Studies on the detection, biology and control of Gaeumannomyces graminis var. tritici
by Monica Elliott Juhnke

A thesis submitted in partial fulfillment of the requirements for the degree of Master in Science in Plant Pathology
Montana State University

Abstract:
Although take-nail disease of wheat (caused by Gaeumannomyces graminis var. tritici) is not new to Montana, there has been no research conducted on the disease in this state. Elsewhere, most of the research concerning take-all has been conducted with winter wheat. These two factors plus the potential severity of the disease prompted this research on the disease and its effect on irrigated spring wheat in Montana.

Four objectives were accomplished. One was the determination of the range of the disease in Montana via an informal field survey. Samples were collected on field trips to various counties and obtained from the Plant Disease Clinic at Bozeman, MT. Take-all was identified in 11 counties located throughout Montana including Sheridan, Gallatin, Lake, Hill and Treasure counties.

The second objective was the development of a selective medium (SM-GGT3) which would facilitate diagnosis and confirmation of the disease. The active ingredient amounts for SM-GGT3 are 10 mg dicloran, 10 mg metalaxyl, 25 mg HOE 00703, 100 mg streptomycin sulfate and 500 mg L-DOPA per liter of autoclaved Potato Dextrose Agar (39 g PDA in 1 L distilled water). There was a 32% increase in take-all isolations using SM-GGT3 when compared with PDA.

A third objective concerned the development of a quick, easy and accurate method to use for disease assessment that would correlate well with yield loss observations. This was accomplished using 1000 kernel weight and sub-crown internode disease severity ratings. The r% values for two field sites were -0.917 and -0.906 respectively.

Determining the effect of different forms of nitrogen fertilizer and various levels of chloride containing fertilizer on take-all severity and spring wheat yields was the fourth objective. The only conclusion which can be safely made based on the results is that the addition of excess phosphorus when adequate nitrogen is present did allow spring wheat to tolerate the disease and so produce somewhat higher yields.

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Monica Elliott Juhnke

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date 12/3/83

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ABSTRACT

Although take-all disease of wheat (caused by Gaeumannomyces graminis var. tritici) is not new to Montana, there has been no research conducted on the disease in this state. Elsewhere, most of the research concerning take-all has been conducted with winter wheat. These two factors plus the potential severity of the disease prompted this research on the disease and its effect on irrigated spring wheat in Montana.

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Chapter 1

Introduction

Take-all disease is a root and crown disease of wheat and barley grown with moist soil conditions produced by irrigation or high rainfall. It is caused by the soil-borne fungus Gaeumannomyces graminis (Sacc.) Arx & Olivier var. tritici (Walker) (Ggt). If the plants have mild infections and exhibit no visible symptoms, yield losses often go undetected. However, when symptoms become obvious (stunted plants, sterile heads, rotted roots), yields can be reduced by more than 50% (Wiese,1977). The disease occurs worldwide and is especially important in western Australia, Europe, South Africa, Japan and areas of North and South America. A recent monograph provides a complete report concerning the history and current status of research on the biology of the disease and methods for its control (Asher and Shipton,1981).

Although take-all disease of wheat is not new to Montana, there has been no research conducted on the disease in this state. The last published occurrence of take-all in Montana was 24 years ago (Sharp,1959). In

Sharp's report, it was indicated the disease had caused considerable crop damage in certain areas within the state. The lack of recent information on take-all in Montana is not due to its dissipation but rather because it is primarily an irrigated wheat disease. Only a small percentage of wheat production in Montana is irrigated. The irrigated wheat hectarage in Montana has doubled, though, in the past 10 years to 51,760 ha in 1981 (Montana Agricultural Statistics, 1982). Along with this increase in hectarage there has been an increased awareness that irrigated wheat has the potential to yield up to 6700 kg/ha when fertility and water resources are properly managed. In the past 2 years, I have observed that the next most important yield limiting factor for irrigated wheat is take-all. In 1980 Ggt was isolated via baiting techniques (Mathre, personal communication) from a well managed irrigated spring wheat field which sustained severe yield loss due to take-all. These were the first Ggt isolates collected from Montana.

An extensive amount of research has been done on Ggt throughout the world including the adjacent states of Washington, Idaho and Oregon (Asher and Shipton,1981; Walker,1975). Cook et al. (1968) stated that most of the yield loss due to Ggt in the Pacific Northwest occurs in winter wheat fields rather than spring wheat. This is

due to the low percentage of irrigated spring wheat in those states. For this reason most of the field research conducted with take-all in the Pacific Northwest has been with winter wheat. In Montana, however, winter wheat accounts for only 17% of the irrigated wheat. This factor plus the potential severity of the disease prompted me to initiate research on the disease and its effect on irrigated spring wheat in Montana.

Four objectives were outlined. First was to determine the range of the disease in Montana via an informal field survey. The second was to develop a selective medium which would facilitate diagnosis and confirmation of the disease. A selective medium would also assist in a variety of Ggt research problems ranging from pathogenicity tests to take-all decline experiments. The third objective concerned the development of a quick, easy and accurate method to use for disease assessment that would correlate well with yield loss observations. The fourth objective was to examine the effect of different forms of nitrogen fertilizer and various levels of chloride fertilizer on take-all severity and spring wheat yields. Research from Idaho (Huber et al.,1968) and Washington (Smiley and Cook,1973) have shown that ammonium based fertilizers effectively decrease take-all severity in winter wheat. Christensen et al. (1981)

showed that chloride containing fertilizers also produced some control of the disease in winter wheat. I wished to determine if the experimental results and recommended controls which were developed from these experiments were applicable to Ggt infected irrigated spring wheat in Montana.

Chapter 2

Survey of Montana for Take-all

INTRODUCTION

Sinceno research has been conducted on take-all in Montana, it was considered prudent to conduct a field survey to provide information on Ggt distribution, crops infected and the cropping history of infected fields. The latter could be particularly important in determining if the take-all decline phenomenon occurs in Montana. Take-all decline is defined "as the spontaneous reduction in take-all and increase in yield with continuous cropping of wheat and barley" (Rovira and Wildermuth,1981). This specific antagonism or suppression develops in the presence of Ggt. Cropping histories could also provide a method of predicting which fields have the potential of being severely infected with Ggt and sustaining a substantial yield loss.

MATERIALS AND METHODS

Field samples were collected in 1981 and 1982 during field trips to northcentral, northeasten Montana and in Gallatin County. The majority of samples were collected from irrigated small grain fields - especially those

exhibiting field symptoms of take-all. A few samples were obtained from dryland fields to determine if the fungus was colonizing crop roots grown in this environment. Farm samples sent to the Montana State University Plant Disease Clinic in Bozeman, MT. which were suspected of being infected with Ggt were given to me for disease confirmation.

When possible the following information was obtained for each sample: field location, county, crop, type of irrigation (if irrigated) and field cropping history. Information concerning soil type, fertility, disease pattern in the field and yield was also noted for some fields. All samples in Table 1 were confirmed as take-all by isolating Ggt from the tissue using techniques described in Chapter 3.

RESULTS AND DISCUSSION

Table 1 is a summary of information concerning each sample from which Ggt was isolated. The majority of the samples were irrigated spring wheat as was expected. These fields did sustain yield reductions due to the take-all disease - up to 50% for some fields. Ggt was isolated from barley in three irrigated fields and from winter and spring wheat in four dryland fields. The grain yield in these seven fields was not effected by take-all. This indicated the host range of Ggt among cereals grown

Table 1. Results of 1981 and 1982 survey of take-all disease in Montana by county, host, field climate and field cropping history.

County & Field No.	Host[1]	Field Climate[2]	Field Cropping History[3]
Pondera			
#1	B	I	1977=dryland grain; 78=B[4]; 79=SW; 80=SW
#2	SW	I	1977=dryland grain; 78=B; 79=SF; 80=SW
#3	SW	I	1977-81=SW
Gallatin			
#1	SW	I	long term irrigation; crops unknown
#2	SW	I	long term irrigation; crops unknown
#3	SW	I	long term irrigation; crops unknown
#4	SW	I	long term irrigation; crops unknown
Cascade			
#1	SW	I	1977=dryland native grass; 78=FB; 79=SW; 80=SW
#2	B	I	1977=dryland native grass; 78-81=SW
#3	DW	I	1977=dryland grain; 78=B; 79-80=SW; 81=B
#4	B	I	1977=dryland grain; 78=B; 79-80=SW; 81=B
Teton			
#1	SW	I	1975-80=alfalfa
#2	SW	I	1976-80=spring grains
#3	SW	I	1976-80=spring grains
#4	SW	I	1972-77=alfalfa; 78-80=B; 81=SW
#5	SW	I	unknown
Lake			
#1	WW	I	unknown
Treasure			
#1	WW	I	unknown

Table 1 - continued

County & Field No.	Host[1]	Field Climate[2]	Field Cropping History[3]
Hill			
#1	SW	D	1977=SFL; 78=WW; 79=SFL; 80=WW; 81=SFL
#2	SW	D	summer fallow/grain rotation
#3	WW	D	summer fallow/grain rotation
Chouteau			
#1	WW	D	continuous dryland grain
Blaine			
#1	SW	I	1981=dryland grain
Park			
#1	SW	I	1977=dryland native grass; 78-81=spring grains
Sheridan			
#1	DW	I	1980=dryland grain; 81=DW
#2	DW	I	1980=dryland grain; 81=DW
#3	DW	I	1980=dryland grain; 81=DW
#4	DW	I	1980=dryland grain; 81=DW

1 B=barley; SW=spring wheat; DW=durum wheat; WW=winter wheat.
2 I=irrigated; D=dryland.
3 B=barley; SW=spring wheat; DW=durum wheat; WW=winter wheat; SF=sunflowers; SFL=summer fallow; FB=faba beans
4 Unless indicated otherwise, the crop was irrigated.

in Montana. Although barley becomes infected it usually sustains minimal yield loss due to a lower incidence and severity of infection than wheat (Shipton,1975). However, barley may permit better survival of Ggt than winter and spring wheat as indicated by studies in Australia (Chambers and Flentje,1968) and England (Shipton,1981).

Isolation of Ggt from plants grown under dryland conditions demonstrates that the pathogen is probably present and surviving in all dryland soils in Montana including native pastures and small grain fields. MacNish (1973) determined that a low soil water potential and a low temperature were optimum for survival of Ggt. This was primarily due to the inactivation of Ggt competitors. No lower limit of water potential has been found for Ggt survival. Consequently, a wet soil with a high water potential favors parasitic activity of the fungus and therefore disease incidence and severity, but the opposite soil conditions favor its long term survival.

This helps to explain why take-all can become so severe in newly irrigated wheat fields which were previously dryland grain fields or dryland native sod or pastures. The fungal organism is present under dryland conditions, as shown by this survey, and does parasitize

cereal grains and/or grass weeds. However, few symptoms develop and there is no evident yield loss. When these same fields are put under irrigation, the organism's parasitic activity is stimulated, especially in the presence of a susceptible host, resulting in increased disease severity. As can be noted in Table 1, a large percentage of the fields were previously dryland fields. Some of these fields which had been in irrigated spring wheat for 3 years or longer were determined to be suffering 50% yield loss. Take-all symptomatic plants were easily identified even in fields which had been irrigated spring wheat for only one or two seasons.

Two fields in Cascade County (#1 and #2) have suffered yield losses of 30% to 50%. This is not unusual for fields plowed from dryland native grass and cropped to irrigated small grains for 2 or 3 years. Studies from Washington, Oregon and Idaho (Cook et al.,1968; Shipton et al.,1973) have reported observations of the same phenomenon. However, Shipton et al. (1973) noted that fields in eastern Washington and in the Columbia Basin with a history of dryland grain production converted to irrigated grain production developed little or no take-all. This was due to a biological factor antagonistic to Ggt. Based on my survey this is not true for Montana.

Severe take-all with up to 50% yield loss developed in fields with the history Shipton et al. (1973) described.

Whether this bio-control factor exists in Montana fields of long term irrigated wheat is not known. I have not located a producer who has knowledge of a given field's cropping history for the past 10 to 15 years. Where long term irrigation does occur in Montana, for example Teton County, alfalfa is often incorporated into the crop rotation. It has been shown that alfalfa can cause a take-all suppressive soil to become take-all conducive (Cook,1981a). Cook et al. (1968) in their survey of Washington, Oregon and Idaho, noted the "common occurrence of take-all in wheat immediately following alfalfa". Because alfalfa is regularly grown in long term irrigated rotations, take-all suppressive soils will be rare in these fields of Montana.

Walker (1975) defined the six conditions under which the different forms of take-all suppression may develop.

> It now seems possible to distinquish at least six conditions of disease suppression, some or all of which may turn out to be related, or grouped together with similar underlying causes. The distinction is made here only to try to clarify the literature on the phenomena and does not imply a real distinction between them, although it may exist in some cases. The conditions are:
>
> (i) suppression developed within a few seasons in the presence of the severely diseased host (both pathogen and diseased host present) - this is take-all decline (TAD) as studied by Shipton (1972a) and other British workers.

(ii) suppression developed over a long period in the presence of the diseased host (both pathogen and diseased host present) - this is the long term decline of take-all in older agricultural areas discussed by Zogg (1969).

