The potential for the occurrence of Rhizoctonia root rot in cereal crop production areas of Montana
by Joseph Michael Hudak

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


Abstract:
Rhizoctonia root rot symptoms have been seen on small grain cereals in Montana. Isolation of Shizoctonia solani AG 8, the cause of Rhizoctonia root rot, has not been achieved from Montana soils. The focus of this study was to investigate the potential of Rhizoctonia root rot to occur in Montana and its effects under Montana environmental conditions.

In inoculated field plots, it was determined that the disease can cause root rot and yield losses up to 23%.

Field isolations were performed and R. solani AG 8 and AG 4 were identified.

Downy brome, jointed goatgrass, green foxtail, and wild oat were secondary hosts of the pathogen. Volunteer winter wheat also acts in a similar manner. All of these can subsequently serve as sources of infection for cereal crops.

Isolation of the pathogen is difficult from infected plants beyond five weeks after planting. The use of ELISA as a diagnostic tool was useful in detecting R. solani in infected plants more than five weeks after planting.

Rhizoctonia root rot was more prevalent in inoculated fumigated or inoculated pasteurized soils initially, but rapidly declined over time. This may be due to the activity of soilborne microorganisms.

Incidence of Rhizoctonia root rot in relation to timing of seeding after glyphosate application showed less incidence of root rot on barley planted two weeks after spraying for volunteer and weed control versus barley planted at one or seven days after glyphosate application.

The best time to sample for Rhizoctonia root rot was from 3 to 5 weeks, when isolations can be made and spear tipped roots are noticeable.

# THE POTENTIAL FOR THE OCCURRENCE OF RHIZOCTONIA ROOT ROT IN CEREAL CROP PRODUCTION AREAS OF MONTANA

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A thesis submitted in partial fulfillment of the
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of

Master of Science

in

Plant Pathology

MONTANA STATE UNIVERSITY
Bozeman, Montana

December 1992

APPROVAL

of a thesis submitted by

Joseph Michael Hudak

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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ACKNOWLEDGEMENTS

I would like to thank the Montana Wheat and Barley Committee and Monsanto Agricultural Products Company, whose financial support made this research possible.

I also extend my thanks to Dr. Jack Riesselman, Dr. Donald E. Mathre, Dr. Jack Martin, and Dr. Doug Ryerson for serving on my graduate committee and offering their guidance and support throughout the duration of this project.

A special thanks goes to Dr. Bill Grey, Bob Johnston, and Joanne Jennings, whose expertise in plot design and statistical analysis was greatly appreciated. Also, I acknowledge their help during planting and harvesting which could not have been done alone.

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## ABSTRACT

Rhizoctonia root rot symptoms have been seen on small grain cereals in Montana. Isolation of *Rhizoctonia solani* AG 8, the cause of Rhizoctonia root rot, has not been achieved from Montana soils. The focus of this study was to investigate the potential of Rhizoctonia root rot to occur in Montana and its effects under Montana environmental conditions.

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# INTRODUCTION

Plant pathogens are a major yield limiting factor in cereal crops throughout the world. The most ubiquitous of all the pathogens are the soilborne organisms, which are present in all cereal growing regions.

The fungi *Rhizoctonia solani* Kühn and *Rhizoctonia oryzae* Ryker and Gooch, cause a root rotting disease of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.). Rhizoctonia root rot is currently a major problem in the Pacific Northwest United States, Australia, and parts of Europe (23,27,28,32,41,50,59,60). The pathogen causes localized patchy, stunted areas in the field and is sometimes referred to as bare patch or purple patch (8,29,32,36). In Scotland, severe Rhizoctonia root rot is called barley stunt disorder (31,42).

Rhizoctonia root rot has been identified as a problem in Australia since 1928 (14,23,26,27,41,43,49,59), but has only recently been identified in the United States in the states of Oregon, Washington, and Idaho (36,41,59). Since 1984, it has become the most severe root rotting disease of wheat and barley in the Pacific Northwest (52,53).

The reduction in conventional farming methods has led to the increase in the incidence of Rhizoctonia root rot (13,26,41,43,44,46,47, 51,59). As growers move away from traditional tillage with farm implements to chemical tillage with a non-selective herbicide, the soil in fields is not disturbed and turned over. This creates optimum conditions for certain soilborne pathogens to develop. It is especially

true for *Rhizoctonia* which establishes itself by mycelial growth throughout the soil in and on plant debris within and on the soil surface (4,6,10). Planting within two weeks after spraying for weed control has been demonstrated to increase the incidence of Rhizoctonia root rot if the pathogen is present in the soil (13,22,25,26,41,43,44,45,47,51,53,59).

In Montana, during 1989, field symptoms of Rhizoctonia root rot developed in barley fields seeded shortly following application of glyphosate (Roundup). Analysis of root samples revealed the presence of the diagnostically characteristic spear tip symptom associated with this pathogen.

*Rhizoctonia* had previously been overlooked as the primary pathogen causing these symptoms. It is not uncommon to find more than one root rooting organism associated with plant decline (12,14,61). This has been described as a disease complex, which occurs in a number of other cereal diseases. This includes take-all caused by *Gaeumannomyces graminis* (Sacc.) von Arx & Oliver var. *tritici* Walker, crown rot caused by *Fusarium graminearum* Schwabe, common root rot caused by *Drechslera sorokiniana* (Sacc.) Subram & Jain, bare patch caused by *Rhizoctonia solani* Kühn, and Pythium root rot caused by *Pythium spp.* (61).

The reason *Rhizoctonia* has not been examined closer is due to the difficulty in isolating the pathogen. Above ground symptoms of Rhizoctonia root rot are not always noticeable early in the growing season. It is extremely difficult to isolate the pathogen from mature cereal plants due to secondary attack by other root colonizing fungi.

These tend to mask or override the isolation of *Rhizoctonia* (1,14,29,37,53).

Weeds have been shown to harbor *Rhizoctonia spp.* as saprophytes or parasites on their root system (5,15,36,38,39,44,45,47,49). They can provide the fungus with a nutrient source for survival in fields utilizing crop/fallow management systems. This may induce a high inoculum level of *Rhizoctonia* in the soil prior to planting, thus increasing probability of disease.

Montana producers are concerned about the potential effect of Rhizoctonia root rot. This concern is associated with the increased acreages receiving some form of herbicide application in place of tillage for weed or volunteer control. Estimates by weed science personnel at Montana State University (personal communication, Dr. Pete Fay) suggest that some form of chemical fallow will be used on nearly one million acres in Montana by 1994. These estimates are based on increased usage due to price reductions of herbicides, ease of weed control, moisture conservation, and residue compliance programs.

With the increase of chemical fallow, it is necessary that Montana grain producers understand the potential interactions that may exist between root rotting pathogens and herbicide usage. The purpose of this investigation is to develop a better understanding of Rhizoctonia root rot and its potential effects on Montana cereal production. The objectives are:

- to determine which organisms are associated with stunted plants in barley in various Montana locations.

