Microbiological Characterization of Montana Soils Supressive and Conductive to Take-All Disease of Wheat Caused by gaeumannomyces graminis var. tritici

by ORLANDO ANDRADE

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology

Montana State University

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Abstract:

Three soils from different wheat growing areas of Montana were characterized as being conducive or suppressive to the take-all disease of wheat caused by the fungus Gaeumannomvces graminis var. tritici (Ggt). Bozeman PF soil where wheat was grown in rotation with green manure exhibited conducive properties toward the disease. Larslan 1 and Toston LS soils where wheat was grown as a monoculture for more than 10 years showed suppressive properties against take-all. Experiments to determine the organisms most probably involved in the biological suppression of take-all exhibited by these soils were conducted.

In vitro and in vivo tests of antagonism involving modified methodology to that commonly described, indicated that two different mechanisms of suppression are involved in both soils. Mycoparasitism is believed to be the main mechanism involved in the suppression exhibited by Larslan 1 soil against Ggt. Two fungi with exceptional ability to reduce the severity of take-all were isolated from this soil. This ability to protect wheat plants from Ggt infection was corroborated in at least six separate experiments, including natural soil conducive to take-all. Neither bacteria nor actinomycetes isolated from the same soil and selected for their in vitro antagonism towards Ggt were as effective in reducing the severity of the disease.

Antagonistic actinomycetes (Streptomyces spp.) plus the likely involvement of Pseudomonas spp. in antagonism and/or iron depletion is believed to be involved in the suppression exhibited by Toston LS soil against take-all. Actinomycetes, either individually or in mixture, consistently increased the plant shoot dry weight when grown in sterile Ggt-infested soil. The magnitude of this increase was similar to that observed when the plants were grown in natural Ggt-infested Toston LS soil. Pseudomonas spp. also reduced the severity of the disease when added in mixtures, but to a lower degree as compared to the actinomycetes.

An association between antagonism and unidentified abiotic factors in soils was also suggested by the results to be involved in the suppression exhibited by Toston LS soil.

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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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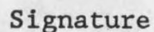

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Date July 24, 1992

I dedicate this thesis to my wife, Alma Cecilia, and to my sons, Daniel Alejandro and Jorge Nicolas, for their tireless support, encouragement, and understanding. This degree is in great part a result of our strong and supportive relationship as a family.

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ABSTRACT

Three soils from different wheat growing areas of Montana were characterized as being conducive or suppressive to the take-all disease of wheat caused by the fungus Gaeumannomyces graminis var. tritici (Ggt). Bozeman PF soil where wheat was grown in rotation with green manure exhibited conducive properties toward the disease. Larslan 1 and Toston LS soils where wheat was grown as a monoculture for more than 10 years showed suppressive properties against take-all. Experiments to determine the organisms most probably involved in the biological suppression of take-all exhibited by these soils were conducted.

In vitro and in vivo tests of antagonism involving modified methodology to that commonly described, indicated that two different mechanisms of suppression are involved in both soils. Mycoparasitism is believed to be the main mechanism involved in the suppression exhibited by Larslan 1 soil against Ggt. Two fungi with exceptional ability to reduce the severity of take-all were isolated from this soil. This ability to protect wheat plants from Ggt infection was corroborated in at least six separate experiments, including natural soil conducive to take-all. Neither bacteria nor actinomycetes isolated from the same soil and selected for their in vitro antagonism towards Ggt were as effective in reducing the severity of the disease.

Antagonistic actinomycetes (Streptomyces spp.) plus the likely involvement of Pseudomonas spp. in antagonism and/or iron depletion is believed to be involved in the suppression exhibited by Toston LS soil against take-all. Actinomycetes, either individually or in mixture, consistently increased the plant shoot dry weight when grown in sterile Ggt-infested soil. The magnitude of this increase was similar to that observed when the plants were grown in natural Ggt-infested Toston LS soil. Pseudomonas spp. also reduced the severity of the disease when added in mixtures, but to a lower degree as compared to the actinomycetes.

An association between antagonism and unidentified abiotic factors in soils was also suggested by the results to be involved in the suppression exhibited by Toston LS soil.

INTRODUCTION

Take-all of wheat, caused by the fungus *Gaeumannomyces graminis* [Sacc.] von Arx & Olivier var. *tritici* Walker (*Ggt*), is probably one of the most important root diseases affecting this cereal worldwide. It also attacks barley, but its effect on this host is less severe than on wheat.

It has been approximately 147 years since the causal agent was first described, and because of the effort, the time, the number of researchers that have been involved in the study of this disease, its worldwide distribution, and economic importance, take-all is considered as a classical plant disease. However, despite all of the above efforts, no effective method of controlling the disease is yet available short of crop rotation.

Little or no resistance to take-all has been found after decades of screening of thousands of wheat lines and cultivars. It is believed that due to the complexity of the plant-pathogen interaction, the chances of finding a substantial degree of resistance seem to be very remote. Occasionally, a modest level of resistance has been reported, but even so, take-all remains one of the most difficult cereal diseases to control by genetic resistance.

Chemical control by the use of fungicides is not showing much promise either, with efforts to date being unsuccessful in providing consistent and effective control of take-all. *Ggt* is very sensitive to

a number of fungicides when tested in vitro. However, a lack of correlation between sensitivity in vitro and control in the field is commonly reported. The effectiveness of sterol-inhibiting fungicides used as a seed treatment to control the disease is widely reported, but for none of them has the yield been close to the non-inoculated checks. Furthermore, in most of the cases the results are not consistent, are seldom reproducible under different environmental conditions, and commonly show high variability among localities, soil conditions, and crop species. This inconsistency of results with chemical treatments has been explained by the discontinuous distribution of the inoculum throughout the soil profile and at various depths in the soil, the variations in the biology of the fungus in different areas, and by the response from the fungicides due to differences in climate, soil physical and chemical characteristics, and soil microbiology. It has also been claimed that most of the tests showing a yield increase and/or reduction of infection involve the use of artificial inoculum which is added in close proximity to the treated seed.

So far, the only recommended method for control of take-all is crop rotation. This method is based on the low competitive ability of the fungus, and its low capacity of survival between seasons in the absence of the host. However, crop rotation is not a feasible alternative in many parts of the world because of economic factors, soil and environmental conditions, and technological aspects.

An interesting alternative for the control of take-all arose after it was observed that some soils were suppressive to the disease. This natural phenomenon, first noticed in the late 1920s, was found to be of

a biological nature from experiments carried out in The Netherlands by Gerlagh in 1968. This finding opened a whole new area of study due to its great potential for the biological control of the disease.

Take-all decline, the term used to describe the natural suppression of the disease, develops under wheat monoculture. This type of suppression is also termed specific suppression, to differentiate it from general suppression (sensu Gerlagh) found in all nonsterile soils.

After determining the biological nature of the suppression of take-all, many theories have arisen to explain the phenomenon. These theories have been grouped into two general categories: microbiological changes in the soil suppressive to the pathogen, and changes within the pathogen itself resulting in a loss of virulence. Even though most of the evidence found so far points to the first category, conclusive results to explain the nature of the phenomenon under all conditions and in all locations do not yet exist.

Of the different groups of microorganisms that have been reported to be involved in take-all decline, the fluorescent pseudomonads have attracted most of the attention of researchers. Their strong inhibition of Ggt when tested in vitro, and some control of the disease in the field when applied to the seed, have made this group of organisms one of the most probable candidates responsible for the suppression of take-all. The fungus Phialophora radicicola is also known to protect wheat crops in a cross-protection type of mechanism, in England, when planted after a grass ley. However, it is not considered to be associated with take-all decline. Bacillus spp.,

Trichoderma spp., and giant vampyrellid amoeba are other microorganisms that have been reported to be associated with soils suppressive to take-all.

Despite the lack of success in explaining the true nature of take-all decline, a considerable amount of effort is still being directed to understand this phenomenon. Efforts to solve the many unknowns underlying take-all decline could considerably increase the chances of obtaining an effective and durable control of the disease through biological methods.

There are still many questions regarding take-all decline that remain to be answered. Is take-all decline a phenomenon in which the same mechanism of suppression is involved worldwide? Due to the fact that soils suppressive to the disease have been found widely distributed around the world under very different environments, it seems unlikely that the same mechanism could be responsible everywhere. Does take-all decline arise as a consequence of the build-up of an antagonist microflora composed of only one group of organisms? The microbial interaction in soils is so complex that it seems unlikely that only one group of microorganisms could be involved in this phenomenon. The finding that different organisms are associated with take-all decline soils is perhaps an indication that the same mechanism of suppression may not be operating in all soils. Are antibiosis, iron depletion by siderophore-producing organisms, and competition for nutrients likely explanations for take-all decline? It is known that antibiosis *in vitro* has little or no correlation with suppression of

the pathogen in the soil, and also that siderophore-producing organisms are present in soils not suppressive to take-all.

More research and newer approaches to the study of this problem in areas with different environments are needed. This would facilitate an analysis based on similarities and differences among soils. It not only could help to accumulate more and new information about this fascinating phenomenon, but eventually it could benefit those areas where the application of results may be specific for those environments.

Based on these assumptions, research on take-all suppressive soil was conducted in Montana. The objectives of this study were:

- To determine if soils suppressive to take-all occur in Montana,
- To characterize microbiologically suppressive Montana soils,
- To determine the organisms most probably involved in the suppression of the disease in the soils under study.

SCREENING AND SELECTION OF MONTANA SOILS SUPPRESSIVE AND CONDUCIVE TO TAKE-ALL OF WHEAT

Introduction

The diminution in the severity of take-all under monoculture of wheat is reported to have first been noted by Glynne, in England, about 1935 (Rovira and Wildermuth, 1981; Cook and Weller, 1987). But it was the work of Slope and Cox in 1964 (Hornby, 1979) which provided convincing experimental evidence that the decline of take-all under wheat monoculture definitely occurs.

After the biological nature of take-all decline was experimentally demonstrated for the first time by Gerlagh in 1968 (Rovira and Wildermuth, 1981; Cook and Weller, 1987), great attention was attracted to this new field, which seemed to offer an innovative approach in the control of this disease.

Following the work of Gerlagh, many theories have arisen to explain the nature of the take-all decline phenomenon (Hornby, 1983; Cook and Weller, 1987). However, most of the evidence found so far points to microbiological changes in the soil suppressive to take-all as the most probable explanation of the nature of the phenomenon (Cook and Weller, 1987; Rovira and Wildermuth, 1981). Changes in the virulence of the pathogen were found not to be responsible for take-all decline (Cook, 1981; Cook and Naiki, 1982). Among the groups of organisms found associated with soils suppressive to take-all,

pseudomonads are believed to play the major role in the decline of the disease (Cook and Rovira, 1976; Kloepper et al.,1980; Smiley, 1979; Weller and Cook, 1983; Wong and Baker, 1984).

Most of the evidence that supports the theory that wheat monoculture builds up an antagonist microflora comes from numerous studies on soils suppressive to take-all in which a decline in the severity of the disease has been consistently observed to be associated with continuous cropping of wheat (Shipton, 1975; Brisbane and Rovira, 1988). The opposite has been reported to occur in virgin soils where severe take-all is recorded after two or three consecutive years of wheat cropping. The suppression of the disease is also absent in double-cropped fields (Shipton et al., 1973; Rothrock and Cunfer, 1985).

After the work of Shipton et al. (1973), most experiments conducted to demonstrate the suppressive properties of soils to take-all are based on the transferability of the suppressiveness, commonly to steam treated or sterile soils (Gerlagh, 1968; Shipton et al., 1973; Cook and Rovira, 1976). This assay has been found to correlate well with what is observed in the field, providing a background soil with constant chemical, physical, and biological characteristics, into which a minute amount of test soil can be introduced without changing those properties, except for the biological properties (Rovira and Wildermuth, 1981). This methodology has also been used to differentiate general suppression (sensu Gerlagh) from specific suppression, by using differential temperature treatments. General suppression is present in all nonsterile soils in the absence of take-

all, is sensitive to heat treatment at 121°C but not to steam treatment at 70°C for 30 min, and is not transferable. On the other hand, specific suppression, or antagonism, arises under wheat monoculture, is eliminated by 60°C steam heat, and is transferable to sterile soils when added at levels as low as 1% (Shipton et al., 1973; Cook and Rovira, 1976).

A disease index based on root blackening, or the dry weight of wheat leaves are the two most common indices used to measure the expression of the disease in pot assays for suppression of take-all (Cook and Rovira, 1976; Shipton et al., 1973; Rothrock and Cunfer, 1985; Sivasithamparam and Parker, 1978; Weller et al., 1985; Brisbane and Rovira, 1988; Asher, 1972; Manners and Myers, 1981). While root blackening is the most characteristic symptom that allows the identification of the pathogen and the disease, stunting and reduction in shoot weight are the direct result of the infection of the plant (Asher, 1972; Fitt and Hornby, 1978; Sivasithamparam and Parker, 1978). A strong correlation between level of infection and decrease in shoot weight has been reported to occur (Powelson, cited by Kollmorgen, 1985). Despite the subjectivity of the disease index methodology, it and dry weight reduction both have been observed to accurately reflect the effect of the disease (Poplawsky and Ellingboe, 1989).

The main objective of this study was to determine if soils suppressive to take-all occur in Montana, which could be used for further microbial characterization of this phenomenon.

Materials and Methods

Soil Sampling

Soils suspected to have suppressive (SS) characteristics to take-all were collected from different Montana wheat growing areas, from fields with a history of wheat monoculture, and where low level of the disease had been observed. Conducive soils (CS) to take-all were collected from fields where wheat is grown in a system of crop rotation, and where high levels of the disease had been observed.

Bulk soil was randomly collected from the top 20 cm, placed in plastic containers, and stored without being dried in a cool room (3°C) until use.

Inoculum Preparation

A single pathogenic isolate of Ggt collected from artificially infected plants was utilized in all the experiments directed to assess SS and CS soils.

Ggt was grown in a regular strength PDA medium (6 g of smashed potatoes which were boiled for 2 min and filtered through 4 layers of cheese cloth, 12 g Dextrose, and 18 g Difco Agar [Sigma Co.]) for 10 days, prior to inoculation of flasks containing sterile oat kernels prepared as follows:

A volume equivalent to 900 ml of oat kernels of homogeneous size (3.35-4.0 mm) were placed in 1 l Erlenmeyer flasks, washed twice with warm tap water, and rinsed once with distilled water. The flasks were filled with distilled water and left 10 hr at room temperature.

Thereafter, the excess water was poured out and the flasks were autoclaved twice at 121°C for 20 min, with 24 hr elapsing between autoclaving.

When cool, the flasks were inoculated with the fungus by using a 20 cm sterile glass tube to place 1-2 PDA discs 5 cm above the bottom of the flask, and in 5 to 6 different points around the flask. After 30-35 days at room temperature, the axenically colonized oat kernels were removed and distributed in layers 2 cm thick on disinfected plastic trays and allowed to dry for 48-72 hrs at room temperature, in an environment of low air movement.

Once dried, the kernels were blended for 12 sec at low speed and 10 sec at high speed (Waring commercial blender, Waring Prod. Division, Conn., USA), sieved to a particle size of 0.5-1.0 mm, and stored in sterile flasks at 5°C for further use as inoculum.

Experimental Protocol

All experiments were carried out in a glasshouse in the MSU Plant Growth Center at Bozeman, Montana.

Soils to be used for tube assay tests were sieved (2 mm), placed in a 2 cm thick layer on large, clean, plastic trays, moistened with tap water, and air-dried for 48 hrs in the greenhouse prior to autoclaving.

Soils were autoclaved at 121°C for 30 min, in 2 l, autoclavable, plastic containers, covered with aluminum foil.