(iii) suppresion developed in the presence of the healthy host, apparently without severe disease having occurred (healthy host present, pathogen detectable in soil but causing no obvious disease) - this is the antagonism studied in the suppressive wheat soils of eastern Washington by Shipton et al. (1973).

(iv) suppression developed in the presence of non-hosts - this is the antagonism reported by Zogg (1969;1972), and well known from the practical use of crop rotation as a means of minimising take-all in the first following cereal crop.

(v) antagonism to the parasite developed in the absence of the host, but the presence of a virulent isolate of the pathogen (host absent, pathogen present) - this is the phenomenon reported by Gerlagh (1968) and Shipton (1969, quoted in Shipton, 1972a).

(vi) the general non-specific antagonism shown by many soils (Gerlagh,1968) and which seems quite distinct from TAD.

With this survey, a number of questions are raised concerning take-all in Montana. The most important question is why does the biological factor antagonistic to Ggt not seem to exist in Montana soils as has been documented elsewhere in the world (Rovira and Wildermuth, 1981). As will be further discussed in Chapter 5, perhaps the take-all problem in Montana is quite unique from the other areas where Ggt has been extensively studied - Europe, Australia and the Pacific Northwest.

There were other common denominators concerning take-all of wheat in Montana other than the cropping

histories. Take-all usually first appeared in the field as small circular patches. These areas were located on poor soil - both structurally and nutritionally. The soils were coarse textured compared to the rest of the field being either sandy or rocky. This is a common observation world wide and "is probably related to the deficiency of nutrients and the lower water holding capacity of these soils" (Huber,1981b). I also observed that if a nitrogen fertilizer skip occurred, often times take-all appeared in that location first or was more severe there. These take-all patches virtually doubled in size each year covering large portions of a field and accounting for the severely reduced yields.

Chapter 3

A Selective Medium for *Gaeumannomyces graminis* var. *tritici*

INTRODUCTION

Observable field disease symptoms which are characteristic of take-all include blackened roots, stunted plants with fewer than normal tillers and prematurely ripened heads. These symptoms are also characteristic of other small grain diseases such as dryland root rot caused by *Cochliobolus sativus* Ito & Kurib. and/or *Fusarium culmorum* (Smith) Sacc. and *F. graminearum* Schwabe. The one symptom of take-all which is normally not confused with other diseases is the black plate mycelium which occurs on the basal stem area of moderately to severely infected plants. Even this field symptom is not considered adequate for confirmation of the presence of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) as the infectious organism. It is necessary to isolate the fungus to confirm the field diagnosis.

Isolation methods which have been used previously generally involve surface sterilization of the infected tissue followed by plating of the tissue on potato dextrose agar, with or without antibiotics (Davies,1935;

Garrett,1942; Nilsson,1969; Asher,1978; Huber,1981a). Since Gaeumannomyces does not normally produce asexual or sexual spores in culture, isolation and confirmation of take-all is difficult, if not impossible, if one is not familiar with the typical colony characteristics. Also, Gaeumannomyces is a slow growing fungus and is easily overgrown by other common soil and plant tissue organisms. Asher (1980), in examining winter wheat root systems for Ggt, stated that "failure to isolate the pathogen was associated, in general, either with bacterial contamination or, very occasionally, the presence of fast-growing fungi (e.g. Fusarium spp.) in culture". This makes it difficult to acquire a pure culture of the fungus for positive identification and/or to conduct further studies with the organism. Studies concerning nutritional, physical and chemical factors affecting growth of Gaeumannomyces on agar media are described elsewhere (Sivasithamparam and Parker,1981). In general these studies were conducted to determine the growth behavior of the fungus in response to these factors rather than to enhance isolation procedures or to develop a selective medium.

One objective of my work was to develop a medium which would allow one to easily isolate, differentiate and identify Ggt from infected plant tissue. This, of

course, would mean inhibiting many of the saprophytic and pathogenic fungi which are also associated with diseased small grain tissue. Another related objective was to identify a basic medium which could be further developed for use in a soil assay system, antagonism studies, inoculum level surveys and other investigations concerning the fungus in its soil and plant environment. At present there is no such medium.

P. H. Tsao (1970) states that the development of a selective medium "is generally based on the principle of selective exclusion of undesirable microorganisms, thus permitting the preferential establishment of the desired fungi on the isolation medium". Three methods which can be used singly or in any combination to achieve the desired medium are selective inhibition, selective enhancement and selective differentiation by pigmentation (Tsao,1970). Other criteria a selective medium should meet include a long shelf life, an adequate recoverability rate and the use of compounds which are relatively inexpensive and readily available. These criteria plus selective inhibition and selective differentiation by pigmention were used as the foundation for the development of a selective medium for Gaeumannomyces graminis var. tritici.

MATERIALS AND METHODS

Compounds Tested

Test compounds were classified into four categories as carbon sources, anti-fungal compounds, anti-bacterial compounds and general organic compounds. These are listed as such, along with selected results, in Tables 18-23 of the Appendix. Carbon sources were primarily incorporated at 100 and/or 1000 ug/ml into a minimal medium containing 1.0 g KH_2PO_4, 1.0 g $MgSO_4$, 3.0 g $NaNO_3$, 1.0 mg biotin, 1.0 mg thiamine and 20.0 g Difco agar or purified Oxoid agar in 1 liter of distilled water. Anti-bacterial compounds were incorporated into potato broth agar (PBA) for testing. The general organic compounds were screened via incorporation into potato dextrose agar (PDA). The majority of the anti-fungal compounds were tested at 1, 10 and 100 ug/ml in one or more of the following basic media - PBA, PDA or the minimal medium described above. After testing each of the compounds individually for their effect on growth of Ggt, various combinations were tested via incorporation into PDA for their combined effect on growth response of Ggt and various test fungi.

Organisms Tested

Pathogenic isolates of Ggt utilized in this study are listed in Table 2. Gaeumannomyces graminis (Sacc.) Arx & Olivier var. graminis and var. avenae (E.M. Turner) Dennis (Ggg and Gga respectively), Phialophora graminicola (Deacon) Walker and a Phialophora sp.(lobed hyphopodia) were obtained from P.T.W. Wong. These organisms were tested to determine if their growth responses would be similar to that of Ggt. Other fungi which were tested on the media and are hereafter referred to as test fungi are listed in Table 3. These fungi were utilized because they are associated with soil as common saprophytes or are common soil-borne pathogens of small grains in the Pacific Northwest. All of these organisms were maintained on PDA and inoculated onto test medium plates by placing a 4 or 7 mm diameter agar mycelial plug on the medium with a minimum of three replicate test plates per organism. Generally, inoculated plates were maintained at room temperature and light for 5 days before final results were recorded. Results were determined by measuring the linear amount of fungal mycelial growth from the edge of the inoculum plug. Growth comparisons were made with replicated check plates.

Table 2. *Gaeumannomyces graminis* var. *tritici* isolates tested in the development of the selective medium SM-GGT3.

Isolate No.	Source	Location
Mt 1-4	D.E. Mathre	Montana
Mt 5-21	M.L. Juhnke	Montana
Pa 42a	R.J. Cook	Washington
Pa 371	R.J. Cook	Washington
Os 1	R.L. Powelson	Oregon
Pu 53/1	D.M. Huber	Indiana
Pu 53/4	D.M. Huber	Indiana
Ar 1	J.P. Jones	Arkansas
Ar 2	J.P. Jones	Arkansas
Ks 2	W.W. Bockus	Kansas
Ks 12	W.W. Bockus	Kansas
Co 2	P.T.W. Wong	Colorado
Id 1	J.H. Riesselman	Idaho

Table 3. Test fungi used in the development of the selective medium SM-GGT3.

Fungus	Isolate	Source
Penicillium spp.	---	R.V. Miller
Chaetomium sp.	---	R.V. Miller
Aspergillus sp.	---	R.V. Miller
Trichoderma sp.	---	R.V. Miller
Alternaria sp.	---	R.V. Miller
Fusarium sp.	---	R.V. Miller
Rhizopus sp.	A	straw sample
Rhizopus sp.	B	straw sample
Rhizopus sp.	C	soil sample
Cochliobolus sativus	---	W. Grey
Fusarium culmorum	398	W. Grey
Fusarium graminearum	424	W. Grey
Rhizoctonia solani - AG2	302	D.E. Mathre
Rhizoctonia solani - AG4	304	D.E. Mathre
Rhizoctonia sp. (from wheat)	404	D.E. Mathre
Rhizoctonia sp. (from wheat)	---	D. Yount
Pythium sp. (from safflower)	310	D.E. Mathre
Phytophthora cinnamoni	377	D.E. Mathre
Pseudocercosporella herpotrichoides	PH 81-2	T. Murray

Media Preparation

Two media were developed and tested. These were SM-GGT1in 1981 and SM-GGT3 in 1982. The recipes for these media are as follows. The amounts listed for the chemicals are amounts of active ingredient.

SM-GGT1	SM-GGT3
1 mg dicloran	10 mg dicloran
10 mg quintozene	10 mg metalaxyl
30 mg fenaminosulf	25 mg HOE 00703
100 mg streptomycin sulfate	100 mg streptomycin sulfate
500 mg L-DOPA	500 mg L-DOPA
39 g Difco PDA	39 g Difco PDA
1 L distilled water	1 L distilled water

The experimental fungicide HOE 00703 was obtained from the American Hoechst Corp., Somerville, NJ.; dicloran from Aldrich Chemical Co., Milwaukee, WI.; metalaxyl from Ciba-Geigy Corp., Greensboro, NC.; quintozene from Cargill, Inc., Minnetonka, MN.; and fenaminosulf from Mobay Chemical Corp., Kansas City, MO. The remaining compounds were obtained from Sigma Chemical Co., St. Louis, MO. A sample of HOE 00703 may be obtained from the laboratory of Dr. D. E. Mathre, Montana State University, Bozeman, MT.

To prepare either medium, dehydrated PDA is added to distilled water, autoclaved at 121 C for 20 minutes and cooled to 50 C. Liquid compounds are added first. The remaining compounds are dissolved in 10 ml sterile distilled water in a sterile 15 ml tube before being incorporated into the molten PDA. The medium is then agitated by hand to evenly suspend any undissolved compounds and poured into sterile glass or plastic petri plates. Plates are stored upside down in plastic bags or metal tins placed in the dark at 4 C. Another medium, SM-GGT2, was also tested. It contains the same compounds as SM-GGT3 but the amount of HOE 00703 was lowered to 10 mg active ingredient. SM-GGT1, SM-GGT2 and SM-GGT3 were compared with each other and with PDA utilizing naturally infected tissue and in the combat tests and soil assay tests described below.

Plant Material Tested

Mature plants exhibiting characteristic field symptoms of take-all were collected during 1981 and 1982 from irrigated small grain fields in Montana - primarily spring wheat. During 1982 mature plants were also collected from dryland small grain fields. All plants were stored dry in paper bags at room temperature. When used for testing, the basal stem area or sub-crown internode (SCI) was removed, rinsed thoroughly with water

and cut into 1 cm lengths. These pieces were then sterilized for 30 seconds in a 1% silver nitrate solution, rinsed 30 seconds in sterile distilled water and blotted dry on filter paper before being placed on one of the final media. If there was enough material, at least three plates per location were inoculated. Inoculated plates were maintained at room temperatue and room light for a minimum of 5 days and a maximum of 9 days. Results were tabulated by determining the percentage of tissue pieces which fostered Ggt growth, with and without pigment production. When possible the number and identity of any contaminating organisms were also determined using the proper media and the microscope.

Combat Tests

To determine the effectiveness of SM-GGT1 and SM-GGT3 in detecting Ggt and eliminating a common fungus such as Fusarium culmorum, combat tests were conducted. Combat tests compare the ability of two organisms to grow on specific media. The sub-crown internode and basal culm tissue naturally infected with Ggt was thoroughly washed and cut into 1 or 2 cm pieces. Half of the pieces were soaked in sterile distilled water for 1 hour. The remaining pieces were soaked in a dense F. culmorum spore suspension for 1 hour. The pieces were then

blotted dry and incubated for 2 days in sterile petri plates. Upon completion of incubation, all pieces were surface sterilized with a 1% silver nitrate solution for 30 seconds, rinsed in sterile distilled water for 30 seconds, blotted dry on filter paper and plated on SM-GGT1 or SM-GGT3 and PDA.

Soil Assays

Although SM-GGT3 was primarily developed as a medium for isolating Ggt from tissue, preliminary experiments were conducted to determine its value as a soil assay medium for Ggt. SM-GGT3 was compared to SM-GGT2 and PDA. Two soil assay methods were tested - one utilizing soil suspensions and the second utilizing soil organic matter consisting mainly of plant residue. Two different soils were used for both methods. One soil was collected from a field at the A.H. Post Agricultural Research Station, Bozeman, MT. and had been stored in the laboratory for 6 months in a loosely sealed container. The second soil was collected at Ulm, MT. from a field known to be infested with Ggt. This soil had been supplemented with Ggt infected oat kernels and utilized in a number of take-all experiments in the greenhouse for the previous 10 months. To both soils was added ground Ggt infected oat kernel inoculum at a rate of 1 g of inoculum per 100

g of soil (dry weight basis). The inoculum and soil were tumbled for 5 minutes utilizing a rotating seed treater.

