the soil. In addition, the reduction in aeration and in the alteration of the physical structure of the soil increased the suitability of the medium for the development of the plant. This is brought out by the differences in the height of the plants on the different soils in the check series.

The experiment in which equal amounts of water were applied to different soils produced wheat seedlings which were shorter where grown in podsol soil and taller where grown in black soil. In contrast to this, the plants in the second experiment in which the moisture in the soil was maintained at 70% of the water-holding capacity, were approximately equal in height regardless of the soil type.

The lack of differences in infection of the plants grown on the different soils in the last experiment could be explained in several ways: For instance, in the

method of seed inoculation the quantity of spores as inoculum introduced may have been too great to show any differences; the moisture content of the soil may not have been kept at the proper value for the soils to act as differentials; or the differences in the chemical composition of the soils may not have been great enough to reflect themselves in the composition of the seedlings and in their consequent reaction to the pathogenes.

GENERAL DISCUSSION

The development of fungi in the soil as well as in culture, is dependent upon the nutritive value of the substratum in which it grows. Carbon and nitrogen are necessary for their proper development (16) as well as many other elements (43).

The role of carbon as an energy source has been established and is discussed by Waksman (43). Carbohydrates from the simple forms to the more complex can be used, depending upon their immediate availability to the fungus. In the case of Helminthosporium sativum (42) maltose supports a greater growth than xylose. The presence of organic matter in the soil provides a variety of energy materials. Peterson et al (32) observed that of 25 species of molds studied, 16 were able to ferment the pentose sugars,

xylose and arabinose. Later (39) the same authors observed the depletion of pentosans in plant materials during their decomposition in the soil. Norman (29) in a critical study of the decomposition of rye and oat straws concluded that the pentose units and xylan did not play an important part in the total decomposition since they are associated with the hemicelluloses and celluloses, and their rate of loss is superficial and dependent upon the fractions that carry them.

Cellulose has been found to be a ready source of energy in decomposing plant material as well as its closely allied forms, the hemicelluloses (29, 41, 45). Norman (29) observed that with oat and rye straws after a period of 24 days of decomposition, of the total loss of organic matter, approximately 50% was represented by cellulose. Hemicelluloses were found to be decreased in quantity much more rapidly in the early stages of decomposition but soon were surpassed by cellulose.

In discussing the energy sources in plant materials Norman (29) attributed greatest importance to the cellulose fractions, with secondary importance to the hemicelluloses. The lignin fractions are considered as unavailable to ordinary microorganisms and function as barriers to the readily available sources of energy. The importance, however, of hemicelluloses in the early stages of decomposition is clearly realized, for he states:

> "Together with the soluble components of the tissue, such as sugars and starches, they play an important part in the provision of easily obtainable energy, and in consequence promote the rapid growth and multiplication of the organisms in the early stages, thereby paving the way for the general attack on the cellulose. Only to the extent of hastening the incidence of the rapid breakdown of cellulose can the hemicelluloses be said to control the decomposition."

Waksman and Tenny (45), Tenny and Waksman (41) and Martin (26) also have observed in the decomposition of plant material in the soil that the water soluble fractions disappeared most rapidly, followed by the hemicelluloses and then more rapidly by the cellulose fraction. The lignin fraction being relatively resistant, tended to accumulate, while the crude proteins, being synthesized by the developing organisms from the decomposing plant material, also tended to accumulate.

The importance of nitrogen to fungi is generally recognized and appears to function in growth and metabolism, and in the liberations of energy from carbonaceous material (7, 16). The relationship existing between fungal activity and carbon and nitrogen is close, the relative abundance of the latter (C-N ratio) influencing the activity of the former. It has been estimated that from 30 to 50 parts of energy material in the form of cellulose is utilized for every part of nitrogen. Heck (16) has demonstrated with <u>Aspergillus oryzeae</u> that the C-N ratio of a medium influences not only the amount of fungus material produced but also its composition. He

showed that the total carbon of the mycelium remained relatively constant (40-44 percent) in media of C-N values of 6:1, 30:1 and 150:1. However, the C-N ratio of the mycelia were 20.9:1, 10.7:1 and 6.3:1 respectively, the differences being due to the total nitrogen in the tissues. The amount of mycelium produced on a medium of C-N value of 150 was approximately half that produced on the medium of 30:1 ratio. There was no significant difference between media of 30:1 and 6:1 C/N values. On examining fungi from the field he found that, while the carbon content of the mycelium remained relatively constant, the nitrogen values varied from 1.5 to 7%. The importance of these variations is brought out by him in the following excerpt:

> "The energy nitrogen ratio of the substrate is the determining factor, both in the quantity of mycelium produced and the amount of nitrogen it contains. As the available nitrogen in the substrate decreases, the nitrogen content of the mycelium decreases to about 2 or 3 percent. At this point a further decrease in the amount of nitrogen in the substrate causes a decrease in the weight of mycelial tissue, so that the nitrogen content of the tissue seldom falls below 2 percent. The decrease in the amount of fungous tissue produced begins when its carbon-nitrogen ratio reaches 10 or 12 to 1."