Experiments were carried out by using the tube assays as described originally by Wilkinson et al. (1985), and modified later by Weller and

Cook (1985), except that no vermiculite was added. In brief, plastic conetainers (2.5 cm diameter x 12.5 cm long) containing a cotton ball placed at the bottom were filled with an 8-cm-thick column of soil, and distributed in a 200-conetainer plastic rack. Treatments amended with 1% of nontreated soil, were hand-mixed in previously disinfected plastic containers.

A single disinfected seed (18% ethanol + 0.4% sodium hypochlorite for 2 min), of Pondera spring wheat sieved for homogeneous size was planted in each conetainer. A 1-cm-layer of sterile white sand was added over the soil to avoid contamination at irrigation. Conetainers were watered with nutrient solution (20-20-20 NPK Peter's solution + micronutrients made up in distilled water) every 3 days. The purpose of using distilled water in all the experiments was to avoid the probable deleterious effect on the microorganisms of the chlorine contained in the tap water.

After 30 days, the seedlings were washed from the soil. The shoots were excised at the crown, placed into individual paper envelopes, and dried at 70°C for 72 hrs.

Screening for SS and CS Properties of the Soils

Two individual experiments, experiments 1 and 2, respectively, were performed for each soil in an initial screening to determine their suppressive or conducive properties. For each experiment, the treatments were as follows: sterile soil with/without inoculum; sterile soil + 1% of untreated (natural, air-dried) soil with/without inoculum; and untreated soil with/without inoculum. All the treatments within

each experiment were applied to 8-10 conetainers, arranged in a completely randomized design.

The level of disease, or effectiveness of the infestation, was determined by comparing the treatments with sterile soil with/without inoculum. The SS and CS properties of the soils were determined by comparing both the treatments with sterile infested soil with/without 1% of untreated soil, and the treatments involving untreated soil with/without inoculum.

Soils selected during the first screening for the desired characteristics were tested again in two independent experiments, experiments 3 and 4, respectively, with 12 to 35 replications per treatment. The same treatments as described in the first screening were applied in experiment 3. In experiment 4, the treatments with untreated soil were excluded. Both experiments were established in a factorial arrangement and analyzed accordingly.

Transfer of Suppressive Factors Among Soils

Three independent tube assay experiments were conducted to observe the transferability of the suppressive factor to take-all among the selected soils. The treatments were similar to the ones described for the screening tests, except that 1% of each different untreated soil was added to every other soil being tested. Checks without added inoculum were included for each inoculated treatment. The experiments were arranged in a completely randomized design with 12-15 replications per treatment.

The main purpose of these experiments was to determine whether the transferable suppressive factors to take-all may act interchangeably in other foreign soils.

Statistical Analysis

Analysis of variance and multiple comparisons were performed by using the MSUSTAT Program, version 5.01 (R. Lund, 1991), using the dry weight data.

Results

Screening for SS and CS Properties of the Soils

An adequate level of disease was obtained at the rate of 0.1% w/w of inoculum. All the soils showed a similar response to the addition of *Ggt* inoculum to sterile soil (Table 1). A severe rottening of the roots and a significant diminution in dry weight were consistently observed. These results corroborated preliminary experiments performed to determine the most suitable level of inoculum to be added to the test soils (data not shown). After 30 days growth, none to very few of the plants died in the treatments with sterile soil inoculated with 0.1% of *Ggt* inoculum. At higher levels of inoculum (0.2%, 0.4%, and 0.5% w/w) many plants died prematurely. At 0.05%, the level of disease was too low to provide reliable results.

All the tested soils, except Bozeman PF, exhibited a transferable suppressive factor sensitive to heat sterilization. However, the degree of suppressive response showed significant variability among soils (Table 1).

Table 1. Treatment means of wheat plant dry weight data collected from experiments involving Montana soils screened for suppressiveness to the take-all disease of wheat.

		Soil					
		Bozeman PF		Larslan 1		Toston LS	
Treatments	Ggt[a]	Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Sterile soil	−	79[b] A	78 A	71 A	69 A	51 B	62 AB
Sterile soil	+	36 CD	34 D	24 D	27 D	36 C	35 D
Sterile soil + 1% untreated soil	−	75 A	76 A	70 A	69 A	82 A	62 A
Sterile soil + 1% untreated soil	+	33 CDE	46 C	58 B	44 C	73 A	54 C
Untreated soil	−	55 BC	61 B	49 C	58 B	77 A	55 BC
Untreated soil	+	27 DE	49 C	44 C	50 C	72 A	50 C
LSD[c] 0.05		12	6.6	8.3	6.6	12	7

Table 1. (Continued).

Treatments	Ggt	Soil						
		Larslan DL		Larslan EB		Worral 2	Worral 1	Tarum's
		Exp. 1	Exp. 2	Exp. 1	Exp. 2	Exp. 1	Exp. 1	Exp. 1
Sterile soil	–	95 A	76 A	78 A	63 A	65 A	83 A	61 C
Sterile soil	+	46 C	38 D	18 C	31 D	50 B	32 D	34 D
Sterile soil + 1% untreated soil	–	90 A	72 A	80 A	65 A	77 A	74 B	78 B
Sterile soil + 1% untreated soil	+	45 C	53 BC	39 B	39 C	65 A	47 CD	60 C
Untreated soil	–	76 B	59 B	79 A	48 B	71 A	68 AB	96 A
Untreated soil	+	57 C	50 C	77 A	43 BC	71 A	61 BC	36 D
LSD 0.05		14	6	17	7	14	19	10

[a]: Rate of inoculum: 0.1% w/w.
[b]: Shoot dry weight (mg) measured after 30 day growth.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Statistically significant differences were found in all the tested soils between the treatments established to determine the transferability of suppressive factors. Even though plants grown in most of the sterile inoculated soils showed a higher shoot dry weight as compared to sterile inoculated soils without the addition of 1% of untreated soils, the magnitude of this response was also different among the soils (Table 1).

Plants grown in Bozeman PF sterile soil showed the lowest response to the addition of untreated soil. The increase in shoot dry weight observed in this treatment was quite small, and was statistically significant in only one of the two experiments.

Larslan DL and Larslan EB soils showed a slight increase in shoot dry weight in one of the experiments. A stronger response to the addition of untreated soil was observed in a second experiment with these two soils.

Larslan 1 and Toston LS soils consistently showed a significant increase in the shoot dry weight after the addition of untreated soil. The consistency of these results was observed in all the treatments, and in both experiments.

Worral F2 and Worral P1 were tested only once after detecting a high number of plants showing chlorosis and distortion, similar to the damage caused by some herbicide.

Plants grown in Tarum's soil showed an inconsistent reaction, increasing in dry weight significantly after the addition of untreated soil to sterile soil, but showing a dramatic decrease in the same parameter in the treatment with untreated soil infested with Ggt.

In almost all of the experiments no statistical differences were observed in plant dry weight between treatments of sterile-noninfested soil with/without 1% untreated soil. This suggests that no nutritional factors were carried by the minute addition of untreated soil that could explain the increase in shoot dry weight. Statistical differences were observed in some experiments between sterile versus untreated-noninfested treatments, probably due to a general deleterious microflora competing for nutrients, or to subclinical pathogens. Roots infected from natural inoculum of Ggt were rarely found in the untreated-noninfested soil treatments, that could explain the lower values of shoot dry weight referred to above.

Based on the consistency of the results in experiments 1 and 2, and on the level of SS and CS observed, Bozeman PF, Larslan, and Toston LS soils (Table 2) were selected for a second test to corroborate their SS and CS properties against take-all.

Experiments 3 and 4 (Table 3) conducted with these three soils, showed the same pattern as observed in the first screening. The statistical analysis indicated that the three soils responded differentially to the addition of 1% untreated soil, with Bozeman PF showing the least response (Table 4). The same differential response was observed when comparisons are made between untreated soil with/without infestation with Ggt in all 4 experiments conducted with these soils (Figure 1). The shoot dry weight was significantly more affected when Bozeman PF untreated soil was infested with Ggt, than in the case of Larslan 1 and Toston LS soil.

Table 2. Location and soil analysis of three Montana soils selected for characterization of their suppressiveness to the take-all disease of wheat.

	Soils		
Location/Analysis	Bozeman PF	Larslan 1	Toston LS
Location			
County	Gallatin	Valley	Broadwater
Area	Bozeman, MT	Larslan, MT	Toston, MT
Cropping system	wheat alfalfa alfalfa fallow	spring wheat 14 years monoculture	spring wheat 10 years monoculture
Soil Analysis*			
pH 2:1	7.2	7.8	8.3
EC 2:1 mmhos/cm	0.13	0.39	0.16
Organic matter	2.33	2.06	1.75
K mg/kg	488	422	466
NO3-N mg/kg	20.8	67.1	16.6
H20 1/3 Bar %	34.1	21.4	25.5
P Olsen mg/kg	38.7	30.3	19.3
Cu mg/kg	1.9	0.6	1.3
Fe mg/kg	19.2	18.7	2.2
Mn mg/kg	14.4	11.2	10.8
Zn mg/kg	0.6	3.7	1.3
CaCO3 Equiv. %	0.1	0.1	8.3
Texture			
Sand %	9	54	38
Clay %	35	20	21
Silt %	56	26	41
	silty clay loam	sandy loam/ sandy clay loam	loam

* Soil analysis carried out by the Soil Testing Lab, Department of Plant and Soil Science, Montana State University, Bozeman, MT.

Table 3. Treatment means of shoot dry weight data of wheat plants grown in three soils selected for their ability to suppress the take-all disease of wheat.

Treatments/Treatment Combinations		*Ggt*[a]	Exp. 3 Shoot dry weight (mg)	Exp. 4 Shoot dry weight (mg)
Soils	Bozeman PF	+/- [b]	53 C	114 C
	Larslan 1	+/-	74 B	133 B
	Toston LS	+/-	89 A	143 A
	LSD[c] 0.05		3.3	7.9
Treatment Combinations				
	Sterile soil	-	97 B	184 A
	Sterile soil	+	30 E	47 C
	Sterile soil + 1% untreated soil	-	104 A	186 A
	Sterile soil + 1% untreated soil	+	61 D	103 B
	Untreated soil	-	74 C	NT[d]
	Untreated soil	+	65 D	NT
	LSD 0.05		4.7	9.1

[a]: Rate of inoculum: 0.1% w/w.
[b]: +/-: Include *Ggt*-infested/noninfested treatments.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.
[d]: Treatments not tested.

Table 4. Multiple comparisons among the three soils selected for their ability to suppress take-all, in treatments designed to observe the transferability of suppressive factors.

Treatments	Ggt[a]	Experiment 3		Experiment 4	
		Shoot dry weight (mg)	Diff.[b] %	Shoot dry weight (mg)	Diff. %
Bozeman PF	+	28 D	-	51 D	-
Bozeman PF + 1% untreated soil	+	39 C	39	72 C	41
Larslan 1	+	26 D	-	43 D	-
Larslan 1 + 1% untreated soil	+	54 B	108	111 B	158
Toston LS	+	34 CD	-	51 D	-
Toston LS + 1% untreated soil	+	91 A	168	126 A	147
LSD[c] 0.05		8.1		15.4	

[a]: Rate of inoculum: 0.1% w/w.
[b]: Increase in shoot dry weight expressed in percentage as compared to the same soil but without adding 1% untreated soil.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

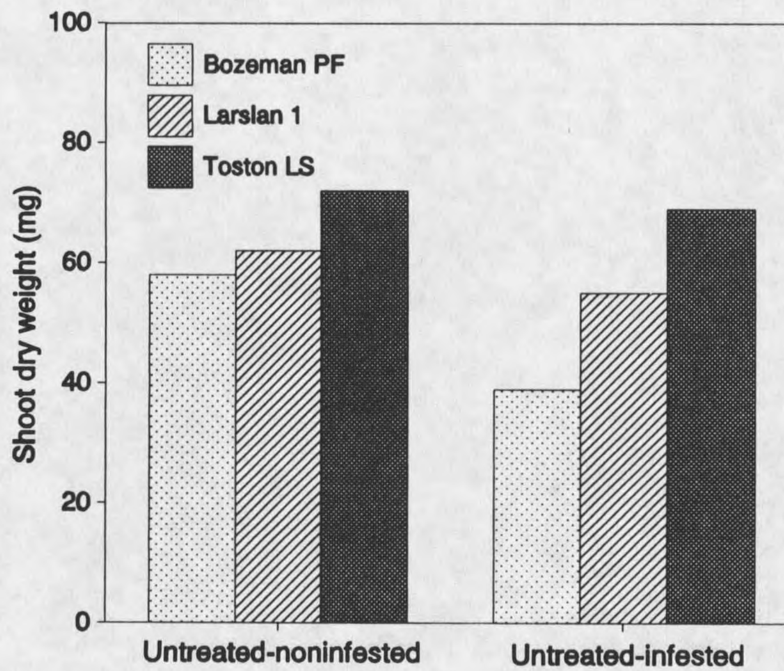

Figure 1. Shoot dry weight of wheat plants grown in untreated Bozeman PF, Larslan 1, and Toston LS soils infested with Gaeumannomyces graminis var. tritici. Average from experiments 1, 2 and 3 (Tables 2 and 4).

Transfer of the Suppressive
Factors Among Soils

Wheat plants growing in all three selected soils showed a significant increase in shoot dry weight when the soils were the recipient of 1% untreated foreign soil. However, the magnitude of the response differed (Tables 5, 6, and 7).

Toston LS exhibited the strongest reaction, with a significant increase in shoot dry weight, when it received either 1% of untreated Bozeman PF or 1% untreated Larslan 1 soil (Table 5). In this experiment, an unexpected response was observed with the addition of 1% of Bozeman PF untreated soil. The significant increase in shoot dry weight observed when untreated Bozeman PF soil was added to sterile Toston LS soil seems to indicate that the expression of antagonism may have an abiotic component, which in the case of Bozeman PF soil is absent, but in the case of Toston LS soil is present. This assumption is based on the lack of response observed when 1% of untreated Bozeman PF soil is added to its own sterile soil but occurs when Bozeman PF soil is added to Toston LS soil.

Plants grown in Larslan 1 soil quantitatively showed the least response to the addition of either Bozeman PF or Toston LS untreated soil (Table 6). Plants grown in Bozeman PF soil had a reaction in between the one exhibited by plants growing in the other two soils when they were a recipient of the other two untreated soils (Table 7).

Analyzing the responses observed in these experiments, and the responses produced in all three soils in the screening tests (Figure 2), it appears that Bozeman PF untreated soil quantitatively

Table 5. Effect of the addition of 1% of sterile and untreated Bozeman PF and Larslan 1 soils to Toston LS sterile soil, on shoot dry weight of wheat plants grown in infested or noninfested soil with Gaeumannomyces graminis var. tritici.

Treatments	Ggt[a]	Shoot dry weight (mg)	Difference[b] %
Toston LS	-	154 A	-
Toston LS + 1% Bozeman PF sterile	-	151 A	-
Toston LS + 1% Bozeman PF untreated	-	143 A	-
Toston LS + 1% Larslan 1 sterile	-	148 A	-
Toston LS + 1% Larslan 1 untreated	-	146 A	-
Toston LS	+	34 E	-
Toston LS + 1% Bozeman PF sterile	+	41 E	21
Toston LS + 1% Bozeman PF untreated	+	113 B	232
Toston LS + 1% Larslan 1 sterile	+	55 D	62
Toston LS + 1% Larslan 1 untreated	+	101 C	197
LSD[c] 0.05		11	

[a]: Rate of inoculum: 0.1% w/w.
[b]: Percent increase in shoot dry weight as compared to Toston LS sterile Ggt-infested soil treatment.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 6. Effect of the addition of 1% of sterile and untreated Bozeman PF and Toston LS soils to Larslan 1 sterile soil, on the shoot dry weight of wheat plants grown in infested or noninfested soil with Gaeumannomyces graminis var. tritici.