For the soil suspension assay method, 1 g of the soil/inoculum mixture was suspended in 100 ml of sterile distilled water to obtain a 10^{-2} dilution. Other dilutions which were made were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}. Approximately 0.1 ml of each dilution was pipetted onto each plate and spread over the plate with a sterile bent glass rod. Four plates of each medium were used for each dilution.

With the sieved soil technique, approximately 150 g of soil/inoculum mixture was dry sieved through a set of four sieves with openings of 2.36 mm, 1.70 mm, 0.85 mm and 0.25 mm. Twelve pieces of organic matter, primarily straw residue, were selected from each set of sieved material, including the material that passed through the 0.25 mm sieve, and placed directly on the test plates. Again, four plates of each medium were used for each size of sieved material. There was some difficulty in selecting pieces of organic matter less than 0.25 mm in size. If this occurred, soil particles picked up with the organic matter were also used for inoculating these plates.

RESULTS

Compounds Tested

None of the carbon sources tested enhanced the growth of Ggt exclusively nor inhibited the growth of the test fungi. Based on these results and the fact that PDA is inexpensive and readily available, PDA was selected as the basal medium in which the other compounds of the selective medium would be incorporated. The effect of each compound on Ggt growth is reported in Tables 18-23 of the Appendix. L-β-3,4-dihydroxyphenylalanine (L-DOPA) was selected as the compound which was noninhibitory to growth of Ggt and caused the production of a dark melanin pigment. Based on streptomycin sulfate's wide antibacterial spectrum (Franklin and Snow,1981), cost and availability, it was chosen as the anti-bacterial compound to be utilized in the selective medium.

Selection of the anti-fungal compounds was based on two criteria. The compound must not significantly inhibit Ggt growth but at the same time should severely inhibit the growth of one or more of the test fungi. Dicloran was selected as an inhibitor of Rhizopus (Henson,1981). It should be noted however that dicloran was not a very effective inhibitor of the isolates of Rhizopus used in this study. It was utilized anyway due to the lack of any other substance which would control

Rhizopus without inhibiting *Ggt*. Fenaminosulf, which inhibits Oomycetes such as *Phytophthora* and *Pythium* (Kreutzer,1963), was later replaced with metalaxyl as fenaminosulf is unstable in light (Hills and Leach,1962). Metalaxyl is an effective systemic fungicide for the control of the Oomycetes at low concentrations (Fisher and Hayes,1982). At 10 ug/ml, Oomycetes were inhibited 100%. Quintozene was initially selected as the third fungicide for use in the selective medium. However, after comparing HOE 00703 with quintozene at various rates, HOE 00703 was easily shown to be the more effective compound (Table 4). HOE 00703 has the chemical name of 1-(3,5-dichlorophenyl)-3-methoxymethyl-pyrrolidin-2,4-dion. Chemically, it belongs to the carboximides.

Table 4. A comparison of quintozene and HOE 00703 in their ability to inhibit common pathogenic soil-borne fungi.

	% of PDA Check Growth				
	HOE00703 (ug/ml)			Quintozene (ug/ml)	
Fungus	10	25	50	25	50
Gaeumannomyces graminis var. *tritici* (Mt 1)	75	75	75	50	50
Fusarium culmorum	17	0	0	83	67
Fusarium graminearum	23	0	0	50	50
Cochliobolus sativus	0	0	0	33	20
Rhizoctonia solani (302)	0	0	0	50	67
Rhizoctonia sp.(404)	17	0	0	67	67

One of the major problems encountered was the identification of a compound or compounds that would effectively inhibit F. culmorum, F. graminearum, C. sativus and Rhizoctonia solani Kuhn without inhibiting Ggt. HOE 00703 was the only compound tested which gave at least 90% to 100% inhibition of these fungi and only 25% inhibition of Ggt at the same rate. It should be noted that a number of fungicides inhibited Ggt by greater than 50% at 10 ug/ml or less, including thiram, triphenyltin hydroxide, prochloraz, CGA-64251, thiophanate-methyl, thiabendazole, carboxin, captan, methfuroxam and imazilil (Appendix Table 18).

Selective Medium SM-GGT1

To determine the practical use of SM-GGT1,16 irrigated wheat plant samples from 13 different fields in Montana were procured in 1981. Samples were collected based on characteristic field symptoms of the take-all disease. Using silver nitrate as a surface sterilant and SM-GGT1 as the isolation medium, 73% of the pieces tested allowed Ggt growth, with 96% of that growth producing a black pigment (Table 5). All 16 samples were confirmed as take-all disease. There was never a problem in delineating Ggt from contaminants on the plate as Ggt was distinguishable by colony morphology and pigment formation. Fusarium species and C. sativus, although

Table 5. Results of isolations from 1981 irrigated wheat tissue suspected of being infected with Gaeumannomyces graminis var. tritici (Ggt) utilizing the selective medium SM-GGT1.

Sample	Tissue Pieces per Sample	No. Tissue Piece Isolations Yielding				
		Ggt	Fus[1]	Cs[2]	Other[3]	None[4]
1	12	8	0	0	0	4
3	16	14	0	1	0	2
6	16	15	0	0	1	0
7	12	9	0	0	2	1
8	16	11	3	0	0	2
9	16	13	1	0	2	0
10	12	8	2	0	0	2
11	12	9	2	1	0	0
12	16	14	1	0	1	0
20	16	11	0	0	0	5
21	12	3	0	0	6	3
22	16	13	4	0	0	0
23	17	12	1	1	2	2
24	16	13	1	0	2	0
26	12	10	2	0	0	1
29	16	8	3	2	0	4
Total	233	171	20	5	16	26
%	---	73	9	2	7	11

1 Fusarium species - primarily of the F. roseum group.
2 Cochliobolus sativus.
3 This group consisted of sterile mycelia - septate and non-septate hyphae.
4 No growth of any organism.

present on some plates, did not usually interfere with obtaining a pure culture of Ggt.

Since the F. roseum group was the most common non-Ggt group isolated using SM-GGT1, a combat test experiment was conducted to determine the efficiency of SM-GGT1 in detecting Ggt and F. culmorum using the technique described in the Materials and Methods. Those pieces of naturally infected Ggt plant tissue soaked only in sterile distilled water yielded 72% Ggt with pigment and 5% F. culmorum whereas the naturally infected tissue soaked in F. culmorum spores yielded 54% Ggt with pigment and 46% F. culmorum. Fusarium isolation did increase with the F. culmorum inoculated samples but after 5 days incubation Ggt was still easily distinguished from F. culmorum via pigment production and gross morphology. Even with this heavy contamination of Fusarium, Ggt could be transferred to obtain a pure culture.

Selective Medium SM-GGT3

Further testing of SM-GGT1 using pure culture inoculations of F. culmorum, F. graminearum, C. sativus and R. solani illustrated that if the Ggt infected material was also heavily colonized by these fungi it was quite probable that these fungi would prevent isolation of Ggt from the infected material. Continued work with

SM-GGT1 culminated in 1982 in a new medium designated as SM-GGT3. Three different tests were conducted to compare PDA or PDA containing 100 ug/ml streptomycin sulfate with SM-GGT2 and SM-GGT3. They were combat tests, soil assay tests and plant tissue isolation tests. SM-GGT3 was selected over SM-GGT2 because the 25 ug/ml of HOE 00703 in SM-GGT3 was more effective in inhibiting non-Ggt fungal growth without significantly decreasing Ggt growth.

Thirty-four small grain plant samples were collected in 1982 (Table 6). The irrigated small grain samples were collected based on characteristic symptoms of the take-all disease from fields located throughout Montana. The dryland samples were randomly collected from fields in northcentral Montana. With all samples, if the sub-crown internode or basal stem area appeared darkened, that plant tissue was used for isolation purposes.

Table 6. Small grain plant samples collected in 1982.

Field Source	No. Fields Sampled
Irrigated Barley	4
Dryland Barley	4
Irrigated Spring Wheat	15
Dryland Spring Wheat	7
Irrigated Winter Wheat	1
Dryland Winter Wheat	3

The results are given in Table 7. Figure 1 illustrates the differences between PDA and SM-GGT3. Silver nitrate was used as the surface sterilant. Using SM-GGT3 as the isolation medium, 31% of all the pieces tested produced Ggt growth and pigmentation, 16% produced other fungal growth and 53% produced no growth of any organism. Ggt was isolated from four of the dryland wheat samples. Six of the irrigated small grain samples were not confirmed as being infected with Ggt - three barley and three wheat samples. Separating the irrigated samples from the total sample group (Table 8), 51% of the tissue pieces yielded Ggt and pigment, 7% produced other fungal growth and 43% produced no growth of any organism. Tables 9 and 10 show the results obtained on SM-GGT3 and PDA using the same isolation techniques. It is apparent that SM-GGT3 would be the superior medium in isolating Ggt from plant tissue. Seven of the 16 samples collected in 1981 and tested on SM-GGT were also tested on SM-GGT3 using the same isolation techniques. Ggt was isolated from all seven samples.

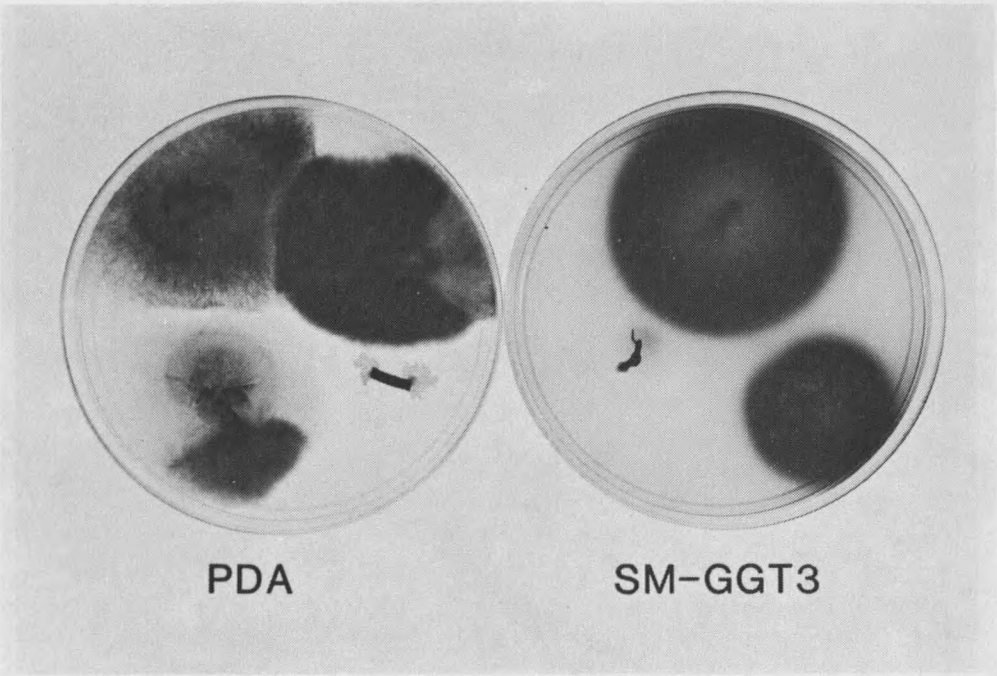
Not all Ggt colonies isolated with SM-GGT3 produced a dark melanin pigment within 9 days. However, after 1 or 2 additional days or transfer to a fresh plate of SM-GGT3, pigmentation occurred with all isolates. There were two reasons for not obtaining pigmentation within 9

Table 7. Results of isolations from 1982 irrigated and dryland wheat and barley tissue suspected of being infected with Gaeumannomyces graminis var. tritici (Ggt) utilizing the selective medium SM-GGT3.

Sample	Field Source[1]	Tissue Pieces per Sample	No. Tissue Piece Isolations Yielding				
			Ggt	Fus[2]	Cs[3]	Other[4]	None[5]
1	IB	10	0	0	0	5	5
2	ISW	10	4	0	0	1	5
3	ISW	10	9	0	0	0	1
4	ISW	10	9	0	0	0	1
5	DB	10	0	5	0	1	4
6	ISW	10	9	1	0	0	0
7	IWW	10	0	0	0	0	10
8	IB	10	0	0	0	0	10
9	DB	10	0	0	0	4	6
10	DSW	8	0	0	0	0	8
11	ISW	10	9	0	0	0	1
12	DB	10	0	0	0	0	10
13	DB	10	0	0	0	6	4
14	ISW	10	0	0	0	0	10
15	ISW	10	0	0	0	0	10
16	ISW	10	7	0	0	2	1
17	ISW	10	9	0	0	0	1
18	ISW	10	7	0	0	0	3
19	ISW	10	8	0	0	0	2
20	ISW	10	5	0	0	0	5
21	DSW	10	2	0	0	2	6
22	DSW	10	0	0	0	3	7
23	DSW	10	0	1	0	2	7
24	DSW	10	0	7	0	0	3
25	DSW	10	1	0	0	1	8
26	DWW	10	0	0	0	1	9
27	IB	10	7	0	0	0	3
28	DSW	10	0	0	0	2	8
29	DWW	10	1	0	0	0	9
30	ISW	10	5	0	0	0	5
31	ISW	7	2	0	0	0	5
32	IB	4	0	3	0	0	1
33	ISW	4	4	0	0	1	0
34	DWW	4	1	3	0	0	0
TOTAL		317	99	20	0	31	168
%		---	31	6	0	10	53

1 I=irrigated; D=dryland; B=barley; SW=spring wheat; WW=winter wheat.
2 Fusarium species - primarily of the F. roseum group.
3 Cochliobolus sativus.
4 This group consisted of sterile mycelia and a few bacteria.
5 No growth of any organism.