- to determine the interaction of *R. solani* and other root colonizing fungi with glyphosate in relation to timing of seeding and herbicide application.
- to determine the role of common grassy weeds in the epidemiology of the disease.
- to determine if weed and volunteer density have an effect on disease severity and yield reduction.
- to identify the best time and method of sampling for Rhizoctonia root rot.
- to design effective disease management practices for Montana cereal producers.

# LITERATURE REVIEW

## Rhizoctonia

*Rhizoctonia* is an imperfect fungus in the Deuteromycete family. It rarely forms its sexual state in a natural setting (60). Its teleomorph, a basidiomycete, *Thanatephorus cucumeris* (Frank) Donk is the perfect stage for many other anamorphs (Table 1). There are many plant diseases on a wide range of hosts which are caused by various species within the genus (7,31,48,54).

Table 1. Anamorphs of *Thanatephorus cucumeris*.

| Isolates of *Rhizoctonia solani* have been described under the following: |
|---|
| Anamorphs: *R. alba, R. aderholdii, R. anomalla, R. betae, R. brassicarum, R. dauci, R. dichotoma, R. dimorpha, R. fusca, R. gossypii var. aegyptica, R. gossypii var. anatolica, R. lupini, R. macrosclerotia, R. melongena, R. microsclerotia, R. napae, R. napaeae, R. napi, R. potomacensis, R. practicola, R. rapae, R. solani var. cedrideodare, R. solani var. cichorii-endiviae, R. solani var. fortensis, R. solani var. lycoperisacae, R. solani var. typica.* |
| Teleomorph for all above: *Thanatephorus cucumeris* |

The genus concept of *Rhizoctonia* was established in 1815 by de Candolle and *R. solani* was first described by Kühn in 1858 (39,54). The original description of *R. solani* lacked specificity, which caused taxonomic confusion. The current species concept was established by

Parmeter and Whitney in 1970 (39). The new description stipulates that isolates of *R. solani* possess the following characteristics:

- some shade of brown hyphal pigmentation.
- branching near the distal septum of cells in young vegetative hyphae.
- constriction of hyphae and formation of septa a short distance from the point of origin of hyphal branches.
- dolipore septa.
- multinucleate cells in young vegetative hyphae.

At present the classification of *Rhizoctonia spp.* is based on cytomorphology of hyphae and the morphology of cultures. Supporting evidence for classification is provided by the morphology of teleomorphs, affinities for hyphal anastomosis, and more recently DNA base sequence homology (8,9,54). Other methods include serology (1) zymograms of pectic enzymes (34,57), isozyme analysis (21), fatty acid analysis (56), and analysis of restriction fragment length polymorphisms (RFLP's) (11,58).

Determination of the number of nuclei in vegetative hyphal cells is an important process in the identification of *Rhizoctonia spp.* The genus is divided into species with binucleate vegetative hyphal cells and those with multinucleate hyphal cells (39,54).

## Anastomosis Grouping

*R. solani* is multinucleate (20) and is subdivided into host specific subgroups and intraspecific groups. These have been termed anastomosis groups (AG's) and are categorized by their host plant pathogenicity and the ability of hyphal cell fusion or anastomosis between two strains of

the fungus (7). Anastomosis is based on the affinity for hyphal fusion with members of designated anastomosis groups. Division of the species into AG's is based on the ability of hyphae of different isolates to anastomose. When hyphae fuse, parent isolates are said to belong to the same AG (7).

The anastomosis reaction in *R. solani* is a sign of somatic incompatibility (7) and, despite the localized cell death (38) that often accompanies anastomosis, is indicative of relatedness between anastomosing isolates. Members of the same AG are said to be more closely related to each other than to members of other AG's (7). At present, there are twelve known anastomosis groups of *R. solani* (8) associated with a wide range of host plants (48,49), some having intraspecific groups (Table 2) (7,37,54).

The AG 8, which is host specific to wheat, barley, and oats, is the organism on which this study concentrates.

## Rhizoctonia Root Rot

*Rhizoctonia solani* Kühn AG-8 and *Rhizoctonia oryzae* Ryker and Gooch are the cause of Rhizoctonia bare patch and/or root rot (35,36,41,46). They are able to survive in or on plant debris on or near the soil surface (5,43). *R. solani* is most damaging at root zone temperatures below 15°C and *R. oryzae* prefers root zone temperatures above 15°C (36,49,53). The cool temperatures during planting season in Montana suggest that *R. solani* is the most probable organism associated with bare patch symptoms in Montana.

Table 2. Plant diseases associated with *Rhizoctonia solani* anastomosis groups.

| Anastomosis Group | Related Host Diseases |
|---|---|
| AG 1-IA | Sheath blight and sheath spot of rice<br>Sclerotial disease of corn<br>Leaf blight of corn, sorghum bean, and soybean<br>Banded leaf of sorghum and corn<br>Summer blight of crimson clover<br>Southern blight of camphor seedlings<br>Brown patch of turfgrass |
| AG 1-IB | Web blight and leaf blight of rice, bean, and soybean<br>Web blight of larch seedlings<br>Leaf blight of hortensia<br>Rot of cabbage<br>Bottom rot of lettuce |
| AG 1-IC | Damping off of buckwheat, carrot, soybean, and pine |
| AG 1-Unspecified | Foliar blight of cabbage<br>Leaf blight of sugar beet<br>Root rot of bean and soybean<br>Seedling blight of soybean |
| AG 2-1 | Damping-off of crucifers<br>Bud rot of strawberry<br>Leaf blight of tulip<br>Foot rot of Japanese radish and subterranean clover |
| AG 2-2 IIIB | False sheath blight of rice<br>Sheath blight of mat rush, ginger, and gladiolas<br>Black scurf of edible burdock<br>Brown patch of turfgrass<br>Crown and brace rot of corn<br>Damping-off of sugarbeet, tree seedlings, and chrysanthemum<br>Root rot of conjak and chinese yam |
| AG 2-2 IV | Root rot and leaf blight of sugarbeet<br>Large patch of turfgrass |
| AG 2-2 Unspecified | Crater rot of carrot<br>Brown patch of St. Agustinegrass<br>Root rot of subterranean clover, Chinese radish, and snapbean |

Table 2. Continued.

| Anastomosis Group | Related Host Diseases |
|---|---|
| AG 3 | Black scurf of potatoes<br>Leaf blight of tomato<br>Leaf spot of tobacco<br>Brown spot of egg plant |
| AG 4 | Tomato fruit rot<br>Stem rot of pea<br>Foot rot of spinach<br>Stem canker of potato<br>Damping-off and root rot of onion, pea, potato, and snap bean |
| AG 5 | Black scurf of potato<br>Brown patch of turfgrass<br>Root rot of bean and soybean |
| AG 6 | Nonpathogenic<br>Form mycorrhizal associations with orchids |
| AG 7 | Nonpathogenic |
| AG 8 | Bare patch or root rot of cereals |
| AG 9 | Infect crucifers and potatoes |
| AG 10 | Nonpathogenic |
| AG BI | Nonpathogenic |

Symptoms of the disease include stunted plants with yellowing of the lower leaves, which result in bare spots within the field. In some instances, the crop achieves normal height, but stunted plants may be scattered throughout the field only to be hidden by healthy plants (23). Infected plants display a characteristic "spear tip" root due to the cortical rotting effects of the pathogen (53,59,60). Plants may recover

from this infection, but early tillers abort and yield can be significantly reduced due to the reduction in tiller numbers (27,43,53).