Treatments	Ggt[a]	Shoot dry weight (mg)	Difference[b] %
Larslan 1	-	139 B	-
Larslan 1 + 1% Bozeman PF sterile	-	151 A	-
Larslan 1 + 1% Bozeman PF untreated	-	153 A	-
Larslan 1 + 1% Toston LS sterile	-	157 A	-
Larslan 1 + 1% Toston LS untreated	-	148 AB	-
Larslan 1	+	52 E	-
Larslan 1 + 1% Bozeman PF sterile	+	48 E	0
Larslan 1 + 1% Bozeman PF untreated	+	74 D	42
Larslan 1 + 1% Toston LS sterile	+	48 E	0
Larslan 1 + 1% Toston LS untreated	+	87 C	67
LSD[c] 0.05		11	

[a]: Rate of inoculum: 0.1% w/w.
[b]: Percent increase in shoot dry weight as compared to Larslan 1 Ggt-infested treatment.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 7. Effect of the addition of 1% of sterile and untreated Larslan 1 and Toston LS soils to Bozeman PF sterile soil, on the shoot dry weight of wheat plants grown in infested or noninfested soil with Gaeumannomyces graminis var. tritici.

Treatments	Ggt[a]	Shoot dry weight (mg)	Difference[b] %
Bozeman PF	-	118 BC	-
Bozeman PF + 1% Larslan 1 sterile	-	134 A	-
Bozeman PF + 1% Larslan 1 untreated	-	120 BC	-
Bozeman PF + 1% Toston LS sterile	-	126 AB	-
Bozeman PF + 1 Toston LS untreated	-	112 C	-
Bozeman PF	+	29 G	-
Bozeman PF + 1% Larslan sterile	+	49 E	69
Bozeman PF + 1% Larslan untreated	+	73 D	152
Bozeman PF + 1% Toston LS sterile	+	39 F	34
Bozeman PF + 1% Toston LS untreated	+	82 D	183
LSD[c] 0.05		9.6	

[a]: Rate of inoculum: 0.1% w/w.
[b]: Percent increase in shoot dry weight as compared to Bozeman PF Ggt-infested treatment.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

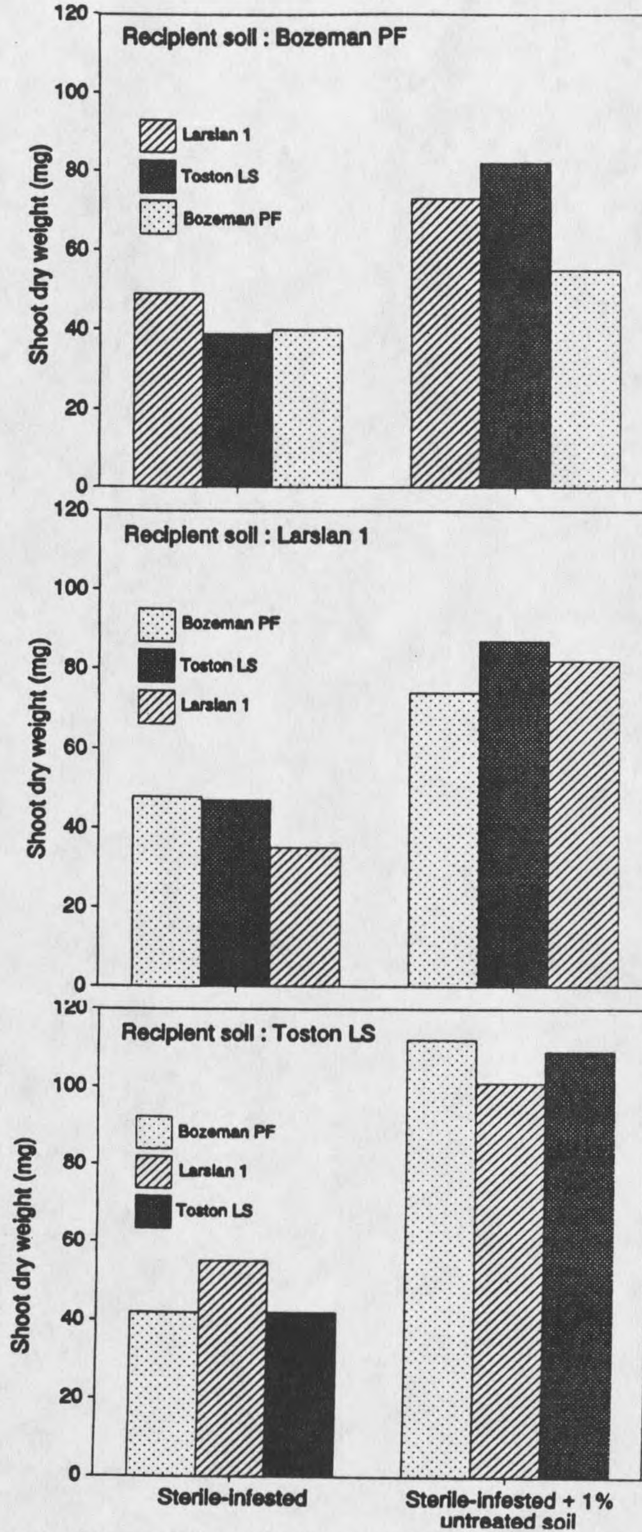

Figure 2. Shoot dry weight of wheat plants grown in sterile Bozeman PF, Larslan 1, and Toston LS soils infested with Gaeumannomyces graminis var. tritici, affected by the addition of 1% of different treated soils.

induces a lower suppression of take-all in its own soil, as compared to the response induced by the addition of the other two soils in the same Bozeman soil. Larslan 1 soil expressed in its own sterile soil a stronger response than did the other two soils, and Toston LS soil induced essentially the same high response in its own soil, as did the addition of the other two soils. These results suggest that the biotic factors involved in the suppression of take-all may have an abiotic component that affects the expression of biotic antagonism against the fungus, since the conducive Bozeman PF soil appears to have an antagonistic microflora against Ggt that is activated when transferred to a different soil.

Little or no effect on shoot dry weight of plants grown in the recipient soils was observed when amended with 1% of sterile foreign soils. Plants growing in Bozeman PF and Toston LS soils showed a slight increase in dry weight when 1% of sterile Larslan 1 soil was added to them. It is difficult to determine which factor transmitted in such a minute amount of soil could induce this difference. The soil analysis given in Table 1 indicates that NO_3-N and Zn would probably be the only two candidates to explain these differences.

Discussion

From eight Montana soils used for wheat production analyzed to determine their suppressive or conducive properties toward take-all, two of them, Larslan 1 and Toston LS from fields in wheat monoculture for many years, were selected for their suppressive characteristics. A third soil, Bozeman PF where wheat is grown in rotation with a green

manure crop, was selected because of its conducive characteristics against take-all.

All three selected soils carry a microflora suppressive against take-all, which when eliminated by sterilization is unable to express antagonism against the fungus, resulting in severe symptoms of the disease. Soils under continuous wheat cropping, as is the case of Larslan 1 and Toston LS, were more suppressive to take-all than Bozeman PF, which came from a wheat/alfalfa rotation. Similar results have been observed by Cook (1981), Rothrock and Cunfer (1985), Shipton (1975), Shipton et al. (1973), and Simon et al. (1987).

The results from all the experiments conducted with Bozeman PF, Larslan 1, and Toston LS soils, suggest that the former carries only a minimal suppressive ability against take-all. In contrast, Larslan 1 and Toston LS soils carry a highly transferable suppressive ability which significantly reduced take-all severity when sterile soils were amended with 1% of untreated soil. Furthermore, plants grown in untreated Larslan 1 and Toston LS soils infested with Ggt were significantly less affected than those grown in untreated Bozeman PF soil.

Because the treatments were not directed to determine the form of suppression present in the soils under study, I can not define their suppression as either general or specific. However, because of what was observed in all of the experiments with these soils, and also due to the fact that the results obtained correlate with the observations and information obtained from these fields (Dr. D. E. Mathre and Dr. W. Grey, Dept. of Plant Pathology, MSU, personal communication),

Toston LS and Larslan 1 can be defined as a SS soils, and Bozeman PF as a CS soil to take-all.

It is worthy of mention that many different forms of suppression to take-all have been described as existing under different conditions, and also, using even the gross categorization of general or specific suppression, neither of them can be precisely described because their nature remains unknown (Rovira and Wildermuth, 1981). Alabouvette (1986), and Cook and Rovira (1976), postulate that both types of suppression seem to be complementary rather than opposed, acting at different levels in the rhizosphere and in the rhizoplane.

The results observed in the case of Bozeman PF soil seems to accommodate to the definition of general suppression (sensu Gerlagh) when the response in its own soil is analyzed. However, when untreated Bozeman PF soil is transferred to another soil, the magnitude of that response changed completely. While little or no effect on the expression of the disease was observed after the addition of untreated Bozeman PF to its own sterile soil, a dramatic decrease in the expression of take-all was observed when Bozeman PF soil was added to Toston LS soil. Similar results have not been described in the literature reviewed. Shipton et al. (1973) were able to transfer suppression of the disease from two different soils into a fumigated conducive soil. When the conducive soil was added to a fumigated suppressive one, little or no effect was observed. Pope and Hornby (1975) reported an unexpected increase in the disease when a minute amount of a conducive soil was added to a suppressive one. No clear explanation was provided for those results.

My results suggest that there is in Bozeman PF soil a suppressive factor against take-all, which is not active in Bozeman PF soil, but which becomes activated when transferred to a chemically and physically different soil. Because no biological forms were present in the sterile soil, the evidence suggests that an abiotic factor may be related to the expression of antagonism under different environments.

The effect of different nutrients and chemical elements on take-all has been widely reported (Huber, 1981), with evidence that pathogenicity of the fungus is not affected. However, changes in the rhizosphere microflora could be responsible for the different expressions of the disease. Smiley and Cook (1973), and Hornby and Brown (1977) report changes in the microflora antagonistic to take-all after soils were amended with different forms of nitrogen. The variable response of selected antagonists to take-all tested in different soils has also been abundantly reported (Thomashow and Weller, 1990)

The results I obtained in the transference of suppressive factors among the soils under study, seems to indicate in the case of Bozeman PF soil that factors of an abiotic nature could be involved in the activation or inactivation of a specific microflora antagonistic to take-all.

Changes that I introduced during the course of this research in the methodology first used by Shipton et al. (1973), appeared to work well in decreasing the high variability commonly observed in take-all studies. The homogeneity and consistency in the results may be a result of the combined use of homogeneous inoculum particle size,

homogeneous seed size, use of distilled water without chlorine for irrigation, and the 30 day period for growing plants.

The use of shoot dry weight as a parameter to measure the expression of the disease, proved to be an objective method of measurement. After analyzing a number of experiments, it was observed that shoot dry weight did reflect the level of infection in roots. The effect of the disease on shoot weight has been widely reported, showing that Ggt greatly affects it, mainly after colonizing the crown roots (Sivasithamparam and Parker, 1978; Asher, 1972; Fitt and Hornby, 1978). The precision of the shoot dry weight in reflecting the effect of the disease has also been reported by Poplawsky and Ellingboe (1989), and Powelson (cited by Kollmorgen, 1985).

MICROBIOLOGICAL CHARACTERIZATION OF MONTANA SOILS SUPPRESSIVE AND CONDUCIVE TO TAKE-ALL OF WHEAT

Introduction

An antagonistic microflora that arises in soil after wheat monoculture has been suggested as the main factor involved in the suppression of take-all under such conditions (Shipton et al., 1973; Cook and Rovira, 1976; Rovira and Wildermuth, 1981; Cook and Weller, 1987). The discovery of the biological nature of the suppression observed in soils showing take-all decline has greatly attracted the interest of researchers, because of the potential to control a disease which, so far, has escaped most, if not all, traditional plant disease control methods.

Searching for suppressive soils to take-all and attempting their microbial characterization has been the methodology commonly used to both determine the true nature of the suppression, and to look for potential biocontrol agents. Even though no final success has occurred yet to explain the nature of the suppression for most of the soils and conditions under which these studies have been conducted, they provide an indispensable source of information of where to look for factors in common. Furthermore, these studies appear to have narrowed the spectrum of possible causes of the suppression of take-all and the organisms likely to be involved in it.

Organisms from different taxonomic groups have been reported as being associated with take-all suppression. The variability of these findings seems to suggest that different mechanisms might be involved in different wheat growing areas. However, the lack of success in demonstrating their specific role in the suppression of the disease, has reduced their general acceptance as being "the" cause of suppression.

Amoebae have been found to cause perforations in hyphae of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in suppressive soils (Homma et al., 1979; Chakraborty, 1985). However, their role in suppression of *Ggt* has been questioned (Cook and Baker, 1983) because their action is too low and their water requirements too restrictive to account for the suppression of the take-all fungus.

Fungi showing antagonism to *Ggt* have been reported in a number of papers indicating their possible association with suppression of the take-all fungus. Siegel (1961) observed that the severity of take-all was reduced about 50% when wheat seedlings were grown under sterile conditions in soil infested with *Ggt* together with the fungus *Didymella exitialis*. Mangan (1967) observed in *in vitro* tests that of numerous soil fungi isolated from wheat fields, *Penicillium megasporum*, *Penicillium nigricans*, *Gliomastix murorum* var. *felina*, and *Paecilomices carneus* were strong antagonists of *Ggt*. Another fungus shown to be involved in suppressive soils to take-all, mainly in Australia, is *Trichoderma* spp. (Simon and Sivasithamparam, 1988; Dewan and Sivasithamparam, 1988). It was later reported that *Trichoderma koningii* produces a pyrone compound with antibiotic properties against

Ggt (Simon et al., 1988). Even though not associated with take-all decline soils, avirulent isolates of *Gaeumannomyces graminis* var. *graminis* and *Phialophora* sp. were effective in controlling Ggt and *Gaeumannomyces graminis* var. *avenae* in bentgrass turf (*Agrostis* spp.) (Wong and Siviour, 1979).

Actinomycetes, specially *Streptomyces* sp., is another group of organisms that are commonly reported as associated with take-all decline soils (Sivasithamparam and Parker, 1978; Smiley, 1978; Zogg and Jaggi, 1974). Actinomycetes are known to be active producers of numerous and different types of antibiotics, and *in vitro* tests clearly show their strong ability to inhibit the growth of the take-all fungus. However, their role in suppression has not been conclusively demonstrated.

Much research regarding the role of soil bacteria in suppression of take-all has occurred. After the work of Pope and Jackson (1973) suggesting that bacteria were the organisms most probably involved in the protection against Ggt observed when wheat seedlings were dipped into a soil suspension from suppressive soils, an increasing number of reports appeared in the literature relating take-all decline with bacteria. Within this group of organisms, pseudomonads appear to be one of the most probable candidates. *Bacillus* spp. antagonistic to Ggt have also been isolated from suppressive soils (Poplawsky and Ellingboe, 1989), but lack of correlation between the antibiosis observed *in vitro* and plant protection in the field has also been commonly observed (Capper and Campbell, 1986). Smiley (1978), after performing *in vitro* tests for antagonism to Ggt found that

pseudomonads and streptomycetes, but not Bacillus spp. were antagonistic to Ggt in Australian soils.