Figure 1. A comparison between potato dextrose agar (PDA) and the take-all selective medium (SM-GGT3) in the efficiency of isolating Gaeumannomyces graminis var. tritici (Ggt) from naturally infected wheat tissue.



PDA: (upper left) Ggt
(upper right) Fusarium roseum; Ggt
(lower left) sterile septate hyphae
(lower right) bacteria

SM-GGT3: (upper center) Ggt
(lower left) no growth
(lower right) Ggt

Table 8. Results of isolations from 1982 irrigated wheat and barley tissue suspected of being infected with Gaeumannomyces graminis var. tritici (Ggt) utilizing the selective medium SM-GGT3.

Sample No.	Tissue Pieces per Sample	No. Tissue Pieces Yielding				
		Ggt	Fus[1]	Cs[2]	Other[3]	None[4]
1[5]	10	0	0	0	5	5
2	10	4	0	0	1	5
3	10	9	0	0	0	1
4	10	9	0	0	0	1
6	10	9	1	0	0	0
7	10	0	0	0	0	10
8[5]	10	0	0	0	0	10
11	10	9	0	0	0	1
14	10	0	0	0	0	10
15	10	0	0	0	0	10
16	10	7	0	0	2	1
17	10	9	0	0	0	1
18	10	8	0	0	0	3
19	10	8	0	0	0	2
20	10	5	0	0	0	5
27[5]	10	7	0	0	0	3
30	10	5	0	0	0	5
31	7	2	0	0	0	5
32[5]	4	0	3	0	0	1
33	4	4	0	0	1	0
TOTAL	185	94	4	0	9	79
%	---	51	2	0	5	43

1 Fusarium species - primarily of the F. roseum group.
2 Cochliobolus sativus.
3 This group consisted of sterile mycelia and a few bacteria.
4 No growth of any organism.
5 Barley tissue.

Table 9. Comparison between the selective medium (SM-GGT3) and potato dextrose (PDA) tissue isolation results using 1982 irrigated and dryland wheat and barley tissue suspected of being infected with Gaeumannomyces graminis var. tritici (Ggt).

Medium	% Total Tissue Pieces Yielding				
	Ggt	Fus[1]	CS[2]	Other[3]	None[4]
PDA	12	22	12	29	25
SM-GGT3	31	6	0	10	53

1 Fusarium species - primarily of the F. roseum group.
2 Cochliobolus sativus.
3 This group consisted of sterile mycelia (septate and non-septate) and bacteria.
4 No growth of any organism.

Table 10. Comparison between the selective medium (SM-GGT3) and potato dextrose agar (PDA) tissue isolation results using 1982 irrigated wheat and barley tissue suspected of being infected with Gaeumannomyces graminis var. tritici (Ggt).

Medium	% of Total Tissue Pieces Yielding				
	Ggt	Fus[1]	Cs[2]	Other[3]	None[4]
PDA	19	21	6	29	25
SM-GGT3	51	2	0	5	43

1 Fusarium species - primarily of the F. roseum group.
2 Cochliobolus sativus.
3 This group consisted of sterile mycelia (septate and non-septate hyphae) and bacteria.
4 No growth of any organism.

days. First was the slow and minute amount of growth which was produced in 9 days from some tissue pieces. Once these colonies began to enlarge more rapidly, pigmentation occurred. Second, some colonies of Ggt were inhibited from pigmentation production, but not growth, by bacterial contaminants immediately around the tissue piece. Once the Ggt colony grew further from the bacterial contaminant or was transferred to a fresh SM-GGT3 plate, pigment production occurred. All colonies of Ggt isolated thus far have produced a melanin pigment when cultured on SM-GGT3. It should be noted that Rhizoctonia species may also produce a melanin pigment on SM-GGT3 but they are usually severely inhibited by the selective medium. If Rhizoctonia should grow, it can easily be differentiated from Ggt based on macroscopic and microscopic characteristics.

Comparisons were made of the amount of fungal growth on PDA and SM-GGT3 originating from plant tissue. Ggt growth was inhibited slightly, if at all, on SM-GGT3. C. sativus did not grow on SM-GGT3 even though the average colony size on PDA was 24 mm in diameter. The F. roseum group produced colonies of 60 mm in diameter on PDA but were reduced by 85% to 90% on SM-GGT3, providing F. roseum colonies grew at all. Rhizoctonia species were isolated only once on PDA and were never isolated on

SM-GGT3. Although unidentified sterile mycelia grew on SM-GGT3, the growth was restricted to less than 15 mm in diameter. Rhizopus contaminated 1 plate of PDA and 1 plate of SM-GGT3. Bacterial growth was usually completely inhibited on SM-GGT3. If bacteria were present, they were restricted to a small area immediately surrounding the tissue piece and did not spread.

As with SM-GGT1, a combat test experiment was conducted to determine the efficiency of SM-GGT3 in detecting Ggt and F. culmorum. The naturally infected take-all plant tissue soaked only in sterile distilled water yielded 67% Ggt with pigment and 0% F. culmorum. The naturally infected tissue soaked in F. culmorum spores yielded 17% Ggt and 17% F. culmorum on SM-GGT3 versus 100% F. culmorum and 0% Ggt on PDA. The F. culmorum colonies on SM-GGT3 were severely inhibited in growth to less than 10 mm in diameter versus 60 mm in diameter on PDA.

Preliminary tests were conducted to determine the feasibility of SM-GGT3 for use in a soil assay system. Using the soil suspension assay method, Ggt was not isolated from either soil with either PDA containing streptomycin sulfate (PDA+), SM-GGT2 or SM-GGT3. Rhizopus and bacterial colonies were the only organisms growing on PDA+ using the soil/inoculum mixture from Ulm,

MT. Only Rhizopus grew on SM-GGT3. With the Bozeman, MT. soil/inoculum mixture, a greater variety of organisms grew on PDA+ but only a sterile fungus with non-septate hyphae and a bacterial species grew on SM-GGT3. Both of these organisms were few in number and small in size.

With the sieved soil technique, Ggt was isolated from the Bozeman mixture using all three media and all five sieve sizes. With PDA+, Rhizopus, Fusarium species, Penicillium species and a sterile fungus with non-septate hyphae were also isolated while only Rhizopus, a sterile fungus with non-septate hyphae and, a bacterium were isolated on SM-GGT3. Ggt was not isolated from the Ulm, MT. soil/inoculum mixture as all plates were covered 100% by Rhizopus.

DISCUSSION

Two of Tsao's suggestions (1970) were used to develop SM-GGT3 - selective inhibition and selective differentiation by pigmentation. Gaeumannomyces utilizes L-DOPA to produce a melanin pigment which easily differentiates it from most other soil organisms. Selective inhibition was accomplished with the compounds dicloran, metalaxyl, HOE 00703 and streptomycin sulfate. Streptomycin sulfate is an inhibitor of gram positive and gram negative bacteria which makes it the single most

useful anti-bacterial compound to add to a selective medium.

SM-GGT3 selectively inhibited Oomycetes due to its use of metalaxyl. It was necessary to inhibit Oomycetes such as Pythium species when isolating from root tissue or soil as they can be parasitic on plants and are usually common soil inhabitants.

Dicloran was incorporated in the medium because of its reported ability to inhibit Rhizopus, a fungal genus commonly encountered in soil. Rhizopus was never isolated from any of the plant tissue cultured but was a contaminant once on SM-GGT3 and on PDA. However, growth from pure culture inoculations of Rhizopus on the selective medium were not extensively inhibited nor were colonies of Rhizopus which originated from soil dilutions or soil particles. One reason for this discrepancy could be the use of a 1% silver nitrate solution as a surface sterilant when isolating from plant tissue. The addition of 10 ug/ml silver nitrate to PDA inhibited Rhizopus by 25% to 30% versus no inhibition with 75 ug/ml dicloran. This would suggest it was the silver nitrate surface sterilant which inhibited growth of Rhizopus on SM-GGT3 and not neccessarily the anti-fungal compounds.

At 25 ug/ml, HOE 00703 completely inhibited pure cultures of F. culmorum, F. graminearum and C. sativus

with only minor inhibition of Ggt. This showed the selective inhibition of the fungicide as all four fungi are Ascomycetes. It also completely inhibited R. solani which is a Basidiomycete. F. culmorum, F. graminearum, C. sativus and R. solani are common root and basal culm pathogens of small grains, and in general, these and other species of these fungi are common soil inhabitants. The probability of one of these organisms being present with Ggt can be high. Also, plant tissue isolation studies with SM-GGT1 indicated that F. roseum and C. sativus accounted for the largest percentage of non-Ggt isolations. This necessitated complete inhibition of Fusarium as it grows at a significantly faster rate than Ggt. Asher (1980) stated that fast-growing fungi, such as Fusarium species, were a leading cause in failing to isolate Ggt from infected plant tissue. Cunningham (1981) also noted that when isolating from stored cereal roots, only a few hyphae would emerge and great care was taken to transfer the Ggt hyphae "from the midst of more rapidly growing colonies". Without the addition of HOE 00703, the value of SM-GGT3 as a selective medium would be greatly diminished.

The second of Tsao's suggestions used in SM-GGT3 was selective differentiation by pigmentation. Noting that Ggt infection of wheat is associated with dark runner

hyphae called macrohyphae (Walker,1975; Nilson,1969) and that melanin has been extracted from Ggt hyphae (Teschudi and Kern,1979), an attempt was made to enhance this natural hyphal pigmentation. L-tyrosine, an amino acid precursor of melanin, did induce Ggt to produce a brown pigment in the medium. This allows for selective differentiation of Ggt via pigmentation as is done with the selective medium for Streptomyces scabies (Menzies and Dade,1959). Of the four tyrosine analogues tested, L-β-3,4-dihydroxyphenylalanine (L-DOPA) was shown to be the best compound to induce the darkest pigment formation without inhibiting Ggt.

All isolates of Ggt listed in Table 2 produced a melanin pigment when cultured on SM-GGT3. Gga, Ggg and P. graminicola also produced a melanin pigment and with the same intensity as Ggt, as did the lobed hyphopodial Phialophora species. This was not surprising, however, considering the close taxonomic relationship of these fungi (Walker,1981). Some R. solani isolates also produced a melanin pigment but SM-GGT3 completely inhibited Rhizoctonia species. If Rhizoctonia growth occurred, macroscopic and microscopic examinations easily distinguished Rhizoctonia from Ggt.

To distinguish between Ggt, Ggg, Gga and P. graminicola will be more difficult and will probably

require the production of perithecia in culture. However, Ggt is considered to be the main cause of take-all of wheat. Gga is the major cause of oat take-all and take-all patch disease of turf grass although it also attacks wheat and barley. Ggg is only slightly pathogenic causing little root damage to infected grass hosts (Walker,1981). P. graminicola has been identified only once in the United States (personal communication, P.T.W. Wong) and is considered a saprophyte in Europe (Walker,1981). Thus, it would logically be assumed that when working with take-all of wheat, the pathogenic organism which would be isolated would be Ggt.

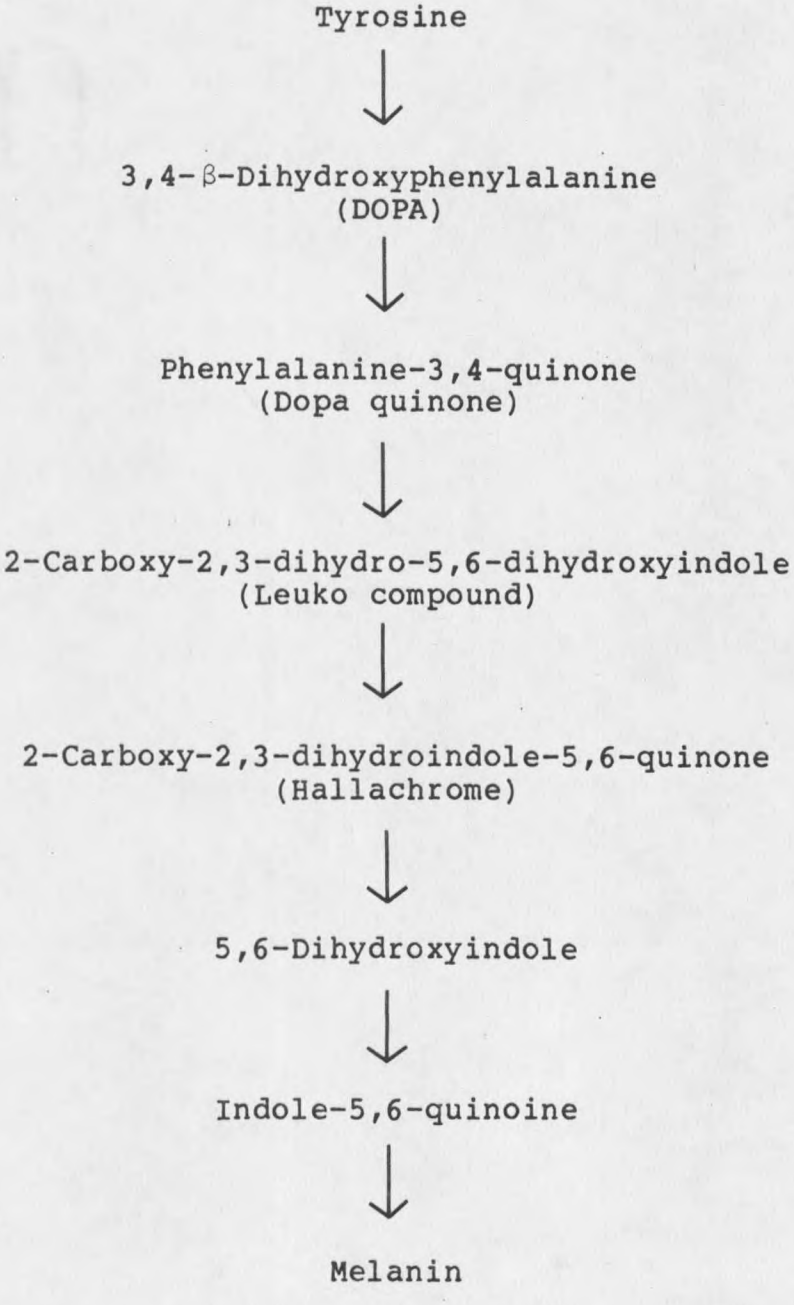
Another possible way to distinguish between varieties of Gaeumannomyces graminis would be with chemical additives to SM-GGT3. The sulfur containing amino acids cysteine and cystine reportedly inhibit Gga growth while stimulating Ggt growth (Turner,1957,1959,1961). Preliminary results utilizing the cultures of Gg obtained from P.T.W. Wong and the defined medium described by Turner (1957;1959) did not follow this pattern. β-escin (aescin) is a triterpeneglycoside similar to avenacin, the factor in oat roots causing resistance to Ggg and Ggt but not Gga (Turner,1960,1961; Olsen,1971). When β-escin was incorporated into PDA at 1 and 10 ug/ml, Ggg, Ggt and P.

