Studies on

the Pathogenicity of Species of Fusarium

A Thesis

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INTRODUCTION

The most widespread and important root disease of small grain crops in Western Canada is Helminthosporium - Fusarium Root Rot, or Common Root Rot. It is present every year throughout the grain-growing area of the three prairie provinces, and affects all cereal crops. This root disease may be caused by several different soil-inhabiting fungi although *Helminthosporium sativum* P.K. and B., *Fusarium culmorum* (W.G. Sm.) Sacc., and other species of *Fusarium*, are most commonly associated with it. These fungi live in the soil and attack the roots, crowns, and lower portions of the stem of cereal plants. Certain of these fungi are, however, also capable of attacking the leaves, heads and seeds of different grain crops, causing diseases which are commonly referred to as "leaf spots", "head blights" and "seed discolorations". The greatest loss, however, from these fungi usually occurs through damage to the underground parts of the plants.

Common root rot attacks both seedlings and mature plants. As a seedling blight it may be very destructive, killing off a large number of seedlings. Affected plants that survive the seedling stage usually lack vigour and fail to tiller properly so that stands of affected crops are usually thin and weedy. In addition to causing serious

direct losses in yield and grain quality, the disease is also responsible for very important indirect losses. The nature of the direct loss from common root rot is twofold. First, there is a loss in yield due to reduction in the number and size of kernels in the heads of grain; and second, a loss in grain quality due to the production of small shrunken kernels of inferior quality. It is important to point out that these inferior kernels, when sown, produce weak seedlings that are likely to be easily and seriously injured by root-rotting fungi inhabiting the soil.

The total actual loss due to common root rot is somewhat difficult to estimate. Judged by the results obtained in experimental plots at Winnipeg, Craigie (5) has estimated that the annual loss in wheat, oats, and barley is not less than five per cent. On a basis of five per cent loss in yield the annual cash loss to Manitoba alone for these three crops amounts to $2,470,000. According to Broadfoot (2) and Simmonds (31), similar important annual losses are caused by common root rot in Saskatchewan and Alberta. The investigations of Henry (19), Dosdall (8), Christensen (6) and McKinney (23), have demonstrated the great economic importance of root diseases in cereal crop production.

A large number of pure-culture isolations from the roots of cereal plants collected in various parts of the

three prairie provinces of Canada by Henry (19), Simmonds (31), Broadfoot (2), Greaney and Bailey (15) and Sanford and Broadfoot (29), showed that fungi belonging to the genus Fusarium were very widely and consistently associated with root rots in Western Canada. These investigators carried out extensive pathogenicity tests to determine whether species of Fusarium found associated with the roots of cereal plants were saprophytic, weakly parasitic, or capable of causing severe injury under certain environmental conditions. Sanford and Broadfoot (29) studied the relative pathogenicity of numerous isolates of Fusarium culmorum obtained from wheat roots and found that the majority of them were only very weakly pathogenic. Samuel and Greaney (27) and Greaney and Johnston (16) studied the progressive invasion of the roots and crowns of wheat plants by fungi and found that Fusarium culmorum, and other species of Fusarium, were commonly associated with wheat roots and generally present in the soil, although in a number of cases they exerted no appreciable parasitic affect on the wheat plant.

Gordon (12) has made a taxonomic study of the species of Fusarium found to be associated with diseased roots and stem bases of wheat, oats, barley and rye in Manitoba. A study of approximately 4,100 isolations of these fungi indicated that twelve species and nine varieties representing seven sections of the genus Fusarium were present.

Studies on the microflora of the soil of permanent grain plots made at Winnipeg in 1936 and 1937 indicated that fungi of the genus Fusarium constituted an appreciable percentage of the total soil fungus flora of grain plots. In 1936, 13,850 isolations of fungi were made from one series of plots and Fusarium spp. accounted for 1,498 of this number, or approximately 10.8 per cent. Species of Fusarium were found to constitute 18.2 per cent of the total fungus flora isolated from the same series of grain plots in 1937. According to Gordon (12) the various isolates obtained from the soil in 1936 and 1937 comprised many species, varieties and forms of Fusarium belonging to eight sections of the genus.

Owing to the fact that Fusarium culmorum and other species of Fusarium are so commonly associated with the roots of cereal plants and so generally present in the soil, it was considered essential that some attempt be made to determine their pathogenicity. It was therefore decided to carry out a comprehensive series of greenhouse and field tests to determine the relative parasitic behaviour of the predominating species, varieties and forms of Fusarium isolated from diseased parts of cereal plants and from the soil.