Pseudomonas spp., the major group of non-differentiating microorganisms producing antibiotics (Leisinger and Margraff, 1979), have been implicated as being involved in suppression of take-all through a number of mechanisms. Cook and Weller (1987) postulate that pseudomonads act by inhibiting colonization and further infection by Ggt in the lesions on wheat roots. In this type of protection, an antibiotic, phenazine (Leisinger and Margraff, 1979), produced by the bacteria is believed to play an active role (Brisbane and Rovira, 1988; Thomashow and Weller, 1990). It has also been postulated that pseudomonads are among the main colonizers of wheat residue, competing with Ggt, thereby reducing its ability to survive (Sivasithamparam et al., 1979; Cook and Weller, 1987). The role of pseudomonads in the sequestration of iron, its depletion for use by plant pathogens and subsequent increase in crop yield appears to be well established (Bakker et al., 1990). Kloepper et al. (1980) were able to induce suppression of take-all by adding a siderophore-producing Pseudomonas spp. to a conducive soil. Similar results were observed by the same author after adding just the siderophore compound to the same soil. Higher populations of pseudomonads isolated from suppressive soils than from conducive ones (Weller and Cook, 1981), the suppression of take-all induced by these organisms when applied to the seed (Weller and Cook, 1983), and the greater ability of pseudomonads isolated from suppressive than from conducive soils to inhibit the take-all fungus (Smiley, 1979; Cook and Weller, 1987) are other explanations commonly

given by the supporters of the role of pseudomonads in the suppression of take-all.

Antibiosis tested _in vitro_ has been the main criterion in the selection of organisms potentially involved in suppression of the disease. However, lack of correlation of _in vitro_ antibiosis with tests in the field is the common denominator in most of this research (Tomashow and Weller, 1990). Despite the finding of Broadbent et al. (1971) that organisms ineffective in showing antibiosis _in vitro_ were also ineffective in soil, numerous reports indicate that mechanisms other than antibiosis, as mycoparasitism, different antibiotics than the ones produced on PDA medium, induction of phytoalexins in roots, and competition, may also be involved in suppression of the pathogen (Poplawsky and Ellingboe, 1989; Kempf and Wolf, 1989; Sivasithamparam and Parker, 1978; Siegel, 1961; Thomashow and Weller, 1990; Fravel, 1988). Furthermore, it has also been reported that antibiosis _in vitro_ is highly affected by the constitution of the medium used to test antagonists (Rovira and Wildermuth, 1981; Fravel, 1988).

In light of the extensive information accumulated regarding natural suppression of take-all, the biological nature of this phenomenon appears to be unquestionable. Therefore, the lack of success in determining the organisms involved in biological suppression of take-all seems to be related to the availability of methods to unravel the complexity of the interaction between soil organisms.

In this report, the microbial characterization of Montana soils previously found to be either suppressive or conducive to take-all of wheat is presented.

Materials and Methods

Soils

Bozeman PF, Larslan 1, and Toston LS Montana soils, with conducive and suppressive properties against take-all of wheat as described in the first chapter of this thesis, were used in an attempt to determine which organisms are most likely involved in the suppression of the disease. Bozeman PF soil exhibited conducive properties to take-all, while Larslan 1 and Toston LS soils were suppressive.

Isolation of Microorganisms

The dilution plating method as described by Rudolph et al. (1990) was used for the isolation of microorganisms from each soil. A 10 g sample of air-dried and 2 mm sieved soil was added to 100 ml of 0.1% of sterile water-agar containing 20 6-mm glass beads, hand-shaken for 4 min, and a set of 6 ten-fold dilutions made in the same water-agar medium. A 0.5 ml-sample from 10^{-3} to 10^{-7} dilutions was removed and evenly distributed with a glass rod on each plate as the plate was rotated. Three plates for each one of seven different media per dilution, were used to selectively grow and isolate microorganisms from each soil.

The media used were as follows: full strength tryptic soy agar (TSA), used as a general medium; modified King's B medium, to selectively isolate fluorescent pseudomonads (Sands et al., 1980); modified soil extract agar, as described by Parkinson et al. (1971), mainly for isolation of *Bacillus* spp.; actinomycete selective medium, as described by Kuster and Williams (1964) to isolate actinomycetes,

mainly Streptomyces spp.; MRS medium to isolate organisms able of utilizing glucose aerobically (Juhnke, 1987); low strength PDA (3 g of potato flakes boiled for 2 min and filtered through 4 layers of cheese cloth, 6 g of Dextrose, 1 l distilled water), 10 ppm triadimenol, 100 mg/l Streptomycin for the isolation of fungi, mainly Trichoderma spp. (modified from Dewan and Sivasithamparam, 1988); and low strength PDA, as described above, plus 50 mg/l of Vancomycin, and 10 mg/l Rose Bengal for the isolation of fungi (modified from Martin, 1950). Media were prepared as described in Appendix A, and allowed to dry for 48 hr before use.

After 48 to 96 hr incubation at room temperature in the case of bacteria, and 4 to 6 days in the case of actinomycetes and fungi, the number of colonies representing a particular organism was counted in all 3 replicates, and a single colony for each different morphological type of organism present on the plates was aseptically transferred to a 1/10 strength TSA medium in the case of bacteria and actinomycetes, and to low strength PDA in the case of fungi. The same procedure was applied for each different medium.

Fungal colonies were aseptically transferred to low strength PDA, allowed to grow for 72 to 96 hr, and hyphal tips removed and transferred to a new plate for purification. Bacteria and actinomycete colonies were reisolated at least 4 times and transferred to new 1/10 strength TSA plates for purification.

Purified isolates of bacteria and actinomycetes were stored on a 1/10 strength TSA slant medium at 5°C for further use in tests of antagonism against Ggt, hyperantagonism, siderophore production, and

identification. Fungi were stored on a low strength PDA slant medium at 5°C.

In vitro Screening for Antagonists of *Gaeumannomyces graminis* var. *tritici*

Tests of Antagonism with Bacteria and Actinomycetes. All isolated organisms were tested for their ability to inhibit *Ggt in vitro*. A technique based on wells made on PDA and filled with sterile soil was used to test antagonism (antibiosis) *in vitro*, in an attempt to bring nutritional factors into the test that may affect the expression of antibiosis. Soil from which the organism being tested was isolated was used in each case to fill the wells.

Approximately 30 μl of an individual, heavy bacterial suspension (>10^9 cfu/ml) prepared in sterile distilled water from 48 hr old cultures grown on 1/10 TSA medium, were placed into wells made in low strength PDA plates in which autoclaved soil was added. Five wells per plate were made with a No. 4 cork borer (Fisher catalogue) equidistant from each other and 15 mm in from the edge of the plates. Then, a 4 mm plug of *Ggt*, grown for 8 days in the low strength PDA medium, was placed in the center of each plate. Sterile soil-containing wells with only the addition of distilled water were used on each plate as a check.

Low strength PDA without soil-containing wells was also used to test antibiosis against *Ggt*, and also to compare its expression with the sterile soil-containing well technique. Bacteria from 48 hr old cultures grown on 1/10 strength TSA medium were aseptically transferred

to PDA plates, to equidistant points 15 mm from the edge of the plates. Then, a 4 mm plug of Ggt grown on low strength PDA was placed in the center of the plate. A total of 5 isolates per plate were tested. Plates were kept at room temperature and the zone of inhibition was measured after 7 days.

Isolates from each soil selected for their ability to inhibit Ggt *in vitro* were retested using the same methodology described above. In this second test the inhibition zone was measured after 7, 14, and 21 days to observe differences in the length of time that antibiosis was expressed.

Due to the fact that antibiosis *in vitro* has not correlated well with suppression in soil, and since other mechanisms may also be involved in antagonism to Ggt, a second test was conducted to observe the effect of different isolates on Ggt mycelium. A single drop of a heavy bacterial suspension in sterile distilled water taken from a 48 hr old culture grown on 1/10 TSA medium was placed on the hyphae from the border of a 6-day old Ggt culture grown on low strength PDA. Three drops from three different isolates, and one drop of sterile distilled water as a check were placed on each plate. After 4 to 6 days of incubation at room temperature hyphal lysis, protoplasm condensation, cessation of growth, or any other abnormality that was microscopically visible was recorded. Isolates showing deleterious effects on Ggt hyphae were also selected for further tests of antagonism.

Tests of Antagonism with Fungi. Four 4 mm plugs of each fungus isolated from Bozeman PF, Larslan 1, or Toston LS soils, grown for 5

days on low strength PDA were placed in a 250 ml Erlenmeyer flask containing 50 ml of sterile, potato-dextrose broth (6 g potato flakes boiled for 2 min and filtered through 4-layers of cheese cloth, 12 g dextrose, 1 l distilled water), plus 10 ml of sterile-filtered (0.2 μm polycarbonate membrane) soil extract. Cultures were grown on a rotary shaker for 5 days, at room temperature. Then, the growth medium in which these fungi had grown was sterile-filtered through a 0.2 μm polycarbonate (Nucleopore) membrane, added to a 15 mm diameter sterile filter disc, and placed on low strength PDA plate 15 mm from the edge of the plate. Immediately, a 4 mm plug of Ggt grown on low strength PDA was placed in the center of the plate. Four discs containing four different fungal extracts were placed on each plate, equidistant from one another. Each plate was replicated 3 times. Plates were maintained at room temperature and inhibition zones were measured after 7 days.

Test of Hyperantagonism Among Ggt Antagonists

To determine whether there was hyperantagonism (organisms that antagonize Ggt antagonists) between the bacteria and actinomycetes isolated from the three test soils that also showed antagonism to Ggt, tests were established as follows.

Approximately 30 μl of a heavy (> 10^9 cfu/ml) bacterial suspension in sterile distilled water from 48 hr old cultures grown on 1/10 TSA, was placed in a single, 7-mm, sterile soil-containing well placed in the center of a low strength PDA plate. After incubation for 4 days at room temperature (25°C), bacterial growth from 48 hr old cultures

antagonistic to *Ggt* and grown on 1/10 TSA was aseptically and radially streaked beginning 2-3 mm from the edges of the well toward the edge of the plate. Plates were incubated at room temperature and inhibition was recorded after 72 hr.

Detection of Siderophore-Producing Organisms

Since siderophore-producing organisms have been found associated with suppression of *Ggt* in soils, the presence and population of siderophore-producing organisms was determined for the three soils under study. All isolated bacteria and actinomycetes were tested for production of siderophores with the exception of the fungi.

Bacterial growth from 48 hr old cultures grown on 1/10 TSA medium was individually, radially, and aseptically streaked on Blue CAS agar plates (Schwyn and Neilands, 1987) (see appendix for media recipes). Production of siderophores, as observed by change of the color of the medium from blue to orange, was recorded after 24 and 72 hr.

Effect of Soil Type on the Expression of Antibiosis in vitro

It was consistently observed in preliminary *in vitro* tests of antibiosis against *Ggt* that the antagonism exhibited by some isolates varied according to the type of soil used in the wells. To corroborate this differential response, and because the reaction may have important implications in the selection of potential biocontrol agents to take-all, selected antagonists to *Ggt* were tested using the same *in vitro* assay described above, but using plates with wells containing all three soils under study. The hypothesis to be tested is that soils contain

abiotic factors which change the expression of antibiosis of associated microbes.

Approximately 30 µl of a heavy bacterial suspension (> 10^9 cfu/ml) from 48 hr old cultures of Ggt antagonists grown on 1/10 TSA, were placed in different sterile soil-containing wells in low strength PDA plates. Three different organisms were tested on each plate. The same volume of sterile distilled water was added to another two wells as checks. Thereafter, a 4 mm plug of an 8 day old Ggt culture grown on a low strength PDA was placed in the center of the plates. Zones of inhibition were recorded after 7, 14, and 21 days.

Identification of Organisms

Selected Gram-negative organisms which showed either antagonism against Ggt *in vitro* or inhibition of the disease in soil tests were identified using the Biolog Microstation System, Release 3.0 (Biolog Inc., Hayward, CA).

The Gram-positive actinomycetes were identified based on morphological characters (Buchanan and Gibbons, 1974).

Bacillus spp. were identified based on staining and physiological tests as described by Leary and Chun, (1988).

The two fungi observed to strongly inhibit take-all have not yet been identified since they have not produced any fruiting structures in culture.

Tests of Antagonism to Ggt in Soil

All experiments involving antagonism tests toward *Ggt* in soil were conducted in a glasshouse in the MSU Plant Growth Center at Bozeman, Montana.

Tests of Antagonism with Bacteria and Actinomycetes. Bacteria and actinomycete isolates selected using both *in vitro* test procedures were tested individually and in mixture for antagonism against *Ggt* in soil using the same soil from which they were isolated. Sterile, *Ggt*-infested soil was used to test antagonism against *Ggt*, and sterile non *Ggt*-infested soil was used to test whether the isolates were able to increase the shoot dry weight of the wheat plants in the absence of the pathogen. The mixture of antagonists was based on different species as a main criterion.

Soils previously sieved to a 2 mm size, moistened with tap water, and air-dried for 48 hr under greenhouse conditions, were autoclaved at 121°C for 40 min in a 2 l, autoclavable plastic container covered with aluminum foil. Immediately after cooling, *Ggt* inoculum, consisting of 0.5-1.0 mm particle sizes from blended, axenically colonized oat kernels, was added at a rate of 0.1% w/w, and thoroughly mixed for 10 min in a disinfected plastic rotary mixer.

Antagonists to be tested were grown in 10 ml of 1/10 tryptic soy broth for 18 hr, on a reciprocating shaker. The bacterial suspension was mixed with 10 ml of sterile distilled water and 15 μl of this suspension were aseptically added to 80 g of soil previously sterilized at 121°C for 30 min in 250 ml Erlenmeyer flasks. After a 5-day

incubation period at room temperature, the flask caps were replaced with 4-layers of sterile cheese cloth thus allowing the infested soil to air-dry in a clean, low air movement environment.

Antagonist-infested soils were then individually hand-mixed at a rate of 2% w/w with sterile Ggt-infested or -noninfested soil, in disinfected 4 l plastic containers. Every container was thoroughly washed with soap and hot water, disinfected with 95% ethanol, and rinsed with tap water before use with the next isolate.

The experiments involving sterile soil were conducted using the tube assay described by Weller et al. (1985), except that no vermiculite was added. In brief, plastic conetainers (Cone-tainer, Canby, OR) (2.5 cm diameter x 12.5 cm long) containing a cotton ball in the bottom were filled with a 8-cm-thick column of soil, and distributed in a 200-conetainer plastic rack in a completely randomized design.

A single disinfested Pondera spring wheat seed, sieved for homogeneous size, was planted into each conetainer. A 1-cm layer of sterile white sand was added over the soil to avoid contamination at irrigation. Conetainers were watered every 3 days with a nutrient solution (Peter's 20-20-20 NPK solution + micronutrients) made up in distilled water.

After 30 days, the seedlings were washed from the soil. The shoots were excised at the crown, placed into individual paper envelops, and dried at 70°C for 72 hr to obtain the shoot dry weight.

Tests of Antagonism with Fungi. Due to the low number of fungi that were observed to inhibit Ggt *in vitro*, all of them were tested in

soil to determine their antagonism against Ggt. The same tube assay procedure as described above was used in these experiments with both small (4 cm diameter x 12.5 cm long) and large (4 cm diameter x 21 cm long) conetainers. Sterile Ggt-infested and -noninfested soil was used to observe antagonism and plant growth promotion in the absence of the pathogen, respectively. Tests with untreated Bozeman PF soil were also performed to observe the ability of the antagonistic fungi to suppress Ggt under natural soil conditions.