graminicola growth was inhibited more than Gga growth. Another compound which was not inhibitory to Gga but was to the other related fungi was salicin (2-[hydroxymethyl]phenyl-β-d-gulcopyranoside) at 100 ug/ml. Arbutin (hydroxyquinone-β-D-glucopyranoside) at 100 ug/ml was not inhibitory towards any of these related fungi. From these results it appears that SM-GGT3 could be made selective for Gga.

A few interesting points should be noted concerning the melanin pigment phenomenon. Melanin is formed via the oxidation of tyrosine (Figure 2) with DOPA being the first compound produced. Tyrosinase, a copper containing protein (Dressler and Dawson,1960a,1960b; Bright et al.,1963), is the necessary enzyme for this step and for the next step in the formation of dopa quinone. Thereafter, the reactions require no enzymes or occur spontaneously. Melanin is a polymer or group of polymers of random structure (White et al.,1973).

Since the production of melanin is an oxidative process, two experiments were conducted to determine if the production of the dark pigment by Ggt could be inhibited when tyrosine was present in the PDA medium. Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-thimethylquinoline), an antioxidant obtained from Monsanto Agriculture Products Co., St. Louis, Mo., was added to PDA containing

Figure 2. Melanin formation from tyrosine. (Mahler and Cordes,1966; White et al.,1973)



500 ug/ml tyrosine. At the rate of 10 ug/ml, ethoxyquin did not inhibit the growth of Ggt but did completely inhibit pigment production. In a second experiment, plates of PDA containing 500 ug/ml tyrosine were inoculated with Ggt. They were then placed in a Brewer's jar and purged with nitrogen. The result was no inhibition of Ggt growth but 100% inhibition of pigment production. After 5 days these plates were removed from the low oxygen environment and placed in the room atmosphere. Within 2 days pigment production occurred and the hyphae turned a dark color also. The results from these experiments, plus the utilization of L-DOPA to initiate pigment production by Ggt, would definitely suggest it is a melanin pigment being produced by Ggt which diffuses into the medium.

Tyrosine is not only a precursor of melanin but also of p-coumaric acid and hordenine which in turn are distant intermediates in the pathways leading to lignin formation (Frank and Marion,1956; Salisbury and Ross,1969; Friend,1976; Barz and Hoesel,1979). Hordenine is a compound found only in barley roots during the first 30 days of growth (Mann and Mudd,1963) just as gramine is the main alkaloid found in the leaves of germinating barley. However, gramine is derived from trytophan (Grower and Leete,1963). Various compounds within these

pathways plus other phenols and quinones were tested (Appendix Table 23) with the rationalization that perhaps one would not be inhibitory towards Ggt but would be inhibitory towards any or all of the test fungi - specifically F. culmorum, F. graminearum, C. sativus and R. solani. Many phenols and quinones can be potent fungistatic compounds such as chlorogenic acid (Salisbury and Ross,1969). Fungal pigments may be final metabolic products such as toxic wastes or may play an active role in the metabolic ativity of the fungus (Rubin and Artsikhorskaya,1964). Rubin and Artsikhorskaya (1964) also stated that quinone forms of pigments are accompanied by corresponding phenols in various fungi and speculated that perhaps pigments possess antibiotic properties. If this was correct, then perhaps one of the general organic compounds tested would induce Ggt to produce a pigment which would inhibit other fungi but not Ggt. While compounds other than the tyrosine analogues induced pigment production, namely dopamine, chlorogenic acid, catechin and gallic acid, none seriously inhibited the test fungi or Ggt.

The advantages of SM-GGT3 over PDA and PDA containing antibiotics are substantial. SM-GGT3 is selective against bacteria, Oomycetes and many common soil and plant parasitic and saprophytic Ascomycetes and

Basidiomycetes - particularly those causing small grain root and stem diseases. This medium allows Ggt to readily out-compete other fungi. It is then easy to differentiate it via formation of a melanin pigment. Ggt growth and colony characteristics do not appear to be altered by the selective medium. Also, when conducting Ggt pathogenicity tests, results using SM-GGT3 agar discs inoculated with Ggt isolates as inoculum were exactly the same as those using PDA discs - i.e. the seedlings were infected. Thus, one could quickly fulfill Koch's postulates using SM-GGT3 as the isolation medium. To improve upon this method, it would be best to mark the Ggt isolate. One nuclear marker which has been used is a specific requirement for p-aminobenzoic acic (Blanch et al.,1981). The compounds for SM-GGT3 are inexpensive and relatively easy to obtain plus it is not difficult to produce. The shelf life of the medium is at least 3 months.

The only disadvantage of SM-GGT3 may perhaps be its inability to completely inhibit Rhizopus. Rhizopus was never a problem in isolating from plant tissue using this medium and silver nitrate as a surface sterilant. However, in soil assays for Ggt, Rhizopus was and will be a problem until a suitable inhibitory compound is identified. Papavizas and Lusmsden (1982) were able to

significantly improve a selective medium for isolation of Trichoderma from soils infested with Rhizopus by the addition of the surfactant Triton-X-100. In their trials, dicloran and metalaxyl did not inhibit Rhizopus even at 200 ug/ml active ingredient. Quintozene reduced Rhizopus but even at 25 ug/ml active ingredient it also inhibited Trichoderma. These results are similar to those obtained in my study where Triton-X-100 was inhibitory towards Ggt.

It is believed that future work concerning Ggt isolation, either from plant tissue or from soils, should be based on SM-GGT3. A selective medium would also help to quantify the amount of Ggt inoculum in the soil and thus give a basis for long-term forecasting of take-all disease. Currently Ggt is commonly detected in soils indirectly via the use of pathogenicity bioassays. This technique therefore does not allow for the detection of avirulent Ggt isolates (Shipton,1981). Use of SM-GGT3 should accomplish this goal. Past experiments by Asher (1980) have shown an absence of any correlation between virulence and natural pigmentation. If this holds true for chemically induced pigmentation in a selective medium (SM-GGT3), virulent and avirulent isolates could be easily detected and isolated for use in pathogenicity studies. An experiment which should be conducted then

is to obtain known avirulent isolates and determine their growth and pigmentation patterns on SM-GGT3. Another interesting experiment would use two isolates (a virulent and an avirulent) which produce a melanin pigment on SM-GGT3. Mutate them such that both isolates grow on SM-GGT3 but do not produce a pigment. Next, determine if their pathogenic characteristics have been altered. This would help to establish a relationship between pathogenicity and pigmentation.

Chapter 4

Relationship between Sub-crown Internode Ratings and 1000 Kernel Weight

INTRODUCTION

Methods of estimating yield losses caused by take-all in the field are numerous but have the common objective of correlating disease severity assessments with yield loss observations. While yield loss measurements are objective and the techniques established, disease severity assessments are subjective and techniques variable. The most widely used take-all severity assessment method entails the visual estimation of the percentage of blackened roots on a plant (Clarkson and Polley,1981). However, as Clarkson and Polley (1981) point out, this method poses a number of problems. The primary one is the inability to obtain the entire root system when digging or pulling up plants. This results in variation between plants, replicates and even experiments in terms of the amount of root system assessed for disease. This is also impractical in the field and/or with a large numbers of samples.

There is also some doubt as to whether the extent of vascular discoloration is directly related to infection intensity. Deacon and Henry (1978) advocated an assessment method which rated plants on their ability to transport eosin dye past infection sites. This would be impractical for a field survey utilizing mature plants. Therefore, the best method at present is to rate discoloration of the root system. In this study, an attempt was made to identify a quick, easy and accurate method to use for disease assessment that would correlate well with yield loss observations without having to rate the entire root system.

MATERIALS AND METHODS

In 1981 two spring wheat fields in which Ggt had been confirmed were selected as sampling sites. Site 1 was located five miles east of Fairfield, MT. This site has nearly level soils with loam to clay loam surface textures and calcareous surface and subsoil horizons. The cropping history of this long term irrigated field was: 1981=Wampum spring wheat; 1980=barley; 1979=barley; 1978=barley; 1977=alfalfa. Site 2 was located four miles east of Ulm, MT. The soils at this site were coarse textured loamy fine sands or sandy loam surfaces underlain by loamy materials between 50-100 cm. The

cropping history was: 1981=Newana spring wheat; 1980=spring wheat; 1979=spring wheat; 1978=faba beans; 1977=dryland native grass. Both fields in 1981 were under sprinkler irrigation with adequate fertility and crop management according to current recommendations. The two fields were opposites in terms of soils, cropping history and field incidence and severity of take-all. Site 1 had only small, infrequent patches of take-all whereas at site 2, 30%-50% of the field had sterile heads indicating a severe take-all problem.

The single plant sampling technique was utilized in which large numbers of plants in the infected fields were sampled at maturity (Nilsson,1969; Polley and Clarkson,1980). In each field, approximately 100 plants were taken from areas of apparently healthy plants and 100 plants from the take-all patches. This insured having all disease assessment categories adequately represented. Plants were pulled from the soil and placed in paper bags to dry. Mature plants can be air dried and stored indefinitely (Clarkson and Polley,1981).

After two months, the root system was cut from the plant two inches above the crown and the remaining heads of the plant threshed. The root system and threshed grain were placed in the same envelope. Later each plant was rated for disease severity. Heads per plant, kernels

per head and 1000 kernel weight were also recorded for each plant. The sub-crown internode (SCI) for each plant was rated after the method outlined for common dryland root rot (Ledingham et al.,1973):

SCI Rating	SCI Symptoms
1 (Clean)	SCI perfectly clean.
2 (Slight)	SCI with minor isolated lesions or with an overall slight discoloration.
3 (Moderate)	SCI with coalescing lesions.
4 (Severe)	SCI with at least 75% totally blackened.

Correlation and regression statistics were run on SCI ratings and mean 1000 kernel weights for each individual field.

RESULTS

The results are summarized in Tables 11 and 12. The mean 1000 kernel weight for each SCI disease severity category was used rather than the individual plant 1000 kernel weight and SCI rating. It was necessary to measure the correlation between the two variables within a plant population rather than between individual plants. With an individual plant, many other factors besides disease effect yield. These factors are not weighted equally for each plant. Therefore a low correlation would be expected between any two variables for a single plant including SCI rating and 1000 kernel weight. The r

Table 11. The number of Wampum spring wheat plants rated and the mean 1000 kernel weight (TKW) for each sub-crown internode (SCI) take-all disease severity category at field site 1 located near Fairfield, MT in 1981.

SCI Rating	No. Plants	Mean TKW (g)
1 (Clean)	51	36.0
2 (Slight)	28	33.9
3 (Moderate)	20	32.7
4 (Severe)	48	27.8

Table 12. The number of Newana spring wheat plants rated and the mean 1000 kernel weight (TKW) for each sub-crown internode (SCI) take-all disease severity category at field site 2 located near Ulm, MT in 1981.

SCI Rating	No. Plants	Mean TKW (g)
1 (Clean)	24	42.3
2 (Slight)	20	40.8
3 (Moderate)	19	28.5
4 (Severe)	55	25.4

value for single plant assessments for site 1 was -.52 (p=.001) and for site 2 it was -.62 (p=.001).

