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

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.

MICROBIOLOGICAL CHARACTERIZATION OF MONTANA SOILS SUPPRESSIVE AND CONDUCIVE TO TAKE-ALL DISEASE OF WHEAT CAUSED BY *Gaeumannomyces graminis* var. *tritici*

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of a thesis submitted by

Orlando Armando Andrade

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.

7-23-92
Date

D E Mathis
Chairperson, Graduate Committee

Approved for the Major Department

7-23-92
Date

T. W. Carroll
Head, Major Department by dem

Approved for the College of Graduate Studies

7-24-92
Date

R L Brown
Graduate Dean

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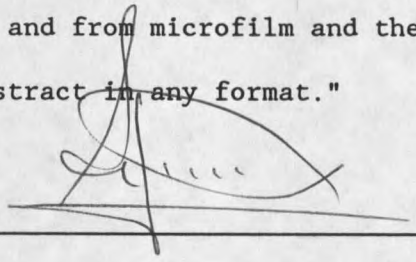
Signature 

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.

Treatments	Ggt[a]	Soil					
		Bozeman PF		Larslan 1		Toston LS	
		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.

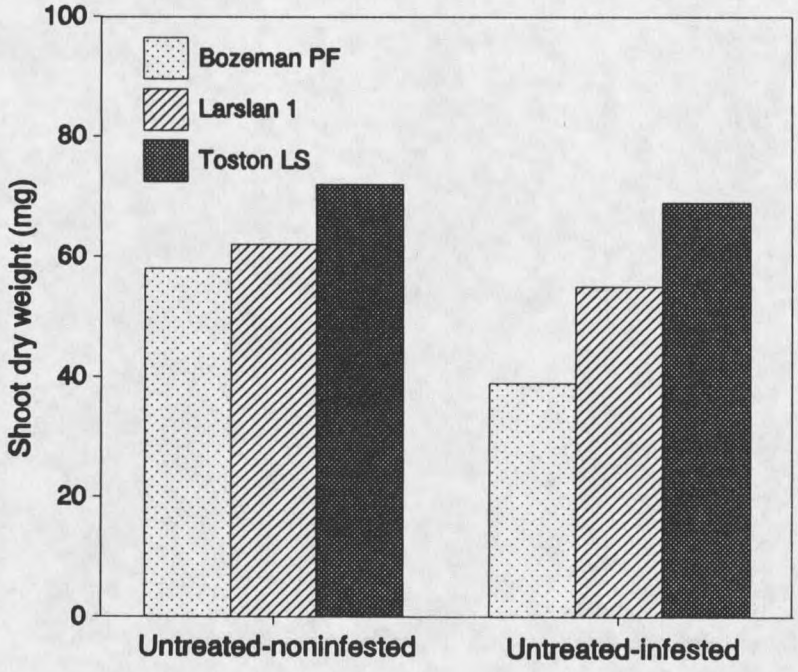

Figure 1. Shoot dry weight of wheat plants grown in untreated Bozeman PF, Larslan 1, and Toston LS soils infested with Gaeumannomyces graminis var. tritici. Average from experiments 1, 2 and 3 (Tables 2 and 4).

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.