Ten 4-mm plugs of fungi cultured for 7 to 12 days on low strength PDA at room temperature, were aseptically transferred into 80 ml of sterilized soil autoclaved at 121°C for 40 min in 250 ml Erlenmeyer flasks. The soils used were those from which the fungi had originally been isolated. Fifteen ml of sterile, low strength potato-dextrose broth (3 g potato flakes boiled for 2 min and filtered through 4-layers of cheese cloth, 6 g dextrose, 1 l distilled water) were aseptically added to the flasks to facilitate the growth of the fungi. After a 10-day incubation period at room temperature, flask caps were replaced with 4-layers of sterile cheese cloth, and the flasks allowed to air-dry for 2 days in a clean, low air-movement environment.

The sterile, fungus-infested soils were added at rates of 1%, 2%, 4%, and 5% as a uniform mixture, or were added as a 1-cm layer under the seed to both sterile and untreated soils to test for antagonism. The same planting procedure as described above was followed. Twelve to eighteen conetainers per treatment were distributed in plastic racks, in a completely randomized design. Plants were watered every 3 days with nutrient solution made up in distilled water.

After 30 days in the case of small conetainers, or 40 days in the case of large conetainers, the plant shoot dry weight was measured.

Results

Isolation of Microorganisms

A total of 50 bacteria, 8 fungi, and 12 actinomycete isolates representing different morphological types were isolated from Bozeman PF soil. From Larslan 1 soil, 48 bacteria, 6 fungi, and 10 actinomycetes were isolated. In the case of Toston LS soil, 50 bacteria, 8 fungi, 12 actinomycetes were isolated based on differential morphology. Some isolates were isolated more than once because of their different characteristics shown in different media.

The total population of isolatable organisms counted on each medium is shown in Table 8. On TSA and MRS media there were higher

Table 8. Population of microorganisms isolated from Bozeman PF, Larslan 1, and Toston LS soils, on different media.*

	Population of microorganisms (cfu/g soil x 10^5)					
Soil	MRS[a]	TSA	MMB'K	MSE	ACT	Fungi
Bozeman PF	81	252	3	221	10	0.43
Larslan 1	9	157	13	122	1	0.23
Toston LS	12	134	5	231	16	0.85

*: See appendix for media recipes.
[a]: MRS: Lactobacillis MRS agar; TSA: Tryptic soy bean agar; MMB'K: Modified King's B medium; MSE: Modified soil extract agar; ACT: Actinomycetes selective medium; Fungi: population of fungi from both low strength PDA medium + Streptomycin + Triadimenol, and low strength PDA + Vancomycin + Rose Bengale.

populations of microorganisms in Bozeman PF soil than in Toston LS and Larslan 1 soils. On the MSE medium both Bozeman PF and Toston LS soils had higher populations than did the Larslan 1 soil. However, in the case of Toston LS one species of bacterium accounted for almost 50% of the total number of colonies on the MSE plates. The "total" population of aerobic organisms is best represented on the TSA medium, as it is a general medium widely used for this purpose. According to these results, the soils under wheat monoculture (i.e., Larslan 1 and Toston LS) exhibited a lower population of bacterial microflora than the one growing wheat in rotation (Bozeman PF). This same pattern has been reported by Simon and Sivasithamparam (1988a), and Smiley (1978a) who found that the "total" number of aerobic organisms in a conducive soil was higher than in a suppressive soil.

Pseudomonas spp., as determined on the MMB'K medium, occurred in higher numbers in Larslan 1, followed by Toston LS and Bozeman PF soils. While Simon and Sivasithamparam (1988) reported a higher population of _Pseudomonas_ spp. in conducive soils, Smiley (1978a) found that the population was fairly stable showing little difference between conducive and suppressive soils.

The population of Actinomycetes spp. in both suppressive soils was quite different, with a high population of these organisms in Toston LS soil and a very low population in Larslan 1 soil. In Bozeman PF soil the population of Actinomycetes was much higher than Larslan 1 soil, but lower than in Toston LS soil. Smiley (1978a) observed a significantly higher population of _Streptomycetes_ spp. in suppressive soil.

The total number of fungi isolated from Toston LS soil was higher than from the other two soils, with the lowest number in Larslan 1 soil. In general, the population of fungi isolated from the three soils was low. Simon and Sivasithamparam (1988) reported higher populations of fungi in suppressive soils than in conducive soils.

In vitro Screening for Antagonists of Gaeumannomyces graminis var. tritici

In vitro Tests of Antagonism with Bacteria and Actinomycetes. Table 9 shows the relative population in the three soils of the different bacteria and actinomycetes found to antagonize Ggt in vitro. The technique of using plates containing sterile soil wells to

Table 9. Population of bacteria and actinomycetes isolated from Bozeman PF, Larslan 1, and Toston LS soils, exhibiting in vitro antagonism to Gaeumannomyces graminis var. tritici.

Organisms	Soils[a]		
	Bozeman PF	Larslan 1	Toston LS
Bacillus spp.	3.3*	6.0	9.7
Erwinia herbicola	0.15	3.7	0
Pseudomonas fluorescens	0.17	5.75	0.96
Pseudomonas spp.[b]	0.2	3.0	0.5
Actinomycetes	8.0	0.2	10.5
Curtobacterium spp.	0	4.0	0
Klebsiella spp.	0	0.9	0
"Total" population of antagonists	11.8	23.5	21.7

[a]: Soils from which the antagonists were isolated.
[b]: Non-fluorescent pseudomonads.
*: cfu/g soil x 10^5.

determine antagonism _in vitro_ to _Ggt_ appeared to be more inclusive than the use of low strength PDA alone. A number of isolates did not show antagonism when tested on PDA alone, but they did when placed in wells with sterile soil. Furthermore, the type of soil placed in the wells also affected antibiosis as will be shown later.

Bacillus spp. and fluorescent pseudomonads antagonistic to _Ggt_ appeared in higher numbers in both suppressive soils than in the conducive soil. The population of actinomycetes antagonistic to the take-all fungus was higher in Toston LS and Bozeman PF, even though in the latter, one _Streptomycete_ spp. accounted for 60% of the population by itself.

Erwinia herbicola, which was as strong an antagonist _in vitro_ as _Bacillus_ spp. and actinomycetes, was isolated from Bozeman PF and Larslan 1 soils but not from Toston LS soil. Other organisms showing antagonism against _Ggt_, i.e., _Curtobacterium_ spp. and _Klebsiella_ spp., were only found in Larslan 1 soil.

The "total" population of bacteria and actinomycetes antagonistic to _Ggt_ as determined in the _in vitro_ tests was higher in the suppressive soils as compared to the conducive soil (Table 9). These results agree with most of the reports regarding microbial studies on soils suppressive and conducive to take-all (Cook and Rovira, 1976; Smiley, 1978a; Simon and Sivasithamparam, 1988a; Cook and Weller, 1987; Weller and Cook, 1985).

The length of time the antibiosis activity was expressed and the effect of soil on its expression is shown in Table 10. The statistical analysis indicated that actinomycetes induced a significantly higher

Table 10. Antibiosis activity of different isolates from Bozeman PF, Larslan 1, and Toston LS soils against Gaeumannomyces graminis var. tritici, observed after 7, 14, and 21 days when placed in different soil-containing wells on low strength PDA.

Soil[a]	Ggt antagonists	Soil in wells: Bozeman PF 7[b]	14	21	Larslan 1 7	14	21	Toston LS 7	14	21
Bozeman PF	Bacillus spp.	12.0[c]	4.5	0.0	11.0	3.5	0.0	5.5	0.0	0.0
	E. herbicola	4.5	1.5	0.0	5.0	2.0	0.0	1.0	0.0	0.0
	P. fluorescens	0.0	0.0	0.0	1.0	0.0	0.0	2.5	0.0	0.0
	Actinomycetes	9.7	9.0	8.5	9.5	9.5	8.5	9.5	9.5	8.7
Larslan 1	Bacillus spp.	14.0	10.0	7.0	14.0	10.5	7.5	8.0	1.5	0.0
	E. herbicola	7.0	2.7	0.7	8.0	4.8	2.3	1.0	0.0	0.0
	P. fluorescens	2.8	1.5	0.5	3.2	2.3	1.2	0.2	0.0	0.0
	Actinomycetes	6.0	5.3	4.7	5.7	5.3	5.0	5.3	5.2	4.3
Toston LS	Bacillus spp.	10.5	3.5	0.0	10.5	3.0	1.0	7.0	0.5	0.0
	P. fluorescens	2.4	1.1	0.4	3.1	1.7	1.4	1.4	0.6	0.0
	Actinomycetes	3.7	4.5	3.7	4.0	4.7	3.2	4.8	6.3	4.2

[a]: Soils from which the Ggt antagonists were originally isolated.
[b]: Days after each reading was made.
[c]: Zone of inhibition of Ggt colonies in mm. Average of 2 plates.
LSD ($P<0.05$) : 0.80.

inhibition of Ggt than the other groups of organisms after 21 days. Furthermore, these organisms appear not to be affected by the soil type contained in the wells. The same analysis showed that Toston LS soil significantly reduced the expression of antibiosis as compared with Bozeman PF and Larslan 1 soils.

Bacteria and actinomycetes exhibiting lysis of hyphae when placed directly on Ggt mycelium were also selected for tests of antagonism in soil. Most of the isolates showing antagonism *in vitro* as described above also exhibited lysis and growth inhibition when placed on the mycelium of Ggt. A few isolates that did not show antibiosis towards Ggt were observed to induce lysis and protoplasm disruption when placed on Ggt mycelium.

Test of Antagonism with Fungi. Only two out of 18 different fungi isolated from the three soils exhibited production of a compound toxic to Ggt. Isolates T-46 and T-55, both obtained from Toston LS, inhibited the growth of Ggt *in vitro* when filter discs embedded with the fungal extracts were placed on PDA plates where Ggt was growing. Neither of these two fungi reduced disease severity when tested in soil.

Test of Hyperantagonism Against Each Other Among Ggt Antagonists

Most of the antagonists to Ggt also exhibited antagonism among themselves, i.e., hyperantagonism (Table 11). In addition, some organisms that did not show antibiosis against the take-all fungus, did inhibit the growth of some Ggt antagonists *in vitro*. The role of hyperantagonists in soils suppressive or conducive to take-all has not

Table 11. Hyperantagonism in vitro detected among Ggt antagonists isolated from Bozeman PF, Larslan 1, and Toston LS soils, using the sterile soil-containing well technique.

Soil[a]	Hyperantagonist	Ggt antagonists*						
		Eh	Pf	Ps	Ba	Act	Ct	Kb
Bozeman PF	Bacillus spp.	+/-	+/-	+/-	-	+/-	NI	NI
	P. fluorescens	+-	+-	-	+	+	NI	NI
	E. herbicola	-	+/-	-	+	+	NI	NI
	Pseudomonas spp	-	-	-	+-	+	NI	NI
Larslan 1	Bacillus spp.	+/-	+/_	+-	-	+	-	+-
	P. fluorescens	+-	+/-	+/-	+	+-	+/-	+-
	E. herbicola	+/-	+	+	+	+	+	+-
	Pseudomonas spp	-	+-	+-	+	+	-	-
	Curtobacterium	-	-	-	+	+-	-	-
	Klebsiella spp.	-	-	+	+	+	+-	-
Toston LS	Bacillus spp.	NI	+/-	-	+-	+/-	NI	NI
	P. fluorescens	NI	-	-	+	+	NI	NI
	Pseudomonas spp	NI	-	-	+/-	+	NI	NI

"Total" population of hyperantagonists:

Bozeman PF: 0.55×10^5

Larslan 1 : 7.80×10^5

Toston LS : 2.10×10^5

a: Soils from where the hyperantagonists were isolated.
*: Eb:Erwinia herbicola; Pf:Pseudomonas fluorescens; Ps:Pseudomonas spp.; Ba:Bacillus spp.; Act: Actinomycetes; Ct:Curtobacterium spp.; Kb:Klebsiella spp.
NI: Not isolated from that particular soil
-: No antibiosis
+: Antibiosis observed
+-: Weak antibiosis or only some strains were affected
+/-: Some strains exhibited antibiosis while others did not.

been established. However, Zogg and Amiet (1980), studying the effect of different root rot pathogens of wheat, observed a significant interference by each pathogen in the build-up of suppression against another.

The population of hyperantagonists in the soils tested was quite different. Contrary to what might be expected the conducive soil exhibited the lowest population of hyperantagonists, while one of the soils suppressive to take-all, Larslan 1, exhibited the highest population. The population of hyperantagonists is associated with the total population of *Ggt* antagonists isolated from each soil.

Erwinia herbicola and fluorescent pseudomonads were the strongest hyperantagonists, inhibiting the growth of practically all other *Ggt* antagonists when tested *in vitro*. *E. herbicola* was not found in Toston LS soil. *Bacillus* spp. showed weaker ability to inhibit antagonists of *Ggt*, and actinomycetes did not inhibit any of the *Ggt* antagonists. These two groups, *Bacillus* and actinomycetes, were usually inhibited by most of the hyperantagonists.

In this study, the role of hyperantagonists in the suppression of take-all in soil could not be established. Due to the fact that most of the hyperantagonists are also antagonists of *Ggt*, this type of study would require one to obtain mutants with opposite features, which was beyond the scope of this research. Hyperantagonism in soil might play an important role in the establishment of potential biocontrol agents, but to date there are no reports on hyperantagonists and their role in the suppression of take-all.

Detection of Siderophore-Producing Organisms

The presence of siderophore-producing organisms was detected in all three soils (Table 12). Toston LS soil had the highest population of siderophore-producing organisms, and Larslan 1 soil exhibited the lowest population.

E. herbicola and fluorescent pseudomonads were the strongest siderophore producers, based on the reaction observed on the blue CAS plates. Non-fluorescent pseudomonads appeared to be weaker producers of siderophores. Some Bacillus spp. exhibited a very weak reaction while others did not grow on the medium. The above might be a consequence of the compound HDTMA (hexadecyltrimethylammonium bromide) used as a detergent in the medium, which is toxic to Gram-positive organisms (Schwyn and Neilands, 1987). No actinomycete grew on the medium. A Klebsiella spp. isolated from Larslan 1 also showed a strong reaction in the blue CAS medium.

Due to the population of siderophore-producing organisms in Toston LS soil, and to the fact that this soil has low iron content (Chapter 1), it seems likely that the high suppression exhibited by this soil to take-all might be due in part to iron sequestration thus decreasing availability of this element to the take-all fungus. The involvement of siderophore-producing organisms in the suppression to take-all has been widely reported (Kloepper et al.,1980; Misaghi et al., 1982; Vandenbergh et al., 1983; Bakker et al., 1990).

Table 12. Population of siderophore-producing organisms in Bozeman PF, Larslan 1, and Toston LS soils.

Soil[a]	Organism	Reaction*	cfu/g soil ($x 10^5$)
Bozeman PF	Erwinia herbicola	+++	0.15
	Pseudomonas fluorescens	+/+++	0.74
	Pseudomonas spp.	++	0.20
	Unidentified G+[b](3 isol.)	+	17.0
	Unidentified G-[c]	++	0.4
	"Total" population		18.5
Larslan 1	Erwinia herbicola	+++	3.7
	Pseudomonas fluorescens	++/+++	4.05
	Pseudomonas spp.	+/++	5.5
	Klebsiella spp.	+++	0.6
	"Total" population		13.8
Toston LS	Pseudomonas fluorescens	+++	0.38
	Pseudomonas spp.	+/++	3.8
	Bacillus spp.	+	6.7
	Unidentified G+ (4 isol.)	+	14.7
	"Total" population		25.6

[a]: Soils where the isolated originated.
[b]: Gram-positive organisms.
[c]: Gram-negative organisms.
*: +++:strong reaction; ++: medium reaction; +:weak reaction;
+/++: some strains with weak and other strains with medium reaction.