Research on Rhizoctonia root rot has been conducted in the Pacific Northwest and further investigation of the disease is still in progress. Researchers have looked at the interaction of non-selective herbicides, tillage methods, timing of planting, and fungicides for controlling the incidence of Rhizoctonia root rot.

Earlier findings in Australia suggest that tillage is a factor in severity and incidence of the disease within a field that is infested with the pathogen (13,23,24,25). This has been reconfirmed in the Pacific Northwest (51,53). Fields managed under a reduced tillage system are almost always highest in incidence and severity of the disease due to little disruption of the mycelial network in the soil and the possible interaction of herbicides (43). However, in some instances, yield is higher under reduced tillage than conventional tillage because of the overriding effects of the reduced tillage systems on water use efficiency (53).

## Herbicide Interactions

Herbicides have been shown to increase the severity and incidence of Rhizoctonia root rot (2,18,19,22,26,43,45,51,53). In Australia, the use of chlorsulfuron (Glean) on high pH soils resulted in increased disease in a field situation. This occurred in areas of high soil pH (> 8.0) where the chemical did not degrade rapidly. In the United States, application of chlorsulfuron was not recommended for use on soils with a

pH of > 7.5. At this time, chlorsulfuron is no longer available for use in the U.S.

Glyphosate is rapidly becoming the non-selective herbicide of choice by cereal growers for weed control before planting. When applied to fields known to harbor *Rhizoctonia solani* it is likely to reduce the crop yield if the spraying and seeding practices are made within two weeks of one another. If the pathogen is not present, there should be no adverse effect from the herbicide on yield or disease incidence (13,25,26,41,43,44,45,47,51,53,59).

The effect of the glyphosate interaction is not completely understood. Its mode of action involves the shikimic pathway, blocking the production of phenolic compounds which help plants deter attack by pathogens. Glyphosate applied at recommended rates is slow acting and rarely kills plants within one week following treatment. Fungal colonization of roots rapidly follows glyphosate application (22). Research suggests that pathogens can increase their inoculum potential on weeds or volunteer treated with herbicides and subsequently cause more disease on crops grown on that site (5,15,17,22,40,42,43,48,49,51,53).

Soilborne fungi can also act as synergists in the herbicidal action of glyphosate, possibly because it blocks the production of phenolics involved in plant disease resistance of plants to these pathogens (22).

Some hypothesize that there is an initiated response by the pathogen to seek a new host plant, the newly emerging seedling, after its alternate weed host has been sprayed with the herbicide. Allowing the plant to senesce for a two week period depletes the nutrient reserve of the Rhizoctonia mycelium, resulting in death of the rapidly growing

pathogen. This may then reduce the possibility of infection of the newly emerging cereal crop (51,53).

## Rhizoctonia Root Rot Control

### Fungicides

Fungicide seed treatments have been proven ineffective in controlling Rhizoctonia root rot because they act as a local protectant around the seed and only translocate systemically up the plant and not down into the root system where the infection occurs (31). Foliar applications and fungicidal soil treatment for control of the pathogen are expected to be highly ineffective and expensive, utilizing currently available products (7,28,50,52,53).

### Biological Control

The use of antagonistic bacteria and fungi has been researched as potential control for pathogenic *Rhizoctonia spp.* in the soil. Binucleate *Rhizoctonia spp.*, *Trichoderma spp.*, and *Aphanomyces spp.* have been shown to reduce infection in controlled environment experiments. There has been limited success with these organisms in a field situation. This is due to the inability to alter the natural environment to maintain conditions optimal for the antagonist to increase and be effective (16,20,30,36,51).

The antagonists need to be in high enough numbers in order to suppress pathogenic strains of *Rhizoctonia spp.*

### Resistance

There are no known resistant varieties of wheat and barley currently available to the grower, although some varieties are known to vary in tolerance to the pathogen (29,33).

## Alternate Weed Hosts

Weed control is essential in cereal crop production. Competition by weeds reduces the amount of available moisture and nutrients to the crop. Weeds have been shown to harbor *Rhizoctonia spp.* as saprophytes or parasites (5,15,36,38,39,44,45,47,49). Over 250 weed species have been reported as hosts of *Rhizoctonia spp.* (17).

Spraying weeds and planting within one week of each other increases the incidence of Rhizoctonia root rot (13,22,25,26,41,43,44,45,47,51, 53,59). Determining which common Montana weeds are secondary hosts for *R. solani* is essential in determining the pathogen's potential in Montana.

## Diagnostic Techniques

### Anastomosis Testing

Determining the anastomosis group of *R. solani* can prove to be very difficult. Environmental conditions need to be perfect for hyphal fusion which is necessary for isolate identification. High humidity, optimum temperature, sufficient light, and an ample nutrient source for hyphal growth are essential.

Various methods have been used for anastomosis testing. One currently acceptable method utilizes potato dextrose agar (PDA) coated

cellophane rectangles which are placed on water agar. Then, a mycelial disk from a tester isolate is placed on one end of the cellophane rectangle and a disk from a unknown isolate is placed on the other end. The mycelial disks are produced on PDA. Plates are covered and incubated at room temperature until hyphae overlap. The area of cellophane upon which hyphae overlap is removed from the agar, placed on a slide, stained, then examined microscopically for hyphal fusion. Identification of at least five fusion sites is necessary for a positive anastomosis reading (9).

The use of PDA mycelial plugs placed directly on water agar or PDA can also be used (16,36,38). They are incubated, stained and examined as in the previous method, but overlapping hyphae are more difficult to remove and transfer to a slide. Disruption of the hyphae can occur during the transfer.

Another method is the clean slide technique. It is rapid and no disruption of hyphae occurs in this process. PDA mycelial plugs are placed on a sterile glass microscope slide and incubated in a moisture chamber. After hyphal overlapping occurs, the plugs are severed from the hyphae and removed. Hyphae are then stained, covered with a glass cover slip, and microscopically examined for anastomosis as previously described (20).

Enzyme Linked Immunosorbent Assay

Enzyme Linked Immunosorbent Assays (ELISA) have been used for several years for the detection and identification of plant viruses and bacteria. Only recently have these rapid, sensitive assays been applied

successfully to the detection of fungi. An ELISA test kit for *R. solani* has recently been developed by Agri-Diagnostics Associates, Cinnaminson, New Jersey.