For a plant population which is randomly sampled, however, these factors do have an equal chance of affecting the total yield. This is the primary reason for using mean 1000 kernel weights for determing the r value. For site 1, the r value for plant population assessments was -0.96 (p=.05). This denotes a very close relationship between the SCI rating and the 1000 kernel weight. The b value was -2.603 indicating there was a 2.6 g loss in 1000 kernel weight for each increase in SCI rating severity. The r^2 value was -0.917 (p=.05) which specifies that 91.7% of the change in 1000 kernel weight was due to the severity of the Ggt disease rating of the SCI. The regression equation and confidence intervals are illustrated in Figure 3. For site 2, the r value was -0.95 (p=.05), b equaled -6.314 and r^2 was 0.906 (p=.05). The regression equation and confidence intervals are illustrated in Figure 4.

DISCUSSION

A very close relationship existed between the SCI take-all severity rating and 1000 kernel weight, which is a yield component. Although the plants in this study were pulled for rating during late dough development and/or ripening, it would be best to rate plants during

Figure 3. Regression equation (▬▬) and confidence intervals (▪▪▪▪▪▪▪▪▪▪▪) for the relationship between take-all sub-crown internode (SCI) severity rating and 1000 kernel weight for Wampum spring wheat in a field located near Fairfield, MT. in 1981. (X=SCI Disease Rating; Y=1000 Kernel Weight)

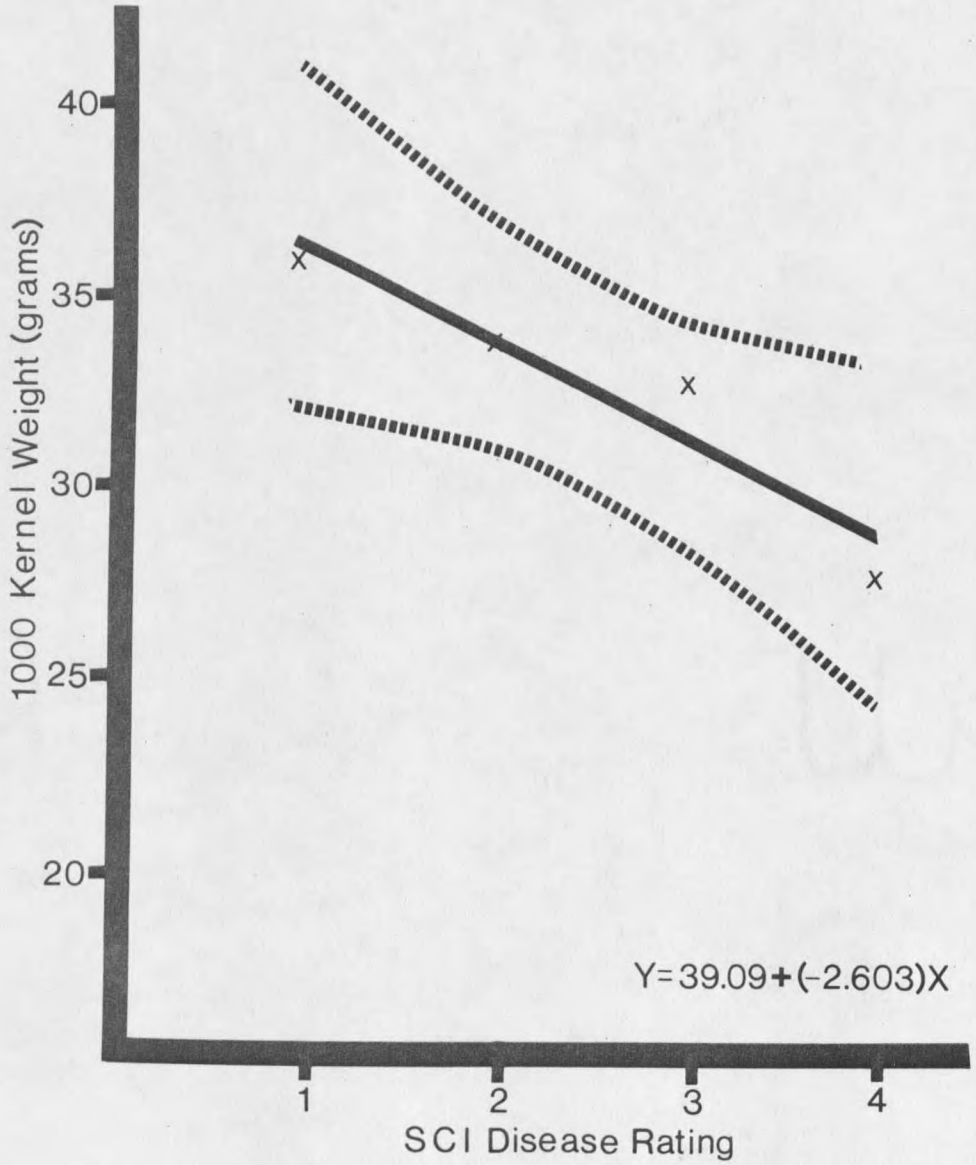
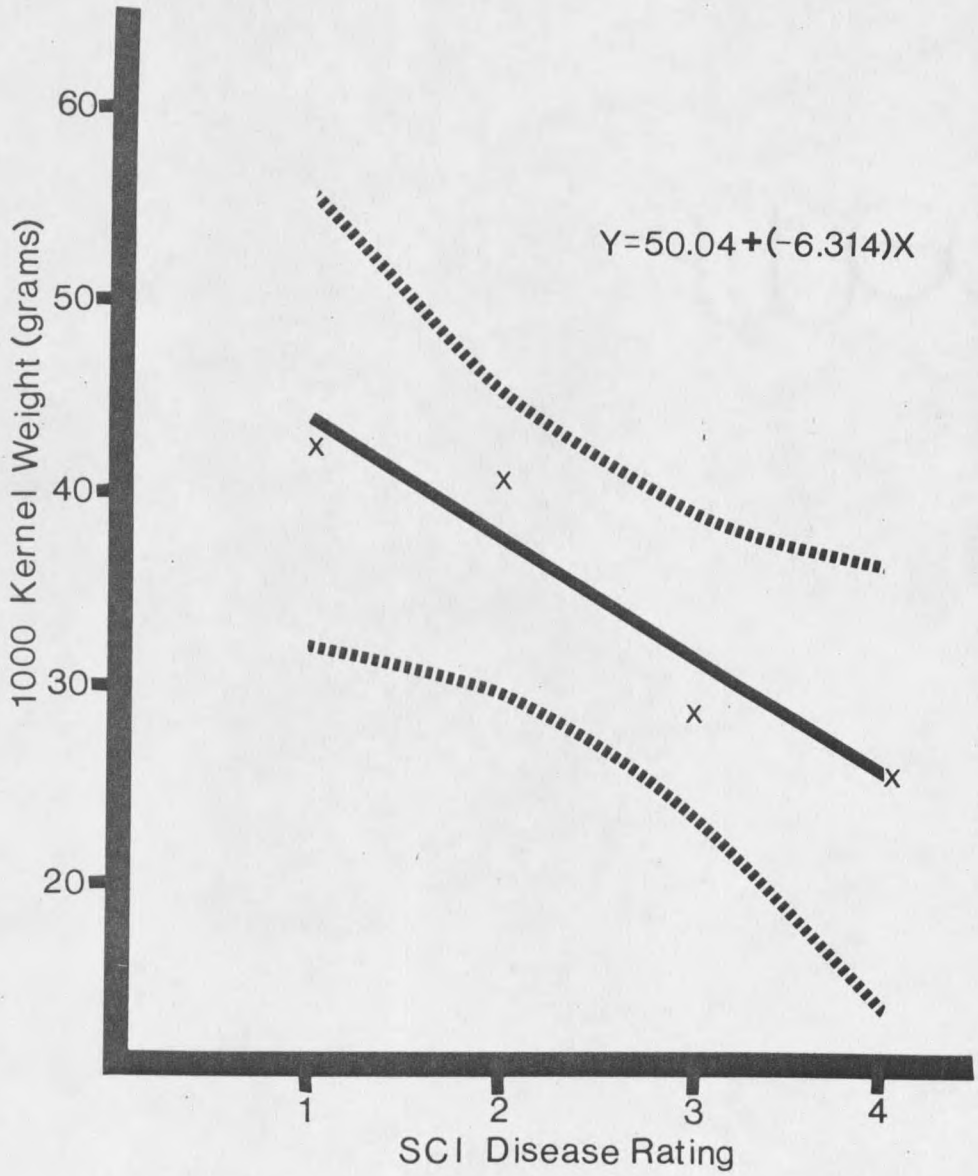


Figure 4. Regression equation (▬▬) and confidence intervals (▪▪▪▪▪▪▪▪▪▪▪▪) for the relationship between take-all sub-crown internode (SCI) severity rating and 1000 kernel weight for Newana spring wheat in a field located near Ulm, MT. in 1981. (X=SCI Disease Rating; Y=1000 Kernel Weight)



anthesis or milk development for two reasons. Late Ggt infections probably have a small effect on the host. Plants rated prior to or at harvest, may have other lesions besides those caused by Ggt due to the ageing of roots or secondary fungal invasion (Clarkson and Polley,1981).

There are a number of reasons why one might advocate the disease assessment method developed in this study. Rating a SCI on a four point scale would be much easier than rating the percentage of blackened roots on a plant and is probably less subjective. Different workers could then adequately compare their results. There was only a 1% difference in the r^2 value for both field sites in my study. Considering the extreme differences between the two fields, these similar r^2 values aid in supporting the usefulness of the SCI disease rating system.

Most workers assess take-all root damage by examining the roots against a white background as the roots float in the water - sometimes with the aid of magnification (Clarkson and Polley,1981). To rate the SCI, it was only necessary to wash the region and observe the extent of lesion development. Although SCI length varied from plant to plant, SCI were quite durable and stood up to much physical handling. Entire plant root systems would be difficult to obtain and would be easily

damaged by transport or handling. This would allow for a considerable amount of variation of assessed roots between plants. To reduce variation with the SCI rating system, it would be best to specify that a SCI to be rated must be at least 2 cm in length. Also, field trials for further evaluation should be planted at a uniform depth of at least 2.5 cm with a reliable planter.

It should also be noted that attack by Ggt on cereals stimulates the production of crown roots (Manners and Myers,1981). The number of additional crown roots varies according to the severity of the attack. The more severe the attack, the more crown roots which develop. Thus, rating the entire root system might not be an accurate assessment of disease severity - especially with young plants.

Other effects Ggt has on the plant are dependent on the severity of the infection. With severe infections, yield, tillering, 1000 kernel weight and the number of kernels per head are all significantly reduced (Cunningham et al,1968; Polley and Clarkson,1980; Manners and Myers,1981). Ggt hyphae are known to invade and disrupt the phloem. This causes a reduction and eventually the cessation of phloem translocation to the root apical meristems and to the shoots. Roots no longer elongate and lesions develop in response to invasion of

the tissue. Damage seems to be related to the rate and frequency at which the lesions develop (Clarkson et al,1975). Extensive plugging of xylem vessels does not occur before ion uptake and translocation ceases to the shoot. Take-all also causes restriction of the water supply to the shoot, especially in severe infections. These physiological effects on a plant infected with Ggt account for the symptoms observed in the field.

Chapter 5

Control of Take-all with Fertilizers

INTRODUCTION

Studies concerning take-all in Idaho (Huber et al.,1968; Huber,1972), Washington (Smiley and Cook,1973) and Oregon (Christensen et al.,1981) have resulted in take-all control recommendations utilizing nitrogen and/or chloride fertilizers. According to their research with nitrogen fertilizers, it is the form of nitrogen (ammonium versus nitrate) which affects the disease severity. Ammonium based fertilizers, such as ammonium sulfate and ammonium phosphate, lower the rhizosphere pH (pH_r) (Smiley and Cook,1973). This has at least three effects on the disease.

One effect may be the direct inhibition of the fungus at a pH_r of less than 5.0 (Smiley and Cook,1973). Secondly, a lowered pH_r may influence the root rhizosphere microflora such that Ggt is subjected to increased antagonism (Smiley,1978a;1978b;1979). A third effect may be the influence of pH on plant nutrients (Cook,1981b). Certain plant nutrients are less available

in alkaline than in acid soils. In general, phosphorus in the soil is most available at a pH of 6.5. Nutrient deficiences of phosphorus, zinc or copper do increase the severity of take-all (Reis et al.,1982). Ammonium fertilizers are also not effected by denitrification or leaching as are nitrate fertilizers. These two processes are most common under high moisture conditions, the result of which can be a crop with a nitrogen deficiency which makes it more susceptible to take-all (Huber,1981b).

With chloride containing fertilizers, it is the chloride ion and not the associated cation which is associated with suppression of take-all. Suppression is proposed to occur via three possible mechanisms. One is the lowering of the chemical potential of water in the roots which in turn reduces the colonization of the roots by Ggt or reduces the host susceptibility (Christensen et al.,1981). This could be accomplished by changing the quantity or nature of root exudates.

This change in root exudates may also account for the second mechanism of suppression - increased antagonism toward Ggt by the root microflora. Halsey and Powelson (1981) indicated that the number of fluorescent pseudomonads increased in soil treated with ammonium chloride and potassium chloride. The third proposed

mechanism of disease suppression by the chloride ion involves inhibition of nitrate uptake by controlling the form of nitrogen absorbed by the plant (Huber,1981b).