Effect of the Soil Type on the Expression of Antibiosis in vitro

It was consistently observed in the *in vitro* tests that the expression of antibiosis towards *Ggt* by antagonists placed in Toston LS sterile soil-containing wells was much weaker than the antibiosis against *Ggt* observed in the other two soils. When 7 different isolates from Bozeman PF soil, 13 from Larslan 1, and 11 isolates from Toston LS were tested in all soil combinations for their antagonism towards *Ggt*, most organisms exhibited fairly low antibiosis in the Toston LS soil (Table 13). It was also observed that many of them did not show any antibiosis against *Ggt* when placed in this soil. The differences between the three soils were statistically significant (Table 14). No difference regarding the origin of the isolates was observed.

In an attempt to determine whether various chemical elements might be responsible for the differences in expression of antibiosis seen above, 25 μl of 13 chemical elements (Ca, Co, Cu, Fe, Li, Mg, Mo, K, Na, Zn, Bo, and Mn) dissolved in sterile distilled water were added to Toston LS sterile soil-containing wells. The hypothesis was that the reaction was due to a lack of one or more elements in the soil. The concentration of the tested elements was an average of the amount reported from an agricultural soil (Barber, 1984) and from a general non-cultivated soil (Sposito, 1989) as follows: Ca: 10,000 ppm; Co: 4 ppm; Cu: 5 ppm; Fe: 30 ppm; Li: 10 ppm; Mg: 6,000 ppm; Mn: 600 ppm; Mo: 4 ppm; K: 8,000 ppm; Na: 6,000 ppm; Zn: 30 ppm; Bo: 20 ppm. No change in the expression of antibiosis from the addition of these elements was observed in this test (data not given).

Table 13. Differential control of *in vitro* antibiosis against *Ggt* by three soils as determined following 7 days of incubation. Selected bacteria and actinomycetes isolated from Bozeman PF, Larslan 1, and Toston LS soils were placed in different soil-containing wells in low strength PDA.

		Soil Contained in Wells		
Soil[a]	Organisms	Bozeman PF	Larslan 1	Toston LS
Bozeman PF	*Bacillus* spp.	12.0[b]	11.0	5.5
	E. herbicola	4.5	5.0	1.0
	P. fluorescens	0.0	1.0	2.5
	Actinomycetes	9.7	9.5	9.5
Larslan 1	*Bacillus* spp.	11.2	12.0	5.5
	E. herbicola	7.0	8.0	1.0
	P. fluorescens	2.8	3.2	0.2
	Actinomycetes	6.0	5.7	5.3
Toston LS	*Bacillus* spp	10.5	10.5	7.0
	P. fluorescens	2.4	3.1	1.4
	Actinomycetes	3.7	4.0	4.8

[a]: Soils from which the organisms were originally isolated.
[b]: Zone of inhibition of *Ggt* in mm.

Table 14: Expression of antibiosis against Gaeumannomyces graminis var. tritici exhibited by organisms isolated from Bozeman PF, Larslan 1, and Toston LS soils, when placed in different sterile-soil-containing wells in a low strength PDA medium, following 7 days of incubation.

Soil	No. of Isolates	Soil Contained in Wells: Bozeman PF	Larslan 1	Toston LS	Average[a] Isolates
Bozeman PF	7	7.1[c]	7.3	5.1	6.5 A
Larslan 1	13	5.8	6.6	2.4	4.9 A
Toston LS	11	4.9	6.0	5.4	5.4 A
Average soils[b]		5.7 A	6.6 A	4.1 B	

[a]: Average for isolates was not significant at $P<0.05$.
[b]: Average soils was significant at $P<0.01$.
LSD 0.05=1.31
Values followed by the same letter are not statistically significant.
[c]: Zone of inhibition of Ggt in mm; Average of 2 PDA plates.

Not only was the expression of antibiosis against Ggt affected by the type of soil, but also the expression of hyperantagonism (Table 15). When five isolates from Larslan 1 soil were challenged by E. herbicola and P. fluorescens isolates from the same soil, and placed in different sterile soil-containing wells, the expression of antibiosis was almost absent when wells were filled with Toston LS soil. However, antibiosis was observed when the wells were filled with either Larslan 1 or Bozeman PF soil.

Table 15. Expression of antibiosis exhibited by two hyperantagonists towards antagonists of Gaeumannomyces graminis var. tritici isolated from Larslan 1 soil, when placed in different soil-containing wells in low strength PDA.

Hyperantagonist[a]	Soil in wells	Ggt antagonists[a] Ba 1-3	Eh 1-7	Eh 1-13	Pf 1-19	Ba 1-38
E. herbicola 1-13	Bozeman	+[b]	+	-	+	+
	Larslan	+	+	-	+	+
	Toston	-	-	-	-	-
P. fluorescens 1-19	Bozeman	+	+	+	-	+
	Larslan	+	+	+	-	+
	Toston	+	-	-	-	-

[a]: Isolated from Larslan 1 soil.
Ba:Bacillus spp. isolates 1-3 and 1-38; Eh:Erwinia herbicola isolates 1-7 and 1-13; Pf:Pseudomonas fluorescens isolate 1-19.
[b]: +:Antibiosis observed; -:No antibiosis observed.

A similar reaction was observed when two media, 1/10 TSA and low strength PDA, were used at two pH values without soil in the wells (Table 16). No antibiosis was observed in TSA at both pH's, while on PDA strong antibiosis was exhibited by the isolates at ph 6.0 and 7.2.

The results observed in the test of two media at two pH values agree with the observations reported by Rovira and Wildermuth (1981), Vidaver et al. (1972), and Weinhold and Bowman (1968) regarding the effect of nutrition on the expression of antibiosis *in vitro*. No reports were found regarding the use of soil-containing wells in tests of antibiosis *in vitro*.

Table 16. Effect of 2 media at pH 6.0 or 7.2 on the expression of antibiosis exhibited by a hyperantagonist isolate toward antagonists of Gaeumannomyces graminis var. tritici isolated from Larslan 1 soil.

Hyperantagonist	Medium	pH	Ggt antagonists[a]				
			Ba 1-3	Eh 1-7	Eh 1-13	Pf 1-19	Ba 1-38
P. fluorescens 1-19[a]	PDA	6.0	+[b]	+	+	-	+
	PDA	7.2	+	+	+	-	+
	TSA	6.0	-	-	-	-	-
	TSA	7.2	-	-	-	-	-
No bacteria	PDA	6.0	-	-	-	-	-
	PDA	7.2	-	-	-	-	-
	TSA	6.0	-	-	-	-	-
	TSA	7.2	-	-	-	-	-

[a]: Isolates from Larslan 1 soil. Ba: Bacillus spp. isolates 1-3 and 1-38; Eh: E.herbicola isolates 1-7 and 1-13; Pf: P.fluorescens isolate 1-19.
[b]: +: Antibiosis observed; -: No antibiosis or normal growth observed.

Tests of Antagonisms in Soil Against Ggt

Tests of Antagonism with Bacteria and Actinomycetes. Bacteria and actinomycetes isolated from Bozeman PF soil, which exhibited either antibiosis in vitro or ability to lyse the hyphae of Ggt were tested in soil to observe their effect on the take-all fungus in vivo. These results are shown in Tables 17, 18, and 19.

Table 17. Tests of antagonisms against Gaeumannomyces graminis var. tritici with bacteria and actinomycetes isolated from Bozeman PF soil, in pots with Bozeman PF sterile soil.

Antagonist	Ggt[a]	Test 1	Test 2
		Shoot dry weight (mg)	Shoot dry weight (mg)
None	-	157 A	126 A
None	+	82 FG	60 BCDE
E. herbicola P-41	+	78 FG	65 BC
E. herbicola P-44	+	101 CD	62 BCD
Bacillus spp. P-3	+	--	54 CDEF
Bacillus spp. P-25	+	88 EF	49 EF
P. fluorescens P-56	+	89 DEF	67 B
P. fluorescens P-209	+	103 C	66 BC
P. fluorescens P-38	+	89 DEF	53 DEF
P. fluorescens P-57	+	--	69 B
P. syringae P-40	+	76 F	44 F
P. fluorescens P-43	+	--	49 EF
Actinomycete P-204	+	98 CDE	--
Actinomycete P-212	+	97 CDE	--
Actinomycete P-28	+	126 B	--
None - Untreated soil	+	107 DE	--
LSD[b]		12	12

[a]: Rate of inoculum 0.1% w/w.
[b]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 18. Tests of antagonism against Gaeumannomyces graminis var. tritici with bacteria isolated from Bozeman PF soil, conducted in pots with sterile Bozeman PF soil.

Antagonist	Test 3		Test 4	
	Ggt	Shoot dry weight (mg)	Ggt[a]	Shoot dry weight (mg)
No organism	-	173 C	+	79 A
E.herbicola P-41	-	169 C	+	78 A
E.herbicola P-44	-	199 AB	+	83 A
P.fluorescens P-56	-	191 ABC	+	68 A
P.fluorescens P-57	-	207 A	+	80 A
P.fluorescens P-209	-	181 BC	+	77 A
LSD[b]		21.2		15

[a]: Rate of inoculum: 0.1% w/w.
[b]: Least significant differences ($P<0.05$). Shoot dry weight values within a column followed by the same letter are not significantly different.

Table 19. Test of antagonism against Gaeumannomyces graminis var. tritici with mixtures of bacteria isolated from Bozeman PF soil, conducted in pots with sterile Bozeman PF soil.

Antagonist	Ggt[a]	Test 3
		Shoot dry weight (mg)
None	-	173 A
None	+	79 C
E. herbicola P-41 + P-44	+	85 C
E. herbicola P-41 + P. fluorescens P-56	+	80 C
P. fluorescens P-56	+	88 C
E. herbicola P-41 + P. fluorescens P-56 + NI[b] P-26	+	83 C
E. herbicola P-44 + P. fluorescens P-57	+	76 C
E. herbicola P-41 and P-44 + P. fluorescens P-56, P-57 and P-209	+	78 C
None - untreated soil	-	132 B
None - untreated soil	+	89 C
LSD[c]		14.2

[a]: Rate of inoculum 0.1% w/w.
[b]: Unidentified organism.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

In only one out of three tests of antagonism conducted with these organisms was a statistically significant increase in shoot dry weight observed (Test 1, Table 17). Three actinomycetes, isolates P-28, P-204, and P-212, isolate *P. fluorescens* P-209, and isolate P-44 of *E. herbicola* produced an increase in the shoot dry weight of wheat plants when added at a rate of 5% to soil infested with *Ggt*. None of the Gram negative (G-) isolates were able to show the same response in a second and third test (Tests 2 and 4, Tables 17 and 18), or when tested in mixtures (Test 5, Table 19). *E. herbicola* and *P. fluorescens* isolates increased the shoot dry weight of plants grown in the absence of the pathogen (Test 3, Table 18). Actinomycete isolates were tested only once.

In the tests of antagonism conducted with bacteria and actinomycetes isolated from Larslan 1 soil, none of the organisms tested, except the actinomycete isolate 1-203 in one test (Test 3, Table 21), was able to significantly reduce the severity of the disease, either in two independent tests with individual isolates (Tables 20 and 21), or when tested in mixtures (Table 22). The only highly significant increase in shoot dry weight was observed with the fungal isolate 1-58, either alone or in mixture with bacterial isolates (Table 22). The protection given by this fungus to the plants grown in *Ggt*-infested soil was so high that the shoot dry weight observed in this treatment was even higher than the one observed in the plants grown in untreated soil without *Ggt*.

Table 20. Test of antagonism against Gaeumannomyces graminis var. tritici with bacteria and actinomycetes isolated from Larslan 1 soil, conducted in pots with sterile Larslan 1 soil.

Antagonist	Test 1		Test 2	
	Ggt	Shoot dry weight (mg)	Ggt[a]	Shoot dry weight (mg)
None	-	223 A	+	123 AB
E. herbicola 1-7	-	223 A	+	113 ABCD
Pseudomonas spp. 1-12	-	206 A	+	110 BCDE
E. herbicola 1-13	-	208 A	+	116 ABC
P. fluorescens 1-14	-	213 A	+	114 ABCD
P. fluorescens 1-17	-	203 A	+	119 AB
Curtobacterium sp. 1-27	-	214 A	+	131 A
NI[c] 1-40	-	218 A	+	114 ABCD
NI 1-42	-	198 A	+	107 BCDE
Curtobacterium sp. 1-43	-	200 A	+	109 BCDE
P. fluorescens 1-15	-	200 A	+	94 DE
P. fluorescens 1-19	-	199 A	+	93 E
NI 1-55	-	203 A	+	104 BCDE
Klebsiella spp. 1-56	-	194 A	+	99 CDE
Actinomycete 1-203	-	211 A	+	118 ABC
Actinomycete 1-205	-	212 A	+	113 ABCDE
Actinomycete 1-207	-	207 A	+	118 ABC
LSD[b]		20.8 NS[d]		20.4

[a]: Rate of inoculum 0.1% w/w.
[b]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.
[c]: Unidentified organism.
[d]: NS: No significant differences at $P<0.05$.

Table 21. Test of antagonism against *Gaeumannomyces graminis* var. *tritici* with bacteria and actinomycetes isolated from Larslan 1 soil, conducted in pots with sterile Larslan 1 soil.

Antagonist	Ggt[a]	Test 3
		Shoot dry weight (mg)
None	-	148 A
None	+	80 CD
Bacillus spp. 1-3	+	77 CD
E. herbicola 1-7	+	80 CD
NI G-[b] 1-11	+	88 BC
E. herbicola 1-13	+	81 BCD
P. fluorescens 1-14	+	85 BCD
Pseudomonas spp. 1-16	+	85 BCD
P. fluorescens 1-17	+	84 BCD
P. fluorescens 1-19	+	86 BCD
Curtobacterium spp. 1-27	+	83 BCD
E. herbicola 1-32	+	75 CD
Bacillus spp. 1-37	+	76 CD
NI 1-40	+	73 D
NI 1-42	+	88 BC
Curtobacterium spp. 1-43	+	87 BCD
Pseudomonas spp. 1-44	+	87 BCD
NI 1-55	+	73 D
Klebsiella spp. 1-56	+	85 BCD
Actinomycete 1-203	+	94 B
LSD[c]		13.9

[a]: Rate of inoculum 0.1% w/w.
[b]: NI G-: Unidentified Gram negative organisms.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 22. Test of antagonism against Gaeumannomyces graminis var. tritici with mixtures of bacteria and actinomycetes isolated from Larslan 1 soil, conducted in pots with sterile Larslan 1 soil.

Antagonist	Ggt[a]	Test 4
		Shoot dry weight (mg)
None	-	217 A
None	+	104 EF
P. fluorescens 1-17 + E. herbicola 1-13	+	114 E
P. fluorescens 1-15 and 1-19	+	98 EFG
Actinomycetes 1-203, 1-205, and 1-207	+	97 EFG
P. fluorescens 1-17 + Klebsiella spp. 1-56 + NI 1-55	+	93 FG
Pseudomonas spp. 1-12 + P. fluorescens 1-14	+	107 EF
Curtobacterium spp. 1-43 + NI[b] 1-40 + NI 1-42	+	91 FG
E. herbicola 1-13 + P. fluorescens 1-14, 1-15, and 1-17	+	90 EFG
Actinomycete 1-203 + P. fluorescens 1-17 + E. herbicola 1-13 + NI 1-55	+	115 E
Fungus 1-58	+	189 BC
Fungus 1-58 + E. herbicola 1-13 + P. fluorescens 1-14 and 1-15	+	202 AB
P. fluorescens 1-17 + Klebsiella spp. 1-56 + NI 1-42	+	100 EF
No organisms-untreated soil	-	178 C
No organisms-untreated soil	+	155 D
LSD[c]		17.8

[a]: Rate of inoculum 0.1% w/w.
[b]: NI: No identified organism.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

None of the bacteria or actinomycetes tested was able to increase the shoot dry weight in the absence of the take-all fungus (Test 1, Table 20).

