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

Chairperson, Graduate Committee

Approved for the Major Department

3-22-83
Date

Head, Major Department

Approved for the College of Graduate Studies

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Date

Graduate Dean

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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