The importance of the energy-nitrogen ratio is undoubtedly applicable to the saprophytic development of *H. sativum*. The soil as a medium is poor in readily available energy materials although a sufficient quantity of nitrogen appears to be present. The addition of plant residue materials results in readily available food materials.

Development then apparently proceeds at a rate determined by the nature of the plant residue.

The establishment of the fungus on plant residues is dependent on the quantity of readily available material such as sugars, starches and hemicelluloses present, and on the presence of a ready nitrogen source. This, apparently, is reflected in the carbon dioxide given off by the fungus in respiration, and varies with different plant residues. The presence of these materials is important in view of the fact that they afford a means for the ready establishment of H. sativum on the plant residue, and the subsequent attack on the less available materials such as celluloses. It is in this respect that the carbon dioxide values obtained for the activity of the fungus are important. This becomes evident when it is recalled that the periods allowed for the development of cultures were from 3-5 days prior to aspiration.

In all of the studies reported it was of course necessary to use sterilized soil. This resulted in changes from the natural, in chemical as well as biological properties. The changes in chemical properties resulted in greater amounts of easily available nitrogenous materials (33, 34). While the organic matter decreases slightly, the ammonia, amino-acids, nitrates, and soluble non-protein nitrogen all increase in quantity. Changes were also expected to have taken place with the plant residue

material resulting in partial hydrolysis and the liberation of easily available energy materials.

Though the changes are not great, the suitability of the substratum will be slightly increased and will result in an increased activity of the fungus introduced (13). Despite these changes, however, the nutritive values of the various substrata are expected to maintain approximately their relative positions. In addition the relative suitability of substrata under sterilized conditions might be expected to be the same when not sterilized.

SUMMARY AND CONCLUSIONS

1. The activity of H. *sativum* as a soil saprophyte was studied using the carbon dioxide evolved in respiration as an index. Sterilized soil was used, and the activity studied as affected by soil type and by the addition of various crop residues and chemical compounds as fertilizers.

2. A respiration apparatus was constructed to measure the carbon dioxide given off and a method developed to study the activity of the fungus.

3. Podsol soil proved a less suitable substratum for H. *sativum* than either brown or black soils, the difference being attributed partly to the nature and partly to the quantity of the organic material present. Water

extracts of these soils did not greatly affect the growth of the organism on agar-agar.

4. Plant residues added to soils increased the activity of H. sativum grown on them in all cases, over that in soil alone, those of western rye grass and brome grass being the most favorable of any tested.

5. The relative values of the residues apparently are not correlated with disease resistance and susceptibility of the living plants.

6. Water extracts of the crop residues contain substances which are toxic and substances which are of nutritive value to H. sativum in agar-agar cultures. The toxicity was readily expressed on a medium rich in food supplies, while the nutritive value was expressed on a medium poor in food supplies to the fungus.

7. No apparent relationship existed between growth of H. sativum on soil extract agar containing plant residue extracts and the relative quantity of carbon dioxide evolved when grown on soil to which plant residues were added.

8. Increased activity of H. sativum was observed to roughly parallel increased nitrogen content of the plant residues.

9. In Duggar's solution nitrate and ammonium compounds were readily used as inorganic nitrogen sources. $NaNO_2$ and $(NH_4)_2CO_3$ did not support any visible growth of the fungus.

10. The addition of inorganic chemical compounds containing N, K and P singly, or in various combinations to soil did not result in a measurable increased activity of the fungus.

11. An energy source is important for the growth of *H. sativum* in the soil. Decomposed plant residues are less suitable energy sources than undecomposed residues. The suitability, however, varies with the nature of the plant residue material.

12. The addition of wheat residue to the three Alberta soil types, brown, black and podsol, removed the differences exhibited bytween them in their suitability as substrata for the growth of *H. sativum*.

13. Sporulation of *H. sativum* was greater on black soil than on either brown or podsol soils. The addition of plant residues greatly increased the number of spores produced. No differences, however, could be detected in the relative values of different plant residues. The addition of chemical compounds to soil as fertilizers did

not increase the number of spores produced over that in soil alone.

14. No significant differences were obtained in the severity of infection of wheat seedlings by H. sativum or F. graminearum due to soil types.

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