In the tests of antagonism against Ggt performed with bacteria and actinomycetes isolated from Toston LS soil it was observed that when a mixture of isolates was used the plants responded much better than when the isolates were used alone. The two tests conducted with individual isolates gave inconsistent results (Tests 1 and 3, Tables 23 and 24) (Note isolates P. fluorescens T-15 and P. corrugata T-16). However, most of the treatments with a mixture of isolates significantly increased the shoot dry weight of plants grown in soil infested with Ggt (Table 25). None of the individual isolates increased the shoot dry weight of plants grown in soil without the take-all fungus (Test 2, Table 24).

Actinomycete isolates produced the greatest reduction in the expression of the disease, either individually or in mixture (Tables 24 and 25). Even though all the treatments involving a mixture of pseudomonads significantly increased the shoot dry weight of plants as compared with the Ggt-infested check without antagonists, the magnitude of the increase was, in all cases, lower than the one observed in the treatments with actinomycetes alone (Table 25).

The analysis of the data obtained in all the tests of antagonism conducted with all isolates from the three soils indicates that the two fungi from Larslan 1 soil, and the actinomycetes and bacteria from Toston LS soil were the only organisms able to significantly (both biologically and statistically) increase the shoot dry weight of wheat

Table 23. Test of antagonism against Gaeumannomyces graminis var. tritici with bacteria and actinomycetes isolated from Toston LS soil, conducted in pots with sterile Toston LS soil.

Antagonist	Ggt[a]	Test 1
		Shoot dry weight (mg)
None	-	129 A
None	+	69 CD
Bacillus spp. T-5	+	59 DEFG
Pseudomonas spp. T-10	+	71 C
P. fluorescens T-11	+	69 CD
Pseudomonas spp. T-12	+	66 CDE
Pseudomonas spp. T-13	+	61 CDEF
P. fluorescens T-14	+	63 CDEF
P. fluorescens T-15	+	89 B
P. corrugata T-16	+	84 B
P. fluorescens T-23	+	76 BC
NI G+[b] T-26	+	62 CDEF
NI G+ T-27	+	55 FG
Bacillus spp. T-37	+	56 EFG
Bacillus spp. T-38	+	54 FG
LSD[c]		10.3

[a]: Rate of inoculum 0.1% w/w.
[b]: NI G+: No identified Gram + organism.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 24. Test of antagonism against _Gaeumannomyces graminis_ var. _tritici_ with bacteria and actinomycetes isolated from Toston LS soil, conducted in pots with sterile Toston LS soil.

Antagonist	Test 2		Test 3	
	Ggt	Shoot dry weight (mg)	_Ggt_[a]	Shoot dry weight (mg)
None	-	178 A	+	72 C
Pseudomonas spp. T-10	-	174 A	+	89 B
P. fluorescens T-14	-	183 A	+	70 C
P. fluorescens T-15	-	189 A	+	69 C
P. corrugata T-16	-	178 A	+	69 C
P. fluorescens T-23	-	191 A	+	80 BC
Actinomycetes T-206	-	184 A	+	117 A
Actinomycetes T-207	-	176 A	+	130 A
LSD[b]		16.6		16.9

[a]: Rate of inoculum 0.1% w/w.
[b]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 25. Test of antagonism against *Gaeumannomyces graminis* var. *tritici* with mixtures of bacteria and actinomycetes isolated from Toston LS soil, conducted in pots with sterile Toston LS soil.

Antagonist	*Ggt*[a]	Test 4 Shoot dry weight (mg)
None	-	159 A
None	+	67 J
Pseudomonas T-10 + *P. corrugata* T-16	+	96 GH
P. fluorescens T-14 and T-15	+	84 HI
Actinomycetes T-206 and T-207	+	120 DE
P. fluorescens T-14 and T-23 + *P. corrugata* T-16	+	77 IJ
P. fluorescens T-14, T-15, and T-23 + *P. corrugata* T-16 + *Pseudomonas* T-10	+	74 IJ
Actinomycetes T-206 + *P. corrugata* T-16 + *P. fluorescens* T-15 and T-23	+	117 DE
Actinomycetes T-207 + *P. corrugata* T-16 + *P. fluorescens* T-15 and T-23	+	113 DEF
P. fluorescens T-14 and T-15 + *P. corrugata* T-16 + *Pseudomonas* T-10	+	84 HI
P. corrugata T-16 + *P. fluorescens* T-14	+	85 HI
Actinomycetes T-206 + *P. fluorescens* T-15 + *P. corrugata* T-16	+	125 CD
Actinomycetes T-207 + *P. fluorescens* T-15 + *P. corrugata* T-16	+	102 FG
None - untreated soil	-	142 B
None - untreated soil	+	109 EFG
LSD[b]		15.3

[a]: Rate of inoculum 0.1% w/w.
[b]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

plants. The degree of increase in this parameter observed in the tests with a mixture of isolates from Toston LS soil was the highest when compared with all the other tests in Bozeman PF and Larslan 1 soils. The only exception was the use of actinomycete P-28 from Bozeman PF soil. Comparing all the tests performed with mixtures of *in vitro* antagonists, Toston LS isolates were the only ones significantly reducing the expression of the disease.

Tests of Antagonism with Fungi. None of the fungi isolated from Bozeman PF and Toston LS soils significantly protected the plants from take-all. However, two fungi isolated from Larslan 1 soil caused a highly significant reduction in the expression of the disease (Table 26).

The same magnitude of protection was observed in two separate experiments conducted with the isolates 1-52 and 1-58 when two rates of antagonist-infested soil were added to *Ggt*-infested or -noninfested soils (Table 27). In experiment 1 the weaker performance of isolate 1-58 was later found to be caused by the contamination of the inoculum by another fungus. The same contaminated inoculum was used in the experiment 1 shown in Table 28. Only in experiment No.1 shown in Table 27, was a differential response to the rate of antagonist-infested soil observed. In the absence of the pathogen, isolate 1-58 increased the shoot dry weight of wheat seedlings.

For the purpose of corroborating these results and to observe whether these two antagonistic fungi could maintain protection for a longer period in sterile soil as well as in natural soil, two other

Table 26. Tests of antagonism against Gaeumannomyces graminis var. tritici with fungi isolated from Bozeman PF, Larslan 1, and Toston LS soils, conducted in pots with sterile Bozeman PF, Larslan 1, and Toston LS soils.

Soil	Isolate	Ggt[a]	Test 1
			Shoot dry weight (mg)
Bozeman PF	None	-	101 A
	None	+	53 CD
	Isol. P-45	+	61 BC
	Isol. P-47	+	61 BC
	Isol. P-51	+	50 D
	Isol. P-54	+	44 D
	Isol. P-56	+	60 BC
	Isol. P-58	+	69 B
	LSD[b]		9.3
Larslan 1	None	-	146 A
	None	+	40 C
	Isol. 1-48	+	43 C
	Isol. 1-49	+	47 C
	Isol. 1-51	+	41 C
	Isol. 1-52	+	129 B
	Isol. 1-58	+	139 AB
	LSD		11.3
Toston LS	None	-	113 A
	None	+	40 D
	Isol. T-47	+	52 BC
	Isol. T-48	+	46 CD
	Isol. T-54	+	59 B
	Isol. T-55	+	39 D
	LSD		9.8
Toston LS	None	-	124 A
	None	+	64 B
	Isol. T-43	+	63 B
	Isol. T-45	+	58 B
	LSD		13.0

[a]: Rate of inoculum : 0.1% w/w.
[b]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are no significantly different.

Table 27. Tests of antagonism against <u>Gaeumannomyces graminis</u> var. <u>tritici</u> with the fungi 1-52 and 1-58 isolated from Larslan 1 soil, conducted in pots with sterile Larslan 1 soil.

Antagonist	Inoculum[a] rate (%)	Ggt[b]	Shoot dry weight (mg)
Experiment 1			
None	0	-	169 B
None	0	+	79 G
Isol. 1-52	5	-	168 B
Isol. 1-58	5	-	184 A
Isol. 1-52	1	+	136 D
Isol. 1-52	5	+	157 BC
Isol. 1-58	1	+	93 F
Isol. 1-58	5	+	114 E
Isol. 1-52 + 1-58	1	+	151 C
LSD[c]			13.7
Experiment 2			
None	0	-	221 A
None	0	+	90 B
Isol. 1-52	2	+	226 A
Isol. 1-52	4	+	227 A
Isol. 1-58	2	+	215 A
Isol. 1-58	4	+	219 A
Isol. 1-52 + 1-58	2	+	217 A
LSD			16

[a]: Rate of tested isolates mixed into soil based on w/w.
[b]: Rate of inoculum: 0.1% w/w.
[c]: Least significant differences ($P<0.05$). Shoot dry weight values followed by the same letter are not significantly different.

Table 28. Tests of antagonism against *Gaeumannomyces graminis* var. *tritici* with the fungi 1-52 and 1-58 isolated from Larslan 1 soil, conducted on pots with sterile Larslan 1 soil (Experiment 1), and with untreated Bozeman PF soil (Experiment 2).

Antagonist	*Ggt*[a]	Shoot dry weight (mg)	Root dry weight (mg)
Experiment 1[b]			
None	-	760 A	267 A
None	+	167 D	55 C
Isol. 1-52 (5% mixed w/soil)	+	680 B	236 A
Isol. 1-58 (5% mixed w/soil)	+	352 C	139 B
LSD[c]		64	38.2
Experiment 2			
None	-	462 A	201 A
None (1 cm sterile soil under seed)	+	262 C	108 B
Isol. 1-52 (5% mixed w/soil)	+	390 B	135 B
Isol. 1-58 (5% mixed w/soil)	+	346 B	130 B
Isol. 1-52 (1 cm under seed)	+	477 A	213 A
Isol. 1-58 (1 cm under seed)	+	501 A	205 A
LSD		57.3	29

[a]: Rate of inoculum : 0.1% w/w.
[b]: Experiments 1 and 2 conducted in big conetainers (4 cm diameter x 21 cm long).
[c]: Least significant differences ($P<0.05$). Shoot or root dry weight values followed by the same letter are not significantly different.

experiments were conducted in big conetainers with a growth period of 40 days. Two methods of applying the antagonist-infested soil were tested in sterile Larslan 1 soil and untreated Bozeman PF soil. In these two new experiments the high performance of both antagonists was again observed (Table 28). Plants in the treatment where the antagonist-infested soil was added as a 1-cm layer under the seed exhibited the best response showing no statistically significant differences with the Ggt-noninfested check (Experiment 2, Table 28). The most important aspect of the results observed in this experiment is the fact that the same high protection was obtained in a natural soil conducive to take-all. The level of protection provided to wheat plants by both antagonists was also reflected in the root dry weight.

Both fungi were easily reisolated from roots of wheat growing in these assays. Under the light microscope, isolate 1-52 was observed to parasitize Ggt hyphae when both fungi were grown together on low strength PDA.

Discussion

Most of the studies on soils suppressive and conducive to take-all have demonstrated the unquestionable involvement of an antagonist microflora that arises as a consequence of wheat monoculture (Shipton, 1975; Cook and Weller, 1987). Continuous wheat cropping seems to naturally select microorganisms that reduce the severity of the disease. Microbial characterization of these soils has shown that the most noticeable differences among suppressive and conducive soils are the higher populations of aerobic organisms in conducive soils as

compared to suppressive soils, and also the significantly higher populations of organisms isolated from suppressive soils that inhibit Ggt in vitro, as compared to those from conducive soils (Simon and Sivasithamparam, 1988a; Smiley, 1978b; Cook and Rovira, 1976; Weller et al., 1988).

Tests for antagonism in vitro (i.e., antibiosis) have been commonly used to determine the organisms likely to be involved in suppressive soils. Following the selection of organisms based on their in vitro antagonism, soil tests are conducted to corroborate their antagonism to Ggt. However, it is this second step where most of the results show inconsistency with the in vitro tests. No conclusive results have yet been obtained to demonstrate the involvement of a particular organism or group of organisms in the suppression exhibited by all soils to take-all. Therefore, the mechanism of suppression is still a matter of debate. While there is much evidence for the involvement of Pseudomonas spp. in suppressiveness, lack of consistent evidence under various conditions has hindered acceptance of this group as being the sole cause of suppression. Due to this lack of consistency between antibiosis in vitro and reduction in the severity of the disease in soil tests, and the fact that antibiosis has not been proven to be the only factor involved in antagonism toward Ggt, mechanisms other than antibiosis have been suggested for the suppression of the take-all fungus (Thomashow and Weller, 1990; Poplawsky and Ellingboe, 1989; Weller, 1985; Sivasithamparam and Parker, 1978). Hornby (1983), in pointing out the complexity of the microbial antagonism in suppressive soils, indicates that "a major

problem that besets this subject (microbial antagonism) is that many of the mechanisms discussed are presumptive and proof is difficult to come by."

An aspect that seems to have been overlooked in a number of studies is the effect that the environment must exert on the expression of antagonism against the pathogen. Antagonism tests in substrates other than the soil from which the antagonists were isolated have been commonly reported. Coincidentally, these same reports indicate failure of the *in vitro* selected antagonists to reduce the severity of the disease when added to the soil (Broadbent et al., 1971; Maplestone and Campbell, 1989).

The constitution of the culture media and its differential effect on the expression of antibiosis have been abundantly reported (Rovira and Wildermuth, 1981; Fravel, 1988). Therefore, the assumption that what is observed in *in vitro* tests, i.e., type and amount of antibiotic produced in artificial media, is also likely to occur in soil may not be necessarily correct. Changes in antagonism when different nitrogen sources are added to suppressive soils have also been reported (Smiley, 1978b). In light of this observation it seems likely that nutrition may also be involved in the expression of antagonism, and therefore it should be another criterion to consider when carrying out *in vitro* and soil tests for antagonism towards *Ggt*.

The main objective of this thesis was to determine the organisms involved in Montana soils suppressive and conducive to take-all of wheat. The main hypothesis to be tested was that the suppression of take-all is due to an organism or group of organisms with antagonistic

properties toward _Ggt_. A second hypothesis was that lack of consistency between _in vitro_ and _in vivo_ tests could be altered by making some changes in methodology. The _in vitro_ tests of antagonism were conducted in low strength PDA using wells filled with sterile soil from which the isolates to be tested originated. The main purpose of using sterile soil and low strength PDA was to bring into play abiotic factors (i.e., nutrition) affecting the production of antibiotics that might be present in suppressive soils, and to avoid high-nutrient-containing media that could change the expression of antibiosis. The effect of abiotic factors in soils in affecting antagonism was suggested by the results obtained in the first chapter of this thesis, and in Tables 13, 14, 15, and 16 of this chapter.

Measuring shoot dry weight following 30 to 40 days of growth also was found to be an excellent assessment of the effectiveness of the antagonists. Homogeneous inoculum particle sizes, homogeneous wheat seed sizes, the use of distilled water without chlorine for irrigation, and the use of the same soil from which the _Ggt_ antagonists were isolated in all tests conducted for antagonism _in vivo_, were other modifications made to the techniques commonly used.

The results of this thesis agree with the two most consistent differences observed between soils suppressive and conducive to take-all of wheat. Higher populations of isolatable microorganisms in soils conducive to the disease, as well as higher populations of _in vitro_ antagonists to _Ggt_ from both soils suppressive to take-all, were observed. Bozeman PF soil conducive to the take-all disease, exhibited a higher population of "total" microorganisms than the soils

suppressive to the disease. Both Larslan 1 and Toston LS soils suppressive to the take-all fungus exhibited the highest population of organisms antagonistic to Ggt when tested in vitro. Similar results have been reported in other studies (Simon and Sivasithamparam, 1988a; Smiley, 1978a; Cook and Rovira, 1976; Weller et al., 1988).