The double antibody sandwich form of ELISA has been adequate for the detection of *Rhizoctonia solani*. The fungus is selectively trapped and immobilized by a specific antibody adsorbed on a solid surface. The test kit is manufactured as microwell polystyrene plates pre-coated with the antibody. The trapped fungus then reacts with specific antibody to which an enzyme has been linked. After washing, enzyme-labelled antibody that has complexed with the trapped fungus is detected by adding an enzyme substrate that is detected by a shade of green. The color appears darker relative to the amount of the pathogen present in the material sampled.

# MATERIALS AND METHODS

## Inoculum Production

Inoculum was produced according to the method of Smiley and associates (53). The test culture of *R. solani* AG 8 was obtained from Smiley. The culture was grown on Difco Potato Dextrose Agar (PDA) for three to five days, cut into 2 cm cubes and mixed with sterile millet seed prior to inoculation. The millet seed was soaked for eight hours in water, drained overnight to remove excess water, then autoclaved at 121°C for one hour, then cooled. One half of a 100 x 15 mm petri dish culture of *R. solani* was added to 300 ml of sterile millet. This was incubated for three weeks in sterile 1000 ml glass jars with porous lids, to allow moisture to escape. Jars were gently agitated every week to enhance colonization of the millet seed.

After incubation, the millet was air dried at room temperature, then stored at 5°C in dry paper sacks until needed. Pathogenicity tests revealed that the inoculum remained viable for at least one year from the date of preparation.

## Anastomosis Testing

Anastomosis testing was conducted using the clean slide technique as described by Kronland and Stanghellini (20). The *R. solani* tester strains were obtained from Dr. Donald Carling, USDA, Palmer, Alaska. The tester strains and the unidentified Rhizoctonia-like organisms were

cultured on 1/2 strength Difco PDA for three days. Seven millimeter hyphal tip plugs were removed from the edge of each culture and placed approximately 3 cm apart on a glass microscope slide. The slides were sterilized in 95% ETOH for one minute, then allowed to dry on sterile paper towels. The slides with the agar plugs were then placed in a moisture chamber for 12 to 36 hours to allow the mycelium to grow. When intersection of the hyphae growing from each plug was observed, slides were removed from the moisture chamber, the agar plug severed from the mycelium and removed from the slide. The mycelium was then stained with Safranin O (3), covered with a 22 x 22 mm glass cover slip and observed for anastomosis for positive identification of the culture within that anastomosis group.

Further confirmation that an organism was within the *R. solani* group was performed using the Enzyme Linked Immunosorbent Assay (ELISA) technique. The ELISA kit, manufactured by Agri-Diagnostics Associates, Cinnaminson, New Jersey is specific for *R. solani* and *R. cerealis*. *R. cerealis* is binucleate and *R. solani* is multinucleate. Only multinucleate isolates were tested to avoid confusion. *R. oryzae*, the other multinucleate organism responsible for Rhizoctonia root rot is not sensitive to this *R. solani* specific ELISA test kit.

## Laboratory Analysis - 1991

### Root Evaluation

All plant samples collected from all four experimental plot locations were evaluated for symptoms of root rot. Roots were washed free of all soil then visually analyzed and given a root disease rating

ranging from zero to four based on the percentage of seminal roots pinched off: 0 = no pinched off roots, 1 = 1 to 25% of the seminal roots pinched off, 2 = 26 to 50% of the seminal roots pinched off, 3 = 51 to 75% of the seminal roots pinched off, and 4 = 76 to 100% of the seminal roots pinched off. The disease rating values were averaged and statistically analyzed for significance using analysis of variance.

Isolation Procedures

Samples were selected from each lot of roots from each plot, then cultured for identification of the pathogen involved with root deterioration. Roots were selected from plants with the highest disease rating from each plot.

Shoots were severed from the root system just above the sub-crown internode and roots were washed for 12 hours in running tap water to remove surface soil and contaminants. Samples were then blotted dry on paper towels, placed on a medium of 2% water agar plus 50 milligrams per liter (mg/l) rifampicin. Cultures were visually analyzed 24 to 48 hours after transferring to the agar for mycelial growth of Rhizoctonia-like organisms. Hyphal tips were taken from these and transferred to 1/2 strength potato dextrose agar media (PDA) amended with 50 mg/l rifampicin. Identification by microscopic examination was based on hyphal morphology (30,53,59).

The original cultures on 2% water agar plus rifampicin were allowed to continue growth for identification of other soilborne organisms that might be growing on the root system.

Those identified as Rhizoctonia-like cultures were then stained with Safranin O (3) to determine the number of nuclei per cell. This confirmed the organism as *Rhizoctonia spp.* The multinucleate organisms were then tested for anastomosis with known tester strains of *R. solani.*

A straw baiting technique was also used to isolate *Rhizoctonia spp.* (36). Four centimeter (cm) pieces of sterile wheat straw were place vertically into field soil, removed three days later and washed free of soil. These pieces were then placed on 2% water agar plus 50 mg/l rifampicin and examined for hyphal growth after 24 to 48 hours. Hyphal tips were removed and placed on 1/2 strength PDA plus 50 mg/l rifampicin and allowed to grow for three days prior to identification. All Rhizoctonia-like isolates were then used in pathogenicity tests in the greenhouse.

## Laboratory Procedures - 1992

Isolations from field and greenhouse experiments were conducted using the same procedures as in 1991. Anastomosis testing continued, to try to categorize the isolates obtained from the previous years field experiments. Five of the isolates were sent to Dr. Donald Carling, USDA, Palmer, Alaska, for confirmation of those that proved to be difficult to assign to known anastomosis groups.

## Field Observations - 1990

A statewide survey was initiated in Montana during the spring and summer by alerting agronomists, county extension agents, and growers to look for the characteristic symptoms of Rhizoctonia root rot in field

situations. They were encouraged to submit samples for further evaluation in the laboratory.

Field visitation to areas in which symptoms occurred was done to evaluate the situation and collect plant and soil samples. These were evaluated in the laboratory and in the greenhouse for the presence of *Rhizoctonia spp.* and other soilborne organisms which may be contributing to the situation. Sites were also evaluated for use in experimental field research trials.

## Field Study - 1990-1991

Field plots were established in four Montana locations which had high potential for the occurrence of Rhizoctonia root rot. They were established in September of 1990 near Great Falls, Willow Creek, and two Bozeman locations.

### Great Falls Field Plot

The Great Falls site was chosen because of previous crop loss due to bare patches occurring in the field. The cropping history of this location favored bare patch because the site involved continuous cropping with winter wheat followed by recropped spring barley. Minimum tillage was used with a single application of glyphosate sprayed for weed and volunteer control within one week of planting.

Eight 3 meter x 3 meter plots were placed randomly into a recently seeded field of Harrington barley. To control weeds and volunteer winter wheat, the field was sprayed with glyphosate at a rate of 399 grams of active ingredient per hectare (g.a.i./ha) one day before planting.

Ten random plant samples were obtained from each of the plots at three weeks and five and one-half weeks after planting. The plants were dug with a hand spade, keeping as much soil as possible on the root system, placed in plastic sacks and stored in a cooler at 5°C. Samples were later analyzed and evaluated in the lab for root rot and the presence of soilborne organisms that may be associated with the symptoms.