The antagonism of microorganisms in culture media, in the host plant, and in the soil has been observed for many years, but it was not until more recently that the full significance of this factor in the study of root

diseases came to be appreciated by plant pathologists and soil microbiologists. The importance of the reaction of one organism upon another, not only in culture, but also in the soil itself is now well established. A review of the literature on the subject has been made by Fawcett (9), Machacek (22), Greaney and Machacek (17), Wiendling (33), Sanford and Broadfoot (30). The relation of biological antagonism to infection by a cereal root-infecting fungi has been studied by Simmonds (31), Broadfoot (3), Bisby, James and Timonin (1), and Greaney and Machacek (17). Bisby, James, and Timonin showed that under greenhouse conditions Trichoderma lignorum suppressed the virulence of Fusarium culmorum and Helminthosporium sativum. Greaney and Machacek demonstrated that in pot cultures the pathogenicity of Helminthosporium sativum was suppressed by the antagonistic action of Cephalothecium roseum.

The importance of soil temperature in relation to cereal root-rotting fungi has been investigated by McKinney (24), Dickson (7), Sanford (28), Hynes (20), and others. The results of these studies indicate that soil temperature is an important factor in the development of seedling root rot. In some cases, infection was accelerated with an increase in soil temperature, in others depressed, depending on the host and fungus studied.

A number of investigators including Dosdall (8),

Henry (19), McKinney (24), Mitra (25), Dickson (7), and Hynes (20), have studied the influence of soil moisture upon infection of wheat seedlings by parasitic fungi. The results show that both soil moisture and temperature are variable in their effect upon the pathogenicity of cereal root-rotting fungi. Further investigations are necessary to determine the actual effect of soil moisture and temperature on the occurrence of severe outbreaks of root rot of wheat under field conditions in Western Canada.

II. OBJECTS OF INVESTIGATION

The principal objects of the present investigation were: (1) to determine the relative pathogenicity to wheat of five species of Fusarium, and a number of cultures of Fusarium culmorum originally isolated either from diseased parts of cereal plants and grasses or from grain soils; (2) to study the influence of soil moisture and temperature on the pathogenicity of certain species of Fusarium; and (3) to determine the effect of some common soil-inhabiting fungi on the pathogenicity of Fusarium culmorum and Fusarium redolens Wr.

III. PATHOGENICITY STUDIES

Greenhouse Experiments

The results of previous greenhouse tests of pathogenicity of cereal root-infecting fungi by Greaney and Bailey

(15), Greaney and Machacek (17), and others, have emphasized the importance, if reliable and reproducible results are to be expected, of controlling factors influencing the soil environment. In the present investigation, therefore, every effort was made to standardize the conditions under which the various greenhouse pot tests were made.

Material and Methods

Greenhouse pot tests were made to determine the relative pathogenicity of twenty-four cultures of Fusarium culmorum isolated from the diseased parts of cereal and grass plants. A list of these isolates, together with information concerning the date and source of their isolation is given in Table 1.

Inoculum of each isolate of F. culmorum was prepared by growing the fungus on a sterilized sand and cornmeal mixture for about 15 days. This medium gave excellent growth of all isolates and species of Fusarium. When completely overgrown with the sporulating mycelium of the fungus, the sand-cornmeal inoculum was mixed with autoclaved soil, one part of inoculum to 30 parts of soil by weight, and placed in 6-inch pots. All soil for a given experiment was prepared at the same time. In order to allow the various fungi to become well established in the soil to which they were added, three days were allowed

Table 1. Number and source of isolates of Fusarium culmorum

Isolate No.	Year isolated	Host	Location
38	1932	Durum wheat	Rhodes, Man.
1263	1935	Reward "	Bowsman, Man.
1040	"	Common "	Brandon, "
937	"	Durum "	Pilot Mound, "
856	"	Reward "	Whitewater, "
854	"	Barley	Emerson, "
1119	"	Oats	Melita, "
1280	"	Marquis "	Griswold, "
1300	"	" "	Pipestone, "
1193	"	Reward "	Foxwarren, "
1202	"	Marquis "	Shoal Lake, "
1158	"	" "	Binscarth, "
1111	"	Common "	Clearwater, "
1122	"	Durum "	Deloraine, "
1197	"	Ceres "	Virden, "
39	1932	Durum "	Rhodes, "
19 A	1930	Common "	Saskatoon, Sask.
19 B	"	" "	" "
19 C	"	Oats	" "
285	1936	Common "	" "
529	1933	Brome grass	Winnipeg, Man.
6	1936	Marquis wheat	Pope, "
15	"	Common "	New Norway, Alta.
10 E	"	" "	Rothamsted, England.

to elapse between the time of soil preparation and date of sowing of the grain.