However, despite the similarities with other reports, the results of this thesis suggest that organisms and mechanisms of suppression other than the ones suggested to exist for other soils are involved in the suppression of take-all exhibited by the two Montana soils. Mycoparasitism appears to be the most likely mechanism involved in the suppression exhibited by Larslan 1 soil toward Ggt. On the other hand, antagonism due to actinomycete species, and also the presence of Pseudomonas spp., associated with abiotic factors appear as the most likely explanations for the suppression observed in Toston LS soil.

The mechanism of suppression exhibited by Larslan 1 soil is suggested as a consequence of the isolation of two fungi with exceptional ability to suppress the take-all fungus. That ability was demonstrated in at least six different experiments. In all of them, including use of natural soil conducive to take-all, the reduction in disease severity was nearly complete even over a 40 day period of growth.

Due to the fact that both fungi were isolated from a soil suppressive to take-all, and because none of the bacteria and actinomycetes isolated from the same soil exhibited antagonism toward Ggt in vivo, the suppression of the disease in Larslan 1 soil seems to be related to the presence of both fungi. The identification of these

organisms remains to be made since neither produced conidia or other reproductive structures in culture. Both have septate mycelium and isolate 1-52 produces a diffusible yellow pigment in culture. The yellow pigment was useful in identifying the presence of the fungus in wheat roots. Both fungi were easily reisolated from roots of wheat plants grown in infested soil. Isolate 1-52 was observed to parasitize hyphae of Ggt when both fungi were grown together on PDA but this needs further study.

Fungi associated with take-all suppressive soils or with antagonism of Ggt have been reported by Simon and Sivasithamparam (1988b), Dewan and Sivasithamparam (1988, 1989), Siegel (1961), Mangan (1967), Wong and Siviour (1979). The fungus Phialophora radicicola has been reported to control Gaeumannomyces graminis in grasses (Deacon, 1976), and Gaeumannomyces graminis var. tritici in wheat (Wong, 1975). In both cases, cross protection is believed to be involved, but this mechanism is not associated with take-all decline. Reports of a similar level of protection of wheat plants as the one determined during this research were not found.

Successful control of plant pathogens by fungi have been reported in the case of tomato wilt caused by Fusarium oxysporum f.sp. lycopersici using Cephalosporium sp.(Phillips et al., 1967), tomato and peanut diseases caused by Sclerotium rolfsii using Trichoderma harzianum (Wells et al., 1972), white rot of onion caused by Sclerotium cepivorum using Coniothyrium minitans (Ahmed and Tribe, 1977), and cucumber black root rot caused by Phomopsis sclerotioides using Gliocadium roseum (Moody and Gindrat, 1977).

The nature of the mechanism involved in the suppression of take-all in Toston LS soil seems to be different from the Larslan 1 soil, since no fungal antagonists toward Ggt were isolated. Also, Toston LS was the only soil that exhibited some relation between antagonism *in vitro* and reduction of the disease in *in vivo* tests. Actinomycetes are believed to play the main role in suppression of the disease in this soil based on the consistency and magnitude of the reduction in the severity of the disease that they caused in soil tests. Furthermore, the actinomycetes was the only group of organisms in which antibiosis was not affected by the type of test soil. They were also the only group of organisms that maintained their antibiosis activity against Ggt over the 21 day *in vitro* test period (Table 10).

Toston LS was also the only soil in which wheat plants exhibited a significant increase in shoot dry weight when grown in soil infested with mixtures of antagonists other than actinomycetes. These results suggest that *Pseudomonas* spp. might also be involved in suppression. Factors that might better explain this type of response are the variety of different antibiotics produced by members of the genus *Pseudomonas*, and the depletion of iron for the take-all fungus by the high-siderophore-producing organisms in a low-iron environment as is the case of Toston LS soil. Since this soil had the highest population of siderophore-producing organisms and the lowest iron content, the involvement of iron depletion by *Pseudomonas* spp. may also explain in part the suppressive characteristics of this soil.

The results from tests obtained with both the antagonistic actinomycetes and the mixture of *Pseudomonas* species in decreasing the

severity of take-all in Toston LS soil agree with most other studies regarding microbial characterization of soils suppressive to take-all. While the involvement of Pseudomonas species has been widely demonstrated as being one of the factors most commonly associated with soils suppressive to take-all (Weller et al., 1988; Cook and Weller, 1987; Smiley, 1979; Brisbane and Rovira, 1988), actinomycetes have also been reported in a number of studies to be associated with this phenomenon (Smiley, 1978b; Cook and Rovira, 1976; Sivasithamparam and Parker, 1978). Furthermore, actinomycetes successfully involved in the protection against plant pathogens have been reported for cotton wilt caused by Fusarium vasinfectum (Arjunarao, 1971, cited by Mangenot and Diem, 1979), wilt of lentils caused by Fusarium oxysporum (Mehrotra and Caludius, 1972), damping-off of pepper caused by Rhizoctonia solani (Broadbent et al., 1971), and wheat root disease caused by Rhizoctonia solani (Merriman et al., 1974).

The use of the sterile soil-containing wells in tests of antagonism towards Ggt appears to be a very useful technique to detect changes in both the expression of antibiosis exhibited by antagonists against Ggt, and the expression of hyperantagonism among Ggt antagonists. Even though the type of antibiotics produced by the different Ggt antagonists was not determined, my results suggest that different antibiotics may be produced by the same strain of bacteria or actinomycete when placed in different soil-containing wells. Some of the evidence that seems to support this hypothesis is seen in Table 13. The same strain of P. fluorescens isolated from Bozeman PF soil did not exhibit antibiosis towards Ggt when placed in Bozeman PF sterile soil,

but it did show antibiosis against the take-all fungus when placed in Toston LS sterile soil. A similar differential response was observed in the tests of hyperantagonism, a portion of which are presented in Table 15. P. fluorescens isolate 1-19 differentially antagonized 5 Ggt antagonists when placed in any of three soils. Also interesting is the fact that while some P. fluorescens strains isolated from either Larslan 1 or Bozeman PF soils exhibited reduced antibiosis when placed in Toston LS soil, most of the P. fluorescens strains isolated from Toston LS did exhibit antibiosis when placed in the soil from which they were isolated. The latter appears to correlate well with the tests of antagonism in Toston LS soil. This soil was the only one where reduction in the severity of the disease was observed when mixtures of Pseudomonas spp. were added to the soil (Table 25). The association of abiotic factors with a differential expression of antibiosis also seems to be supported by the results presented in the first chapter of this thesis. When untreated Bozeman PF soil was added to sterile Toston LS soil, a dramatic reduction in the severity of the disease was observed. This might be explained by a differential production of antibiotics when organisms present in the conducive soil were exposed to another soil, but which are unable to produce the same reaction in the conducive soil because of an as yet unidentified abiotic factor. Therefore, to the observation made by Cook and Rovira (1976) that specific strains of P. fluorescens must be present in order to obtain suppression, it should be added that an adequate environment is also necessary for these strains to express their antagonism towards Ggt.

Despite the above, the involvement of iron sequestration as a mechanism involved in the suppression exhibited by Toston LS soil can not be discarded, since the highest population of siderophore-producing bacteria was found in this soil, which also shows low iron content.

Both the identification of antibiotics produced under different conditions and the effect of hyperantagonists in soils suppressive to take-all is worthy of further investigation. In the former case, the use of soil-containing wells may be useful. A better correlation between antibiotics produced by antagonists *in vitro* and those produced in soil by the same organisms would greatly improve the selection of potential antagonists and it could also shed new light on role of antibiotics in the suppression of take-all.

SUMMARY

An antagonistic microflora that arises as a consequence of wheat monoculture is postulated to be the main mechanism involved in the suppression exhibited by some soils to the take-all disease of wheat, caused by the fungus Gaeumannomyces graminis (Sacc.) Von Arx & Olivier var. tritici Walker (Ggt). After the discovery in The Netherlands of the biological nature of the suppression of this disease by Gerlagh, in 1968, a number of publications report worldwide the presence of soils suppressive to take-all. This finding opened a new field in the search for biological control of take-all of wheat, a disease that so far has eluded most, if not all, traditional methods of plant disease control.

The determination of the organisms and mechanisms involved in soils suppressive to take-all has received more attention than any other soil suppressive to a soil-borne disease. However, despite these efforts, no conclusive results have been found to explain completely the nature of the suppression in different soils and under different conditions. The need for additional information and different approaches to the study of this phenomenon has been widely suggested.

With this in mind, this research was initiated to determine both the presence of soils suppressive to take-all of wheat in Montana and the organisms most likely involved in the suppression exhibited by these soils.

In an initial screening of eight soils collected from different wheat growing areas of Montana, three soils were selected as being either conducive or suppressive to take-all. The suppressive or conducive properties of the selected soils were corroborated in two separate experiments performed for each soil. Bozeman PF soil where wheat was grown in a 4 year rotation with green manure, exhibited properties conducive to the disease since the addition of 1% w/w of natural Bozeman PF soil to Bozeman PF sterile soil, consistently failed to reduce the severity of the disease. In contrast, Larslan 1 and Toston LS, soils where wheat had been grown as a monoculture for over 10 years, both exhibited suppressive properties toward _Ggt_. In an attempt to determine the exclusiveness of the suppression of a particular soil, a test involving the addition of 1% of natural soil interchangeably among all three soils was conducted. In this test 1% of natural soil conducive to take-all dramatically reduced the expression of the disease when added to a sterile suppressive soil. These results suggest that antagonism may also be associated with abiotic factors that activate the expression or repression of an antagonistic microflora under different conditions.

As a second step, the microbial characterization of these three soils was conducted. The results indicated that two different mechanisms appear to be involved in the suppression exhibited by both soils suppressive to take-all. In Larslan 1, mycoparasitism appears to be the main factor associated with the suppression to take-all since two different fungi with exceptional ability to reduce the severity of the disease were isolated from this soil. In six separate experiments,

including natural soil conducive to the disease, both fungi definitely demonstrated this ability to control take-all. No similar levels of control of take-all with a fungus have ever been reported.

In Toston LS soil, antagonism by actinomycetes and perhaps the involvement of *Pseudomonas* spp. in antagonism and/or iron depletion, appear to be the most likely mechanisms involved in the suppression of take-all. Actinomycetes added individually or in mixtures to *Ggt*-infested soil consistently reduced the severity of the disease to a greater extent than that provided by mixtures of *Pseudomonas* spp.

It is suggested by the results of this thesis that an association between antagonism and unidentified abiotic factors in the soil was consistently involved in the biological suppression of take-all exhibited by Toston LS soil.

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APPENDIX

Media

Antibiotics and Sigma products were obtained from Sigma Chemical Co., St. Louis, MO. Bacto products were obtained from Difco Laboratories, Detroit, MI. BBL products were procured from BBL Microbiology Systems, Cockeysville, MD. Rose Bengal was obtained from K & K Laboratories, Inc., Plainview, NY. Baker products were procured from Baker Chemical Co., Phillipsburg, NJ.

MRS Agar

Bacto Lactobacilli Broth., Difco	55.0 g
$CaCO_3$	3.5 g
Bacto agar, Difco	15.0 g
Distilled H_2O	1.0 l

Autoclave 20 min at 121°C.

Tryptic Soy Broth Agar

Bacto Tryptic Soy Broth, Difco	30.0 g
Bacto Agar, Difco	15.0 g
Distilled H_2O	1.0 l

Autoclave 20 min at 121°C.

1/10 Tryptic Soy Broth Agar

Bacto Tryptic Soy Broth, Difco	3.0 g
Bacto Agar, Difco	15.0 g
Distilled H_2O	1.0 l

Autoclave 20 min at 121°C.

<u>Regular Strength PDA</u>

Potato Flakes (instant potatoes)	6.0 g
Dextrose (anhydrous), Baker	12.0 g
Bacto Agar, Difco	18.0 g
Distilled H_2O	1.0 l

Boil potato flakes for 2 min in 250 ml distilled water, filter through 4-layers cheese cloth, restore to 1.0 l with distilled water, add dextrose and agar, autoclave for 20 min at 121°C.

<u>Low Strength PDA</u>

Potato Flakes (instant potatoes)	3.0 g
Dextrose (anhydrous), Baker	6.0 g
Bacto Agar, Difco	18.0 g
Distilled H_2O	1.0 l

Prepare as above.

<u>PDA ± Streptomycin ± Triadimenol</u> (modified from Dewan and Sivasithamparam, 1988)

Potato Flakes (instant potatoes)	6.0 g
Dextrose (anhydrous), Baker	12.0 g
Bacto Agar, Difco	18.0 g
Distilled H_2O	1.0 l

Boil potato flakes in 250 ml for 2 min, filter through 4-layer cheese cloth, restore to 1 l with distilled water, add dextrose and agar, autoclave 20 min at 121°C; cool to 45°C and add the following:

Streptomycin	100.0 mg
Triadimenol	10.0 mg

PDA + Rose Bengal + Vancomycin (modified from Martin, 1950)

Potato Flakes (instant potatoes)	6.0 g
Dextrose (anhydrous), Baker	12.0 g
Bacto Agar, Difco	18.0 g
Distilled H_2O	1.0 l

Prepare as above, autoclave 20 min at 121°C; cool to 45°C and add the following:

Rose Bengal, K & K Laboratories	10.0 mg
Vancomycin	50.0 mg

Modified King's Medium B (Sands et al., 1980)

Proteose Peptone #3, Difco	20.0 g
$K_2HPO_4 \cdot 3 H_2O$	1.5 g
Mg SO_4 (anhydrous)	1.5 g
Glycerol, Sigma	15.0 ml
Bacto Agar, Difco	15.0 g
Double Distilled H_2O	1.0 l

Autoclave at 121°C; cool to 45°C and add the following:

Bacitracin	10.0 mg
Vancomycin	6.0 mg
Rifampicin	0.5 mg
Cycloheximide	100.0 mg
Benomyl	250.0 mg

Modified Soil Extract Agar (Parkinson et al., 1971)

$K_2HPO_4 \cdot 3 H_2O$	0.2 g
Glucose	1.0 g
Bacto Gelatin, Difco	30.0 g
Bacto Agar, Difco	15.0 g
Soil Extract*	1.0 l

Autoclave 20 min at 121°C; cool at 45°C and add the following:

Cycloheximide	100.0 mg
Polymyxin B Sulfate	5.0 mg

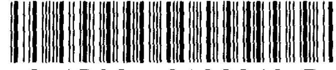
* Soil extract is obtained by autoclaving 1 kg soil in 1 l distilled water for 20 min at 121°C, filtering and restoring to 1 l volume with distilled water. Modified Soil Extract Agar medium was prepared separately for each soil.

Actinomycete Selective Medium (Kuster and Williams, 1964)

Soluble Starch, Sigma	10.0 g
Casein - Vitamin Free, Sigma	0.3 g
KNO_3	2.0 g
NaCl	2.0 g
$K_2HPO_4 \cdot 3 H_2O$	2.0 g
$MgSO_4$ (anhydrous)	0.05 g
$CaCO_3$	0.02 g
$FeSO_4 \cdot 7 H_2O$	0.01 g
Bacto Agar, Difco	20.0 g
Distilled H_2O	1.0 l

Autoclave 20 min at 121°C; cool at 45°C and add the following:

Polymyxin B Sulfate	5.0 mg
Sodium Penicillin	1.0 mg
Cycloheximide	50.0 mg
Nystatin	50.0 mg



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