Willow Creek Field Plot

The Willow Creek location was chosen due to the difficulty in establishing CRP grasses near land which was previously in cereal crop production. The hypothesis was that some type of soilborne pathogen, possibly *Rhizoctonia*, was playing a role in this situation.

The plots were established on land being farmed under crop/fallow management. The crop planted on this land was either winter wheat or spring barley. This area was selected due to the high density of downy brome (*Bromus tectorum*) that was growing in this section. Previous studies have shown that some weeds are an alternate host for many *Rhizoctonia spp.* (5,17). Estimates of the downy brome density was calculated to be forty-five plants per square meter.

A complete randomized block design was chosen and thirty-two, 3 meter x 7.5 meter plots were established using three rates of glyphosate plus a control, applied at ten days and one day before planting. Rates used were 0, 199, 299, and 399 g.a.i./ha. The recommended label rate for control of weeds with glyphosate is 399 g.a.i./ha. All plots were then

seeded with Newana spring wheat at a rate of 67 kg/ha using 0.3 meter row spacing.

Each plot was sampled at 3, 4, and 5 weeks after planting. A 0.5 square meter area of plants was removed using a spade, placed in a plastic garbage sack, then taken to the lab for analysis of the root system.

Post Farm Inoculum Plot

The third location for experimental plots was established at Montana State University's Arthur H. Post Experimental Research Farm near Bozeman. These plots were designed to investigate the interaction of various glyphosate rates in a natural versus fumigated soil relative to the incidence of disease in barley.

Plots at this location were established into an area that had been treated with Basamid (tetrahydro-3,5-dimethyl-2H- 1,3,5-thiaciazine-2-thione), a granular soil fumigant activated by water application after incorporation into the soil at a 5 cm depth. It was applied at the rate of 417 kilograms per hectare (kg/ha) to control residual soilborne pathogens from previous experimental research in the area. Control and inoculated plots were established adjacently in a natural and fumigated soil. Within each treatment, two replications of four glyphosate rates were applied for weed control. Rates used were 0, 199, 299, and 399 g.a.i./ha. Glyphosate was applied one day before planting Clark barley in the spring of 1991. All inoculated plots were inoculated with *R. solani* AG-8, banded with the barley at seeding. Four rows of barley, spaced 0.3 meters apart, were sown at 150 seeds per 3 meter row.

Inoculum, grown on millet seed, was simultaneously banded at a rate of twenty-eight kg/ha using a four row cone seeder.

Each plot was then sampled 3, 4, and 5 weeks after planting. A hand spade was used to remove seedlings at 0.3 meter intervals from the two outside rows of each plot, placed in a plastic sack, then rated for root rot in the lab.

Two 2.44 meter center plot rows were harvested in August 1991 for yield analysis. The barley plants from each plot were cut, then thrashed using a Vogel threshing machine. The grain was then weighed in the laboratory and evaluated by statistical analysis using analysis of variance.

Post Farm Downy Brome Plot

Another experimental plot was established on the Post Experimental Research Farm. The site chosen was an area that was heavily infested with downy brome. The purpose of this experiment was to determine if R. solani was naturally present in Montana soils and if downy brome can act as a secondary host for this organism.

Plots were established using a complete randomized block design. The downy brome was sprayed with glyphosate one day before direct drilling Clark barley at a rate of 54 kg/ha into the area. Four different rates of herbicide were applied to 6 meter x 6 meter plots using 0, 199, 299, and 399 g.a.i./ha. This procedure was replicated four times.

Sampling of the barley was similar to the procedure used at the Willow Creek location. A 0.5 square meter area of the emerging barley

was collected for analysis in the laboratory 3, 4, and 5 weeks after seeding.

## Field Plot - 1992

To investigate the interaction of winter wheat volunteer density, glyphosate, and the incidence of Rhizoctonia root rot, a field test was established at the Post Experimental Research Farm during 1992. Also, identifying the best time to sample for visual symptoms of Rhizoctonia root rot was evaluated.

The plots were organized in a randomized block, split-plot design with four replications. Main plots were *R. solani* inoculum versus no inoculum. An artificial volunteer situation was created by planting Winridge winter wheat in the fall of 1991 at rates of 0, 21.5, 75, and 161 kg of seed per six meter row, which were the sub-plots. Plot size was six meters by three meters for each individual treatment. *R. solani* AG-8 inoculum, grown on millet seed, was banded with the winter wheat at a rate of twenty-eight kg/ha. Control plots were treated with heat killed *Rhizoctonia* inoculum which was also banded with the seed. This was to overcome any additional nutrient source in the soil which may be attributed to the millet seed.

In the spring of 1992, winter wheat plants were sampled from the inoculated field plots. They were tested for the presence of *R. solani* using the ELISA test kit. This was performed to confirm the presence of the pathogen before seeding the plots to barley.

The volunteer winter wheat was then sprayed out with glyphosate at a rate of 399 g.a.i./ha one day before seeding Clark barley. The barley

was seeded perpendicular to the previously seeded winter wheat. The seeding rate was 150 seeds per 3 meter row using a four row cone seeder with 0.3 meter spacing. Eight 3 meter rows were planted per plot. Stand emergence was determined three weeks after planting by counting the center four rows in each plot.

Plant samples were removed from four random 0.3 meter lengths of two of the outside rows. These were dug with a spade, placed in a plastic garbage sack, then taken to the lab for analysis of the root system. Samples were taken at 3, 4, 5, 6, and 7 weeks after planting. Roots were scored using the method previously described.

Four center rows, 1.2 meters long were harvested by hand for yield comparisons. The barley was thrashed, weighed, and statistically analyzed using the method described previously. A count of harvestable heads was taken on one 3 meter row of each plot before harvesting.

## Field Investigation - 1992

Downy brome seedlings were removed from the downy brome experimental plot of 1991 during October of 1992. Visual analysis of the plant root system and a *R. solani* specific ELISA test was conducted to determine if *R. solani* was present in this site after one year of fallow.

## Greenhouse Study - 1991

Rhizoctonia-like organisms isolated from field plots were tested for pathogenicity in the greenhouse using Winridge winter wheat and Clark spring barley. Tapered plastic tubes measuring 4 cm diameter x 20.5 cm long were plugged with cotton, then filled to within 10 cm of the top

with sterile vermiculite. Two cm of pasteurized potting soil mixed in equal parts with pasteurized sand was added to each container. Millet seed inoculum of the cultures obtained was added to each tube and covered with two cm more of the soil mixture. The containers were watered, then covered with plastic film and incubated for three days. Then, three seeds of either wheat or barley were added to each container and grown in the greenhouse for three weeks at 22°C (36). Water was added as needed to keep the soil moist throughout germination and growth. Plants were collected and the root system washed free of soil. Visual and microscopic examination for the incidence of root rot and virulence of the pathogen was performed.

Greenhouse Study - 1992

To determine the best time to sample plants for symptoms of Rhizoctonia root rot and if soil microbes have a role in development of this disease, a greenhouse test was established. Also the incidence of disease relative to seeding date after glyphosate application was investigated.