A purpose of my research concerning take-all disease suppression with nitrogen and chloride fertilizers was to determine if the results observed in Washington, Oregon and Idaho could be duplicated in Montana under natural field conditions with spring wheat. Only one field study in the USA has been completed with spring wheat (Huber,1972) and it only compared spring versus fall application of ammonium sulfate fertilizer. The other field work has all been done with winter wheat, although the greenhouse work conducted by Smiley and Cook (1973) utilized spring wheat. Work has been done in Europe (Shipton,1972b; Hornby and Brown,1977) and Australia (MacNish,1980) concerning control of take-all in spring wheat with nitrogen fertilizers but I felt a better comparison of results could be conducted using the studies of the Pacific Northwest.

MATERIALS AND METHODS

An experiment was established in 1981 at a site under center pivot irrigation located three miles west of Valier,MT. (SE1/4S33 and SW1/4S34, T19N, R2E). Sunburst clay and pendroy clay soils are common in the immediate area. The sunburst soil is classified in the fine,

montmorillonitic (calcareous), frigid family of Ustic Torriorthents. The pendroy soil is classified in the very fine montmorillonitic (calcareous), frigid family of Ustertic Torriorthents (Soil Survey of Glacier County Area and Part of Pondera County Montana). The field cropping history was: 1980=spring wheat; 1979=spring wheat; 1978=barley; 1977=dryland grain/fallow rotation.

Soil samples were taken before planting of the 0.6 ha experimental area using a King tube. Soil depth increments sampled were 0-15 cm, 0-30 cm, 30-60 cm and 60-120 cm. Soil analysis was conducted by the Montana State University Soil Testing Laboratory, Bozeman, MT. (Kresge,1980). The pH was 7.4 and the organic matter was 2.1%. There were 253 kg/ha of nitrate nitrogen present in the top 122 cm of soil. In the top 15 cm there were 8 ppm phosphorus (Olsen method) and 315 ppm potassium.

An air seeder was used to apply 33 kg/ha K_2O and 67 kg/ha P_2O_5 at a depth of 10-15 cm. Wampum spring wheat was planted 23 April 1981 at a seeding rate of 122 kg/ha with a row spacing of 15 cm at a depth of 6 cm. A Haybuster no-till double disc drill was used for seeding. The seed was treated with 125 g Agsco DB Green (50% maneb and 18.75% lindane) per 1 kg of wheat seed. The phosphorus and potassium fertilizer applications and the seeding were done by the farm site cooperator.

The nitrogen and chloride fertilizers were hand broadcast 26 April 1981 using a split plot experimental design (five replications) with nitrogen treatments as main plots (7.3 m by 12.2 m) and chloride treatments as subplots (3.1 m by 12.2 m). The nitrogen fertilizer treatments are listed in Table 13. A no nitrogen check was included also. All nitrogen treatments were applied at 111 kg/ha nitrogen. The ammonium chloride was not applied until May 13 due to its unavailability on 26 April. However, the delayed application had minimal effect on the experiment as the weather previous to this had been cool and moist. At the time of the ammonium chloride application, the crop had not emerged. The chloride fertilizer treatments were hand broadcast at three different rates (56, 139 and 222 kg/ha chloride) using potassium chloride (47% chloride). Within two days, the plots were sprinkler irrigated with 2 cm water to incorporate the fertilizers.

Table 13. Nitrogen fertilizers used in 1981 for the control of take-all.

Nitrogen Fertilizer	Chemical Formula	% N
Ammonium chloride	NH_4Cl	28
Ammonium sulfate	$(NH_4)_2SO_4$	21
Monoammonium phosphate	$NH_4H_2PO_4$	11
Ammonium nitrate	NH_4NO_3	34
Urea	$CO(NH_2)_2$	45
Calcium nitrate	$Ca(NO_3)_2$	15

A urea and ammonium nitrate liquid fertilizer mixture was applied twice through the irrigation system to the entire experiment at growth stages 23 and 43 (Zadoks et al.,1974) to add an additional 69 kg/ha of nitrogen. The total moisture (stored soil moisture, growing season precipitation plus irrigation) for the crop season was 54 cm. Hoelon wild oat herbicide was unfortunately applied twice to the plot. Once at the recommended rate by the farm site cooperator on 28 May 1981 using a pickup truck sprayer. No herbicide damage was observed after this first aplication. Within the week, the plots were again sprayed with Hoelon at the recommended rate by an aerial applicator. This occurred because the rest of the field was too wet to spray via a ground vehicle due to a rain and snow storm which took place shortly after the first application of Hoelon. Although the growth present immediately after the second spraying was discolored and prostrate, the new growth did not show any ill effects. Bromoxynil herbicide was applied for the control of broadleaf weeds on 25 June 1981.

The rhizosphere pH (pH_r) soil samples were collected 20 June (growth stage 41) and 2 September to 9 September (harvest). Samples were collected from the first four replications by pulling approximately 20 plants from the

outside rows per plot and shaking off excess bulk soil. Rhizosphere soil, soil adhereing to the roots, was brushed from the roots into soil bags which were kept cool until frozen within 4 hours. These soils were later dried, ground, bulked and analyzed for pH, nitrate content and ammonium content. The pH was determined by electrode using the saturated paste method. Nitrate and ammonium content were analyzed by the MSU Soil Testing Laboratory using the chromotropic acid method (Sims and Jackson,1971) and the Berthelot reaction (U.S. Dept. Interior,1969) respectively.

To determine disease severity, sub-crown internode (SCI) ratings were determined for all five replications using the procedure outlined in Chapter 4. Approximately 20 plants per plot were pulled from the outside rows and rated 8 July (growth stage 50) and 2 September to 9 September (harvest). The plants were stored dry in paper bags until rated. An area of 1.5 m by 6.7 m was harvested 2 September at growth stage 91 with a Hege plot combine. Yield, test weight and 1000 kernel weight were determined for each plot.

RESULTS

The results for the chloride rate treatments, averaged over the nitrogen treatments, are given in Table 14. There were no observable differences or

statistically significant differences between the chloride rates for any of the measured variables. There were also no significant differences for interactions between nitrogen and chloride treatments for any of the variables measured.

Table 14. Effect of different rates of chloride on Wampum spring wheat grain yield, test weight, 1000 kernel weight (TKW) and sub-crown internode (SCI) mean disease rating (MDR) of take-all measured on two different dates in 1981.

Rate[1]	Yield	Test Wgt.	TKW	SCI MDR[2]	
kg/ha	q/ha	kg/l	g	July	September
0	29.6 a[3]	0.70 a	33.7 a	2.0 a	2.3 a
56	29.6 a	0.70 a	33.8 a	2.1 a	2.5 a
139	29.9 a	0.70 a	33.6 a	2.2 a	2.5 a
222	28.7 a	0.70 a	32.6 a	2.1 a	2.4 a
LSD	2.7	0.01	1.2	0.2	0.2

1 Rates are for chloride applied as potassium chloride.
2 Rating scale: 1=clean; 2=slight; 3=moderate; 4=severe.
3 Those values followed by the same letter are not significantly different at p=.05 utilizing the Duncan's Multiple Range Test and the LSD value indicated.

With the nitrogen fertilizer treatments, differences were observed both visually and statistically (Table 15). Plots treated with ammonium phosphate could be easily discerned as the plants were taller and darker green through the entire experiment. Ammonium phosphate proved to provide the most consistent responses. It produced

the highest yield and 1000 kernel weight and the second highest test weight. The ammonium sulfate, ammonium nitrate, urea and calcium nitrate treatments were all about equal in terms of all of the responses measured.

Table 15. Effect of different forms of nitrogen fertilizer and no nitrogen on Wampum spring wheat grain yield, test weight, 1000 kernel weight (TKW) and sub-crown internode (SCI) mean disease rating (MDR) of take-all measured on two different dates in 1981.

N Form[1]	Yield	Test Wgt.	TKW	SCI MDR[2]	
	q/ha	kg/l	g	July	September
$(NH_4)_2SO_4$	30.3 ab[3]	0.71 a	34.2 ab	2.3 b	2.4 a
$NH_4H_2PO_4$	35.1 a	0.70 a	34.6 a	2.0 ab	2.3 a
NH_4Cl	24.6 b	0.70 a	33.3 ab	1.9 a	2.3 a
NH_4NO_3	29.2 ab	0.70 a	33.9 ab	2.1 ab	2.4 a
Urea	31.2 ab	0.70 a	33.7 ab	2.0 ab	2.6 a
$CaNO_3$	31.3 ab	0.71 a	33.1 b	2.2 ab	2.5 a
No N	24.4 b	0.68 c	31.1 c	2.0 ab	2.3 a
LSD	6.5	0.02	1.3	0.3	0.3

1 Nitrogen was applied at the rate of 111 kg/ha.
2 Rating scale: 1=clean; 2=slight; 3=moderate; 4=severe.
3 Those values followed by the same letter are not significantly different at p=.05 utilizing the Duncan's multiple range test and the LSD value indicated.

As anticipated, the check treatment with no nitrogen fertilizer added had the lowest yield, test weight and 1000 kernel weight. Interestingly, though, the July and September SCI ratings are not significantly different (p=.05) from the SCI ratings of the other treatments.

The SCI disease ratings are the most perplexing response of the results obtained. The average harvest disease rating was 2.4 placing it midway between the slight and moderate severity category, but, the yields and 1000 kernel weights reflect a severe SCI rating of 3 or 4. In this same field in 1980, most spring wheat plants were rated in the severe categrory and had low yield and 1000 kernel weight responses. This was also observed in 1981 in a spring wheat field adjacent to the field location of the experimental plots. It would seem that the SCI ratings correlate with 1000 kernel weight values in field populations but not in small plot populations.

The rhizosphere soil pH, nitrate content and ammonium content results from the nitrogen fertilizer treatments are summarized in Table 16. There are no apparent differences between nitrogen treatments for pH_r. In general, the pH_r had decreased slightly by the end of the growing season. The ammonium phosphate treatment was the only treatment which raised the pH_r slightly. Although there were differences in nitrate and ammonium content for the different nitrogen treatments, the nitrate to ammonium ratios for each date were quite similar.

Table 16. Effect of different forms of nitrogen fertilizer and no nitrogen on spring wheat rhizosphere soil pH, nitrate content and ammonium content on two different dates in 1981.

N Form	pH		Nitrate (ppm)		Ammonium (ppm)	
	June	Sept.	June	Sept.	June	Sept.
$(NH_4)_2SO_4$	7.8	7.6	16.5	8.0	8.0	3.1
$NH_4H_2PO_4$	7.6	7.8	17.8	10.2	10.2	5.8
NH_4Cl	7.8	7.6	29.0	9.8	12.4	4.7
NH_4NO_3	7.8	7.6	19.5	9.5	6.8	3.7
Urea	7.8	7.5	22.0	8.2	9.1	4.5
$CaNO_3$	7.9	7.8	16.0	8.2	6.9	3.5
No N	7.8	7.8	13.3	6.8	8.7	2.8
Average	7.8	7.7	19.2	8.7	8.9	4.0

DISCUSSION

Neither the grain yield parameters nor the SCI disease ratings were statistically altered by the addition of chloride to the soil. Based on the research and recommendations of Jackson et al. (1980), the most plausible explanation is the fact that the chloride was broadcast over the soil and not banded with the seed at planting. In their report they indicated that 4 years of field experiments showed a reduction in take-all when chloride was banded with the seed.

In an attempt to repeat their results, a field trial was planted at the A.H. Post Agricultural Research Station near Bozeman, MT. in May 1982. Spring wheat plus oat kernel inoculum of Ggt was planted. Potassium

chloride at the four chloride rates used in 1981 was banded one inch above the seed. The Ggt inoculum level was so high, however, that the plants ceased to grow aproximately 30 days after emergence. Visual differences could be observed, though, during the first two or three weeks. Plants in those plots with chloride banded with the seed were a darker green than plants in the check without chloride. The plots with 222 kg/ha chloride had plants with the best appearance.

It should be noted, however, that Christensen et al. (1981) were able to obtain a significant increase in yield and kernel weight by topdressing plots with 101 kg/ha chloride in the spring. Their experiment and those conducted by Jackson et al. (1980) were done on winter wheat in the Willamette Valley of western Oregon where the soil pH averages below 6.0. These two factors, soil pH and crop type, could also account for the deviation of my results from theirs. Additional experiments should be conducted to determine if chloride will reduce take-all in Montana. The experiments should be designed to compare banding alone to broadcasting alone at various rates and to compare rate combinations of banding and broadcasting together.

The primary suggestion gleaned from the nitrogen fertilizer treatment results was that additional

phosphorus, in excess of the recommended amount, increased the yield parameters. The extra phosphorus was obtained by the use of ammonium phosphate. Approximately 212 kg/ha extra phosphorus was added to these plots when 111 kg/ha nitrogen was added as monoammonium phosphate. The responses observed here do agree with those in the literature. Phosphorus fertilizer is most beneficial when adequate nitrogen is present (Stumbo et al.,1942; Syme,1966; Huber,1981b). This situation enhances root growth and thus increases the wheat plant's ability to escape the disease. The plant does not become more resistant to fungal invasion but rather it is, in essence, producing more roots at a faster rate than the fungus can infect them (Huber,1981b).