The seed used was Marquis wheat, selected by hand for uniformity and size. It was surface sterilized by rinsing in 95 per cent ethyl alcohol, immersing for three minutes in 0.1 per cent mercuric chloride solution, rinsing in alcohol, and then washing in sterile water. Before sowing, the surface-sterilized seed was inoculated, where required, by dipping it into a suspension of spores and mycelial fragments of the species of Fusarium to be tested. The control seed was dipped in sterile water. Immediately after treatment, the seed was sown in the prepared soil.

In each fungus series there were 4 pots, twenty-five seeds being sown in each pot. The complete test with 24 organisms and 24 control pots of uninfested soil sown with uninoculated seed consisted of 120 pots. The test was replicated four times. During the course of each test uniform conditions of light, moisture and temperature prevailed. The moisture content of the soil was maintained at a uniform level by adding sterile water at two-day intervals to bring the pots up to their original weight. To minimize place effect, the pots of each test were completely randomized on a large bench in the centre of the greenhouse.

Germination was usually complete 12 days after sowing at which time the number of emerged plants per pot were counted. At the end of the experimental period (28 days), non-emerged plants as well as the young seedlings were

lifted from the pots, washed free of soil, examined individually, and the extent of injury due to pre-emergence blight, seedling blight and root-rot was recorded. The classes and numerical ratings used to record the intensity of disease infection on individual seedlings and the method of computing the disease rating that was used to express the extent of the disease on the plants in each series of pots are given in Table 2.

After the disease data had been secured, the green weight of the plants of each inoculated and uninoculated series was recorded. In all experiments herein reported, plant emergence, disease, and yield data were analyzed according to the procedure described by Fisher (10) as the analysis of variance. To estimate the odds of significance, however, the direct ratio of the variances (the F value of Snedecor (32)) was used. The methods used in all greenhouse experiments with young plants herein reported were essentially similar to these described above.

In addition to the seedling test of pathogenicity with various isolates of Fusarium culmorum, the parasitic behaviour of five different species of Fusarium to adult Marquis wheat plants was studied. The species of Fusarium tested were strain No. 38 of F. culmorum (W.G. Sm.) Sacc., F. redolens Wr., F. avenaceum Fr. (Sacc.), F. oxysporum Schl. v. aurantiacum (Lk.) Wr., and F. equiseti (Cda.) Sacc. The methods used in these tests were briefly as

Table 2. Classes and numerical ratings used to record the degree of infection caused by Fusarium culmorum on wheat.

Class	Degree of infection on individual plants	Numerical rating
1	No infection	0
2	Small, scattered necrotic lesions on sheath, subcrown internode, or roots.	1
3	Distinct dark lesions on basal parts, particularly on subcrown internode and roots.	2
4	Large necrotic lesions on crown, subcrown internode, and roots; with loss of plant vigour.	3
5	Severe rotting of basal parts; plant chlorotic, often stunted; some culms dead.	4
6	Plant destroyed after germination, but before emergence. Dead plant.	5

$$\text{Disease rating} = \frac{\text{Sum of numerical ratings of individual plants}}{\text{Number of plants} \times 5} \times 100$$

follows. Surface sterilized Marquis wheat was sown in sterile quartz sand. When the healthy seedlings were six days old they were transplanted from the sand to 6-inch pots of sterilized soil containing inoculum of the fungus to be tested. At the time of transplanting, the entire root system of each seedling was inoculated by dipping it into a water suspension of spores and mycelium fragments of the respective fungi. Five seedlings were placed in each pot and grown to maturity. After the plants had been transferred, they were inoculated at regular intervals during the growing period (at 10, 20, 50, and 70 days) by means of water suspensions of spores which were added to the soil of each pot.

A complete experiment consisted of five pots (25 plants) of each species of Fusarium and five uninoculated control pots. Soil moisture content was maintained at a constant level in all pots throughout the experiment. The complete experiment was carried out at two different times. At maturity, the intensity of disease on individual plants was recorded according to the methods described by Greaney, Machacek and Johnston (18).

Experimental Results

The data of the tests with isolates of F. culmorum were examined by the analysis of variance method (Table 3). The results of the analyses in Table 3 show that the twenty-four isolates of F. culmorum tested differ greatly in their

Table 3. Complete analyses of variance for percentage of plants emerged, disease rating, and green weight of young Marquis wheat plants.

Variance due to	Degrees of freedom	Sum of squares	Mean square	F	5% point
	Plant Emergence				
Replicates	3	4895.16	1631.72		
Isolates	29	15691.85	541.09	10.43	1.60
Error	87	4512.59	51.86		
Total	119	25099.60			
	Disease Rating				
Replicates	3	4292.65	1430.88		
Isolates	29	20816.39	717.80	15.79	1.60
Error	87	3954.81	45.45		
Total	119	29063.85			
	Green Weight				
Replicates	3	126840.66	42280.22		
Isolates	29	16537.68	570.26	7.19	1.60
Error	87	6900.72	79.31		
Total	119	150279.06			

pathogenic behaviour. In every instance, for plant emergence, disease rating and yield, the variance for organisms greatly exceeded the error variance. The complete results of the experiment, with the standard errors associated with the factors studied are summarized in Table 4.