Plots were established using a randomized block, split plot design. Winridge winter wheat was planted in a natural field soil obtained from the Post Experimental Research Farm. The soil was mixed in equal quantities with sand and placed in 12.7 cm diameter plastic pots. Millet seed infested with *R. solani* AG-8 at a rate of twenty-eight kg/ha and 12 winter wheat seeds were added to each container. They were covered with 3 cm of soil, placed in the greenhouse and watered as needed to maintain adequate soil moisture during growth. A control was simultaneously

planted using inoculum that had been autoclaved at 121°C for one hour. The winter wheat seedlings were thinned to eight plants per pot after emergence, equivalent to a seeding rate of 134 kg/ha. The test thus simulated a natural, heavy volunteer situation.

The above procedure was identically replicated using the same soil mixture as above, but steamed at 77°C for one hour to reduce weeds and soil microbes. The experiment was replicated four times for each of the treatments to be described.

Three weeks after planting the wheat, it was sprayed with glyphosate at a rate of 399 g.a.i./ha. Clark spring barley was planted into the pots at a depth of 3 cm at intervals of one, seven, and fourteen days after the spray date. Plants were thinned to five plants per pot after emergence.

The barley plants were evaluated over six sampling dates at weekly intervals, beginning at three weeks after planting and ending at eight weeks. Visual symptoms for Rhizoctonia root rot were analyzed after washing the soil from the roots. The root system was given a disease score from 0 to 4 using the procedure previously described. Each plant shoot was weighed after drying.

Culturing of representative infected plant samples was conducted using the isolation method previously described. Determination of the presence of *R. solani* was also tested using the Agri-Diagnostics *R. solani* specific ELISA test kit.

## Alternate Weed Hosts

Another series of experiments was conducted to determine if grassy weeds play a role in transmission of Rhizoctonia root rot to barley plants as winter wheat volunteer plants do in a field situation (51,53). Four common weeds known to be a problem in Montana cereal crops were chosen. They were downy brome (*Bromus tectorum*), green foxtail (*Setaria viridis*), jointed goatgrass (*Aegilops cylindrica*), and wild oat (*Avena fatua*).

Seed of the above four grassy weeds was broadcast over a 0.3 meter x 0.6 meter flat of potting soil mix and covered with 2 cm of the same soil. *R. solani* AG 8 inoculum on millet seed was broadcast with the seed at a rate of twenty-eight kg/ha. The flats were placed on a bench in the greenhouse and watered as needed to maintain adequate soil moisture. Plants were grown for six weeks, then analyzed for visual root rot symptoms. The same samples were also used to isolate *Rhizoctonia* from the plants by culturing on 2% water agar plus 50 mg/l rifampicin. They were also tested using the Agri-Diagnostics *R. solani* specific ELISA test kit. A control flat for each weed was also planted using heat killed *R. solani* AG 8 inoculum.

After confirmation of the presence of *R. solani* AG 8, each flat was sprayed with glyphosate at a rate of 399 g.a.i./ha. One day after spraying, three rows of Clark spring barley were planted at a rate of 15 seeds per 0.3 meters. This was replicated two times. Four weeks after planting, barley plant samples were collected and visually analyzed for presence of Rhizoctonia root rot symptoms. These were then cultured

on 2% water agar plus 50 mg/l rifampicin and also tested using the ELISA test kit mentioned above.

# RESULTS

## Field Analysis - 1991

### Great Falls Field Plot

Laboratory analysis of the Great Falls experimental field plot samples revealed the presence of a few soilborne organisms. Three multinucleate *R. solani* species were isolated and one of the isolates was confirmed to be AG 8, the causal organism of Rhizoctonia root rot. Confirmation was provided by Dr. Donald Carling, Palmer, Alaska. Other fungi isolated were *Pythium spp.*, *Cochliobolus sativus*, and pigmented mycelium which resembled *Fusarium spp.* All were isolated from plant roots exhibiting some discoloration.

Yield was not taken at this location due to a Russian wheat aphid infestation which completely devastated the barley field.

### Willow Creek Field Plot

Root samples analyzed in the lab showed no evidence of the characteristic spear tip symptom associated with Rhizoctonia root rot. The root disease rating was zero. There were few samples exhibiting root discoloration. Isolations from the few discolored root samples were made on 2% WA plus Rifampicin.

Identification of the isolates revealed the presence of *Pythium spp.*, *Cochliobolus sativus*, *Fusarium acuminatum*, *F. subglutinans*, and pigmented mycelium which resembled *Fusarium spp.* No further testing was

conducted beyond five weeks of plant growth due to the low root disease rating.

Post Farm Inoculum Experiment

Laboratory analysis of barley root samples was positive for the visual symptoms of Rhizoctonia root rot. Plants were stunted and had spear tipped roots. Isolation tests confirmed the presence of *R. solani* which had been banded with the barley at planting. Isolation of other soilborne organisms from the root samples included *Pythium spp.*, *Cochliobolus sativus*, *Fusarium acuminatum*, and pigmented mycelium which resembled *Fusarium spp.*

The root disease score was significant in all inoculated plots versus control plots ($P < 0.05$). There were no significant differences in the overall root disease ratings of the Basamid fumigated plots versus the non-fumigated plots or in the four glyphosate rates applied. Sampling date was significant for the root disease rating ($P < 0.05$) only in the Basamid fumigated by inoculum interaction (Table 3).

Root disease rating of the Basamid plus inoculum treatment progressively decreased over the three week sampling period. It averaged 3.1 over the four glyphosate rates at three weeks down to an average of 1.4 at five weeks after planting. Root disease rating of the non-fumigated plus inoculum treatment had its greatest disease score at three weeks. But overall, the amount of spear tipped roots remained relatively constant, with an average root disease score of 2.42 (Table 3).

Table 3. Influence of soil fumigation and various glyphosate rates, applied one day before seeding barley, on the incidence of Rhizoctonia root rot over 3 sampling dates at Bozeman, during 1991.

| | Root Disease Rating* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Basamid Fumigated Plots | | | | | | | |
| | Glyphosate Rate (g.a.i./ha) | | | | | | | |
| | 0 | | 199 | | 299 | | 399 | |
| Sample date | INOC | CK | INOC | CK | INOC | CK | INOC | CK |
| 3 Weeks | 3.20 | 0.02 | 3.00 | 0.08 | 2.80 | 0.12 | 3.20 | 0.05 |
| 4 Weeks | 1.80 | 0.00 | 2.00 | 0.06 | 1.90 | 0.02 | 1.80 | 0.00 |
| 5 Weeks | 1.10 | 0.02 | 1.40 | 0.04 | 1.50 | 0.02 | 1.50 | 0.02 |
| LSD= | 0.91 | 0.07 | 0.91 | 0.07 | 0.91 | 0.07 | 0.91 | 0.07 |
| | Non-Fumigated Plots | | | | | | | |
| 3 Weeks | 2.90 | 0.07 | 2.90 | 0.04 | 2.20 | 0.04 | 2.40 | 0.08 |
| 4 Weeks | 2.10 | 0.05 | 2.20 | 0.05 | 2.10 | 0.05 | 1.90 | 0.09 |
| 5 Weeks | 2.30 | 0.06 | 2.00 | 0.10 | 2.20 | 0.10 | 1.70 | 0.06 |
| LSD= | 1.18 | 0.07 | 1.18 | 0.07 | 1.18 | 0.07 | 1.18 | 0.07 |

* % pinched off roots. 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

INOC = Inoculated with *R. solani* AG 8. CK = control. Sample Date = weeks after spraying volunteer with glyphosate and planting Clark barley.