Phosphorus may have another effect on take-all severity. Graham and Menge (1982) stated that the decrease in disease severity associated with phosphorus was not due to an increase in root growth but rather to improved root phosphorus status and decreased root exudation. This reduced pathogen activity. It has been demonstrated that Ggt hyphae have a positive growth response to wheat roots or their exudates (Pope and Jackson,1973). Phosphorus has also been shown to reduce disease severity of dryland common root rot by inhibiting

the rate of lesion development on the SCI (Verma et al.,1975). Perhaps this is also true for take-all.

The results from my nitrogen fertility treatments do not indicate that either ammonium or nitrate based fertilizer would decrease take-all severity in Montana. Smiley and Cook (1973) and Smiley (1974) found a high correlation existed between pH_r and take-all disease severity in greenhouse studies and field studies for a variety of soils with pH values ranging from 5.5 to 8.0. The pH_r was significantly influenced by the form of nitrogen. In the field experiment conducted by Smiley and Cook (1973), ammonium sulfate with N-Serve 24 (a nitrification inhibitor) decreased the pH_r when compared with the check, whereas calcium nitrate caused pH_r to remain the same. They gave no yield results for this field experiment so it can only be assumed the reduced disease severity due to the ammonium sulfate would increase the yield over the no nitrogen and calcium nitrate treatments. Their field experiment was conducted with winter wheat. The pH_r difference between ammonium sulfate with N-Serve 24 and calcium nitrate treated soils shortly after planting was 1.2 but in April the difference was only 0.5 (Smiley and Cook,1973). This suggests why the nitrogen form may influence disease severity in a field of winter wheat but not necessarily

of spring wheat. The ammonium form will remain prevalent in soils which are cool and treated with N-Serve 24. Thus for a 4 to 5 month period, the ammonium will be the dominant form in the ammonium sulfate treated plots but not in the calcium nitrate treated plots. This will decrease the pH_r and will inhibit Ggt infection in the ammonium sulfate plots. However, the practical aspects were never determined since no yield parameters were given and it is not known how well their disease severity ratings would correlate with the yield.

The experiment conducted by Huber et al. (1968) is equally as interesting when it is examined closely. They also used winter wheat but did not broadcast the two fertilizers, ammonium sulfate and ammonium nitrate, until late March. This would eliminate the problem observed with Smiley and Cook (1973). A portion of their results are shown in Table 17. Differences between the two fertilizer forms were first observed at the rates of 120 and 180 lbs/ac nitrogen. It should be noted that at the higher rates of nitrogen (i.e. >240 lbs/ac of nitrogen) there were no differences between disease severity ratings of the two nitrogen treatments and it is stated in the text that the yield differences were not significant. This high rate (>240 lbs/ac nitrogen) would

be comparable to the amount of nitrogen which was available to plants in my plots.

Table 17. Effects of take-all on irrigated Gaines winter wheat with increasing rates of two sources of nitrogen applied 23 March 1967 (Huber et al.,1968).

Nitrogen Rate	% Whiteheads		Test Wgt. (lbs/bu)	
lbs. N/acre	$(NH_4)_2SO_4$	NH_4NO_3	$(NH_4)_2SO_4$	NH_4NO_3
0	45 d[1]	45 d	----	----
60	30 c	37 c	59.3 d	59.3 d
120	11 ab	37 c	59.3 d	59.3 d
180	8 ab	32 c	58.0 c	59.0 d
240	7 ab	4 a	56.3 b	60.3 e
300	3 a	8 ab	54.6 a	60.0 e

1 Means not followed by the same letter differ significantly at the 5% level of probability.

This suggests another reason why my results may differ from the previous experiments mentioned. Due to the extremely poor wheat crop the previous year, there were 253 kg/ha of nitrate nitrogen present at planting in the first 122 cm of soil. If the form of nitrogen effects pH_r, as indicated by Smiley and Cook (1973), the pH_r would probably not be effected by the meager 111 kg/ha of various ammonium nitrogen forms added to the soil. This was indicated by the average 2:1 nitrate to ammonium ratio for all treatments on both dates. This would account for the lack of differences in pH_r values

and disease severity ratings between the nitrogen treatments. At best the only conclusion which can be safely made based on my results is that the addition of excess phosphorus when adequate nitrogen is present does allow spring wheat to tolerate the disease and so produce higher yields. These yields obtained with excess phosphorus, however, will not be equal to those obtained before take-all became a severe disease problem. Without take-all, it is possible for properly managed spring wheat to yield 6700 kg/ha. The grain yield of the diseased plant can not be improved enough to justify the cost of extra phosphorus. At present, a rotation utilizing barley will probably be the most economical control for take-all.

Chapter 6

Summary

Although take-all disease of wheat was not new to Montana, there had been no research conducted on the disease in this state. Most of the research concerning take-all in the Pacific Northwest had been conducted with winter wheat. These two factors plus the potential severity of the disease prompted my research on the disease and its effect on irrigated spring wheat in Montana.

It was determined via an informal survey that take-all was present throughout the state and was identified in 11 counties. This indicated the range of the disease within the state. Although Ggt was isolated from irrigated and dryland wheat and barley, the disease was determined to be yield limiting only to irrigated spring and winter wheat.

Isolations were accomplished by using the selective medium (SM-GGT3) which was developed. A selective medium for Ggt was needed to improve the isolation, differentiation and identification of the fungus from infected plant tissue. Previously, PDA with or without

antibiotics was used for isolation purposes and failure to isolate Ggt was usually due to bacteria and fast growing fungi. SM-GGT3 increased take-all isolations 32% and virtually eliminated contaminants when compared with PDA. Future work concerning Ggt isolation, either from plant tissue or from soils, should be based on SM-GGT3.

High correlations were observed between 1000 kernel weight and sub-crown internode disease severity ratings for field populations of wheat. The disease severity rating developed was also used in the fertility experiment. Different rates of chloride fertilizer did not influence yields or the disease and neither did the different forms of nitrogen. The only conclusion which can be made based on the results is that the addition of excess phosphorus when adequate nitrogen is present does allow spring wheat to tolerate the disease and so produce somewhat higher yields. However, the phosphorus rate necessary to increase wheat yields is not economical.

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APPENDIX

Tables

Table 18. The effects of the addition of anti-fungal compounds on Gaeumannomyces graminis var. tritici mycelial growth.

Compound	Check Medium	% Check Growth		
		Rate (ug/ml)[1]		
		100	10	1
Piomycin	PBA[2]	105	105	100
Piomycin	MM[3]	0	NT[4]	NT
Polyoxin D	PBA	10	75	75
Polyoxin D	MM	NT	42	NT
Validomycin D	PBA	0	80	85
Validomycin D	MM	NT	22	NT
Blasticin S	PBA	115	105	105
Kasugamycin	PBA	105	95	100
Cycloheximide	PBA	0	0	NT
Dicloran	PBA	15	73	98
Dicloran	PDA	NT	68	NT
Thiram	PBA	0	0	26
Triphenyltin Hydroxide	PBA	0	0	0
Benomyl	PBA	0	0	0
Benomyl	MM	NT	NT	0
Rose Bengal	PBA	0	57	100
Rose Bengal	MM	NT	0	48
Maneb	PBA	0	91	114
Maneb	MM	NT	39	227
Maneb (as Granox)	PDA	NT	119	NT
Triadimefon	PBA	18	86	114
Triadimefon	MM	NT	127	153
Triadimefon	PDA	NT	0	NT
Prochloraz	PBA	0	0	0
Captan	PBA	54	100	109
Captan	MM	0	27	NT
Captan	PDA	NT	53	NT
CGA-62451	PBA	0	0	0
Ethiromil	PBA	95	104	109
Quintozene	PBA	36	91	109
Quintozene	MM	NT	39	227
Quintozene	PDA	NT	84	NT
Thiophenate-methyl	PBA	0	0	91
Thiophenate-methyl	PBA	NT	68	221
TCMTB	PBA	0	11	83
TCMTB	MM	NT	0	11

Table 18. continued

Compound	Check Medium	% Check Growth Rate (ug/ml)[1] 100	10	1
Thiabendazole	PBA	0	0	83
Thiabendazole	MM	NT	0	91
Carboxin	PBA	0	0	50
Carboxin	PDA	NT	21	36
Amphotericin B	PBA	74	94	94
Amphotericin B	PDA	NT	69	NT
Nystatin	PBA	55	87	92
Nystatin	PDA	NT	80	109
Ox Gall Powder	PBA	94	100	NT
Pimaricin	PBA	0	0	111
Pimaricin	MM	NT	0	86
Chloramphenicol	MM	84	91	91
Imazilil	MM	0	0	0
Methfuroxam	PDA	NT	20	81
Metalaxyl	PDA	54	86	NT
Iprodione	PDA	38	59	84
CGA-64250	PDA	0	0	NT
HOE 00703	PDA	18	81	NT
Hexachlorobenzene	PDA	102	109	92
Silver Nitrate	PDA	2	73	NT

1 These were the most frequent rates tested.
2 Potato Broth Agar.
3 Minimal Medium (page 17) with Bacto Agar.
4 Not tested at this rate.
5 Potato Dextrose Agar.

Table 19. Gaeumannomyces graminis var. tritici mycelial growth effects due to the addition of 100 ug/ml of various carbon sources to a base medium.

	% of Base Medium Growth		
Carbon Source	PBA[1]	MM[2]	PMM[3]
Asparagine	94	152	NT[4]
Cysteine	119	110	NT
Cystine	94	94	NT
Methionine	106	NT	NT
Sodium Carboxymethylcellulose	NT	110	NT
Corn Starch	NT	142	NT
Gelatin	NT	140	NT
Xylan	NT	91	NT
Dextrin	NT	93	NT
Cellobiose	NT	98	NT
Galactomannon Polysaccharide	NT	102	NT
Agarose (Indubiose - A37)	NT	96	NT
Urea	NT	NT	94

1 Potato Broth Agar.
2 Minimal Medium (page 17) with Bacto Agar.
3 Minimal Medium with Oxoid Purified Agar.
4 Not tested with this basal medium.

Table 20. Gaeumannomyces graminis var. tritici mycelial growth effects due to the addition of 1000 ug/ml of various carbon sources to a base medium.

Carbon Source	% of Base Medium Growth		
	PBA[1]	MM[2]	PMM[3]
Asparagine	88	133	144
Cysteine	0	113	84
Cystine	119	25	123
Methionine	81	NT[4]	NT
Sodium Carboxymethylcellulose	NT	132	52
Corn Starch	NT	142	81
Gelatin	NT	132	134
Glucose	NT	147	NT
Xylan	NT	93	121
Galactomannon Polysaccharide	NT	105	121
Cellobiose	NT	105	142
Dextrin	NT	91	94
Agarose (Indubiose - A37)	NT	91	119
Sorbose	NT	46	NT

1 Potato Broth Agar.
2 Minimal Medium (page 17) with Bacto Agar.
3 Minimal Medium with Oxoid Purified Agar.
4 Not tested with this basal medium.

Table 21. The effects of various basal media on Gaeumannomyces graminis var. tritici mycelial growth.

Basal Medium	% of PDA[1] Check Growth
1/5 Potato Dextrose Agar	100
Potato Sucrose Agar	100
Potato Broth Agar	100
Acidic Potato Broth Agar[2]	0
Crystal Violet Pectin Agar[3]	100[4]
Czapek Solution Agar	25

1 Potato Dextrose Agar.
2 Acidified to pH 5.5 with 25% lactic acid.
3 Cuppels, D., and Kelman, A. 1974. Phytopathology. 64:468-475.
4 Utilization of pectin occurred.

Table 22. The effects of the addition of anti-bacterial compounds on Gaeumannomyces graminis var. tritici mycelial growth.

	% of PBA[1] Check Growth				
	Rate (ug/ml)				
Compound	500	100	20	10	2
Streptomycin Sulfate	100	100	NT[2]	95	NT
Rifampicin	95	100	NT	95	NT
Oxytetracycline	NT	NT	NT	100	89
Bacitracin	NT	NT	114	NT	NT
Neomycin Sulfate	NT	NT	114	NT	NT

1 Potato Broth Agar.
2 Compound not tested at this rate.

Table 23. The effects of the addition of various levels of organic compounds on Gaeumannomyces graminis var. tritici mycelial growth.

	% of PDA[1] Check Growth				
	Rate (ug/ml)				
Compound	1000	500	100	10	1
Tyrosine	95[2]	91[2]	91	91	NT[3]
L-DOPA	75[2]	100[2]	75	100	NT
D-DOPA	55[2]	100[2]	85	75	NT
L-Methoxytyrosine	111	111	100	100	NT
p-Cresol	0	0	50	85	NT
Arbutin	100	NT	100	100	NT
Salicin	NT	NT	10	100	NT
β-Escin	NT	NT	NT	0	100
Dopamine	NT	86[2]	83[2]	83[2]	NT
Tyramine	100	NT	90	90	NT
Gramine	0	NT	73	87	NT
Scopoletin	NT	NT	NT	70	73
Caffeic Acid	NT	NT	100	100	NT
Chlorogenic Acid	NT	NT	100[2]	100	NT
Catechin	NT	85[2]	100[2]	100[2]	NT
p-Coumaric acid	NT	NT	83	100	NT
Gallic Acid	NT	75[2]	NT	NT	NT
Pyrogallol	NT	0	NT	NT	NT

1 Potato Dextrose Agar.
2 Pigment observed in medium.
3 Compound not tested at this rate.

Juhnke, M. E.
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