It is evident from Table 4 that a wide variation exists in the virulence of the 24 strains of F. culmorum studied. On the basis of the standard error a significant necessary difference in disease rating between the organisms is 10.1. Using this as a basis for classification it was found that the various isolates of F. culmorum could be divided into three classes; namely, strong pathogenic, moderately pathogenic and weakly pathogenic. The great majority of those tested were only very weakly or weakly pathogenic to Marquis wheat. These results confirm the findings of Sanford and Broadfoot (29) who tested the comparative pathogenicity of some 219 cultures of F. culmorum isolated from the roots and crowns of wheat plants collected from widely separated fields in Alberta.

In the final analysis of the experiment, disease ratings and total green weight of the plants of the individual pot series were correlated. The significance of the correlation coefficient obtained was determined by the method described by Goulden (13). A highly significant negative coefficient of -0.9259 was obtained. This result substantiates the work of Livingstone (21) who studied the

Table 4. The relative pathogenicity of several isolates of *Fusarium culmorum* to Marquis wheat seedlings.

Fusarium culmorum isolate No.	Percentage of plants emerged	Degree of infection	Green weight of plants in grammes
38	47.0	64.0	42.6
1263	57.2	35.8	63.4
1040	59.2	25.8	62.4
937	59.8	35.9	60.9
856	60.2	20.6	67.6
854	61.2	24.6	66.0
10 E	61.8	27.8	68.5
1119	63.0	25.1	67.3
1280	63.0	24.4	70.0
1300	63.0	32.2	68.5
6	64.0	23.8	69.0
19 B	64.5	16.8	73.3
1193	65.0	21.1	66.7
1202	66.0	28.3	61.0
1158	66.2	22.4	71.6
1111	67.2	19.5	71.2
15	67.5	22.9	74.6
19 C	68.5	34.2	62.5
529	70.0	6.0	80.3
1122	72.2	16.0	76.7
39	74.0	11.9	78.3
19 A	75.2	16.4	78.0
285	77.0	10.0	79.0
1197	78.5	17.6	84.0
Control A	88.7	2.8	91.2
Control B	90.6	3.0	92.1
Standard error	± 3.60	± 3.37	± 4.45

relation between growth of roots and tops in wheat, and found that poorly developed wheat tops were associated with poor root development.

The results of the mature plant study with five species of *Fusarium* are given in Table 5. To economize space, the complete analysis of variance tables for disease ratings, weight of plants and grain are not given. However,

Table 5. The relative pathogenicity of five different species of Fusarium to mature plants of Marquis wheat in greenhouse pot tests.

Organism	Degree of root-rot infection (Disease Rating)	Weight of root and tops of plants (gm.)	Yield of Grain (gm.)
Fusarium redolens	15.6	125.6	19.2
" oxysporum	18.8	119.8	18.7
" equiseti	26.8	124.4	16.7
" avenaceum	32.4	113.2	15.0
" culmorum No. 38 1/	59.0	88.4	11.1
Control (no organism)	13.6	126.1	23.3
Standard error	± 3.73	± 4.22	± 2.05

1/ Plants first inoculated when 10 days old.

the results of these analyses established that the differences in pathogenicity observed between organisms in the experiment were very great. The complete results of the experiment in Table 5 show that of the species of Fusarium tested F. culmorum

(Isolate No. 38) was exceedingly pathogenic to wheat. Fusarium equiseti and F. avenaceum were weakly pathogenic, while F. oxysporum and F. redolens were not pathogenic to Marquis wheat. The comparative pathogenicity of these five fungi to young wheat plants is discussed in a later section of this paper. From Table 5 it is again evident that the intensity of root rot infection caused by species of Fusarium is negatively associated with yield in wheat.

Field Experiments

Field tests to determine the relative pathogenicity of five species of Fusarium; namely, F. culmorum, F. redolens, F. oxysporum, F. avenaceum, F. equiseti and of nine isolates of F. culmorum to adult wheat plants were made in 1938.

Methods and Materials

Seed of Renown wheat inoculated by the spore-suspension method was planted in artificially-infested soil (600 c.c. of oat-hull inoculum per rod-row where required). One part of oat-hull inoculum was used to nine parts of autoclaved soil, by volume. This soil inoculum was incubated for 15 days and then applied at seed level at the rate of 600 c.c. per rod-row.

The complete experiment consisted of four replicates of 16 plots each. Each plot contained two rod-rows, one in which 100 seeds were spaced in the row for estimating