Plant emergence was not significant between the four glyphosate treatments ($P = 0.78$). Significance was observed in the control plots versus inoculated plots ($P < 0.05$). Inoculated plots had 22% less plant emergence than the control plots (Table 4). Germination tests of the barley seed before planting showed a >90% germination rate.

Table 4. Influence of Rhizoctonia root rot on barley seedling emergence at Bozeman, during 1991.

| | Plant Emergence* | | | |
|---|---|---|---|---|
| | Fumigated | | Non-Fumigated | |
| Glyphosate Rate g.a.i./ha | Inoculated | Control | Inoculated | Control |
| 0 | 72 | 110 | 74 | 110 |
| 199 | 88 | 103 | 86 | 100 |
| 299 | 87 | 100 | 88 | 98 |
| 399 | 82 | 111 | 82 | 103 |
| LSD = | 20 | 35 | 27 | 20 |

* Number of plants/square meter. 161 seeds of Clark barley planted/square meter.

Plants were harvested for yield only from the Basamid treated plots due to a reduced stand in the non-fumigated plots. The stand reduction was caused by weed competition where lower rates of glyphosate were applied. Few weeds were present in the fumigated plots as a result of the Basamid treatment.

There was no significant difference in yield between the four glyphosate treatments in the inoculated plots ($P = 0.09$) or in the control plots ($P = 0.57$). Significance was determined for the inoculated versus control plots ($P < 0.05$). Eight plots of each inoculated and control were harvested. The average yield of inoculated plots was 23% less than control plots, yielding 4788 kg/ha and 6209 kg/ha, respectively (Table 5).

Table 5. Influence of Rhizoctonia root rot on barley yield in Basamid fumigated plots at Bozeman, during 1991.

| | Yield (kg/ha)** | |
|---|---|---|
| Glyphosate Rate* | Inoculated Plots | Control Plots |
| 0 | 4129 | 6253 |
| 199 | 4959 | 5936 |
| 299 | 5059 | 6739 |
| 399 | 4975 | 5907 |
| LSD = | 823 | 1960 |

* Grams of active ingredient per hectare.
** Values are means over two replications.

Post Farm Downy Brome Experiment

Plant growth was very slow in this experimental area due to the heavy mat of decaying organic matter after spraying the downy brome with glyphosate. There was no significant difference in root disease score between the various rates of glyphosate applied ($P = 0.55$) (Table 6). There was a significant difference in root disease rating over the three sampling dates ($P < 0.05$).

Plant samples collected during the three week period had a very low root disease rating (<0.25). The root disease score was reduced by 59% and 55% during week 5 in comparison to weeks 3 and 4, respectively (Table 6).

Isolation of *Rhizoctonia spp.* from representative barley root samples was successful in this natural field situation. Twenty-eight *Rhizoctonia spp.* isolates were recovered. Twenty-four of the isolates were multinucleate and four were binucleate. The multinucleate isolates

Table 6. Influence of plant age on the decline of Rhizoctonia root rot on barley planted into downy brome sprayed with various glyphosate rates one day before seeding at Bozeman, during 1991.

| | Root Disease Rating* | | | |
|---|---|---|---|---|
| | Glyphosate Rate (g.a.i./ha) | | | |
| Sample Date** | 0 | 199 | 299 | 399 |
| 3 | 0.22 | 0.19 | 0.21 | 0.21 |
| 4 | 0.15 | 0.20 | 0.25 | 0.20 |
| 5 | 0.10 | 0.10 | 0.15 | 0.10 |
| LSD = | 0.13 | 0.13 | 0.13 | 0.13 |

* = % pinched off roots. 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.
** Weeks after planting Clark barley.

were identified as *R. solani* by microscopic examination and a *R. solani* specific ELISA test (Table 7). Isolates were obtained from all four glyphosate plot treatments. Five were from the zero g.a.i./ha, ten from the 199 g.a.i./ha, six from the 299 g.a.i./ha, and five from the 399 g.a.i/ha treatment plots. These isolates were cultured from approximately 9% of the barley roots which had a root score of greater than zero. Isolation of *Rhizoctonia spp.* was successful only in weeks 3 and 4.

Numerous attempts to place these isolates into specific anastomosis groups proved negative in laboratory testing. Four representative isolates sent to Dr. Donald Carling yielded positive results. Three of the isolates were identified as AG 4 and one was identified as AG 8, the causal organism of Rhizoctonia root rot.

Isolation of other soilborne fungi from root samples included *Pythium spp.*, *Cochliobolus sativus*, and pigmented mycelium which resembled *Fusarium spp.*

Table 7. ELISA assays of *Rhizoctonia solani* using mycelium or infected winter wheat.

| Mycelial Assays* | | Winter Wheat Assays** | |
|---|---|---|---|
| Isolate | Absorbance | Plot Number | Absorbance |
| 91-9 | 1.82 | 2 | 1.19 |
| 91-11 | 0.87 | 3 | 1.35 |
| 91-12 | 1.76 | 4 | 1.00 |
| 91-14 | 1.76 | 14 | 1.11 |
| 91-18 | 1.82 | 15 | 1.05 |
| 91-19 | 1.60 | 16 | 0.91 |
| 91-20 | 1.80 | 21 | 0.57 |
| 91-21 | 1.83 | 22 | 1.20 |
| 91-22 | 1.82 | 23 | 0.73 |
| 91-24 | 1.76 | 25 | 0.92 |
| 91-25 | 1.82 | 26 | 0.62 |
| | | 27 | 1.25 |

Absorbance reading at 410 nm. Value > 0.51 = positive.
Positive control = 0.92. Negative control= 0.17.
* Representative isolates tested based on morphological characteristics. Absorbance is an average of two samples x two replications.
** Volunteer winter wheat planted in the fall of 1991 and inoculated with *R. solani* AG 8. Absorbance is an average of two root samples x two replications.

## Field Analysis -1992

The volunteer winter wheat plants sampled from inoculated field plots in the spring of 1992 tested positive for the presence of *R. solani* using the ELISA test (Table 7). They also exhibited symptoms of root rot, but isolation of *Rhizoctonia* was not achieved from these samples. Fungi isolated included *Pythium spp.*, *Fusarium spp.*, and *Cochliobolus sativus*.

Plant emergence was taken three weeks after planting and there was no significant difference between the inoculated and control plots overall ($P = 0.26$). This was also true for the volunteer rate by inoculum interaction ($P = 0.98$). A significant difference was determined for the various volunteer rates ($P < 0.05$). There was less plant emergence in the two highest rates versus the two lower rates in both the inoculated and control plots.

Table 8. Influence of volunteer density and *Rhizoctonia solani* AG 8 on barley seedling emergence, number of harvestable heads, and yield at Bozeman, during 1992.

| | Plant Emergence* | | Harvestable Heads** | | Yield*** (kg/ha) | |
|---|---|---|---|---|---|---|
| Vol.# Rate | INOC | CK | INOC | CK | INOC | CK |
| 0 | 120 | 124 | 188 | 192 | 5924 | 6327 |
| 21.5 | 120 | 123 | 150 | 146 | 5459 | 5065 |
| 75 | 114 | 115 | 125 | 147 | 5180 | 4988 |
| 161 | 111 | 116 | 149 | 151 | 4976 | 5188 |
| LSD = | 5 | 5 | 18 | 18 | 489 | 489 |

* Number of plants/square meter. Mean of four replications. 161 seeds of Clark barley planted/square meter.
** Number of heads/square meter. Mean of one three meter row by four replications.
*** Mean of four replications.
# Kilograms of winter wheat seeded per hectare.

Barley from all plots was evaluated for visual symptoms of Rhizoctonia root rot. The highest root disease score and best visual symptoms of Rhizoctonia root rot were seen during weeks 3, 4, and 5 after planting. During weeks 6 and 7, root disease scores and visual symptoms were very

low (Table 9). A significant difference in root disease score was determined over the sampling dates ($P < 0.05$) (Table 10).

Table 9. Influence of volunteer density and barley plant age on the incidence of Rhizoctonia root rot in inoculated field plots at Bozeman, during 1992.

| | Root Disease Rating* | | | |
|---|---|---|---|---|
| | Volunteer Rate (kg seeded/ha) | | | |
| Sample Date | 0 | 21.5 | 75 | 161 |
| 3 | 0.44 | 0.98 | 0.82 | 1.04 |
| 4 | 0.83 | 1.04 | 1.47 | 1.54 |
| 5 | 0.32 | 0.57 | 0.71 | 0.83 |
| 6 | 0.37 | 0.30 | 0.33 | 0.53 |
| 7 | 0.01 | 0.19 | 0.14 | 0.27 |
| | LSD = 0.46 | | | |

Sample Date = weeks after planting.
* = % pinched off roots. 0 = 0%, 1 = 1-24%, 2 = 25-49%, 3 = 50-74%, 4 = 75-100%.

Table 10. The influence of barley plant age and volunteer density on the incidence of Rhizoctonia root rot in inoculated field plots at Bozeman, during 1992.

| Sample Date | Root Score | Volunteer Rate | Root Score |
|---|---|---|---|
| 3 | 0.82 | 0 | 0.39 |
| 4 | 1.22 | 21.5 | 0.61 |
| 5 | 0.61 | 75 | 0.70 |
| 6 | 0.38 | 161 | 0.84 |
| 7 | 0.15 | | |
| LSD = | 0.23 | LSD = | 0.27 |

Sample Date = weeks after planting Clark barley.
Volunteer Rate = kilograms of Winridge winter wheat seeded per hectare.
Root Score = % pinched off roots. 0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%.

The root disease score varied significantly ($P < 0.05$) in relation to the volunteer rate in plots containing volunteers before seeding in contrast to those with no volunteers. The zero volunteer plots averaged a 50% less root disease rating over weeks 3, 4, and 5 versus plots containing volunteer averaged over the same weeks. In all cases the disease score was very low seven weeks after planting (Table 10).

Isolation of *Rhizoctonia* on 2% WA plus Rifampicin was achieved only during weeks 3, 4, and 5. Isolation of *Rhizoctonia* was not achieved at weeks 6 and 7 when the root disease rating was low.

A significant difference was determined for the number of harvestable heads ($P < 0.05$) in relation to volunteer density. Harvestable head counts were greater in zero volunteer plots versus plots containing volunteer. This was true for both inoculated and control plots (Table 8).

There was a significant difference in yield ($P < 0.05$) between the zero volunteer inoculated plots versus all other treatments. No significance was observed in inoculated versus control plots overall ($P = 0.45$) (Table 8).

## Field Investigation - 1992

Downy brome seedlings collected from the downy brome experimental site of 1991 exhibited no visual symptoms of root rot. ELISA tests revealed the presence of *R. solani* on the root samples, but it was detected at a very low incidence. The absorbance reading was 0.54 at 410 nm and the threshold for a positive reading was equal to 0.30.

Greenhouse Analysis - 1991

Thirty-one isolates of *R. solani* were recovered from barley root sampled from field plots. Twenty-seven of these were multinucleate and were tested for pathogenicity on wheat and barley seedlings. Sixteen of them produced some symptom combinations of either root rot, sheath rot, or plant stunting on both wheat and barley (Table 11). Three of these were confirmed to be AG 4 and two were AG 8.

Table 11. Pathogenicity, number of nuclei, and anastomosis groups of *Rhizoctonia solani* field isolates obtained from Montana soils.

| Isolate | # of Nuclei | Path Test | AG | Isolate | # of Nuclei | Path Test | AG |
|---|---|---|---|---|---|---|---|
| 91-1 | MULTI | H | - | 91-16 | MULTI | SR | - |
| 91-2 | MULTI | H | - | 91-17 | MULTI | SR | - |
| 91-3 | MULTI | H | - | 91-18 | MULTI | SR | 4 |
| 91-4 | BI | H | - | 91-19 | MULTI | SD | 4 |
| 91-5 | MULTI | H | - | 91-20 | BI | H | - |
| 91-6 | MULTI | H | - | 91-21 | BI | H | - |
| 91-7 | MULTI | H | - | 91-22 | MULTI | SD | - |
| 91-8 | MULTI | H | - | 91-23 | MULTI | SD | - |
| 91-9 | MULTI | SD | 4 | 91-24 | MULTI | SD,SR | 8 |
| 91-10 | MULTI | SR | - | 91-25 | MULTI | H | - |
| 91-11 | BI | H | - | 91-26 | MULTI | SR | - |
| 91-12 | MULTI | H | - | 91-27 | MULTI | SR | - |
| 91-13 | MULTI | H | - | 91-28 | MULTI | SD | - |
| 91-14 | MULTI | SD,RR | 8 | 91-29 | MULTI | SR | - |
| 91-15 | MULTI | RR,SR | - | 91-30 | MULTI | SR | - |
| | | | | 91-31 | MULTI | H | - |

Path Test = pathogenicity tests on wheat and barley at 3 weeks after planting.
H = healthy plant. SR = sheath rot. SD = stunted growth. RR = root rot. AG = anastomosis group.

Greenhouse Analysis - 1992

This experiment examined barley seeding date after spraying volunteer with glyphosate, and the relation of soil microbial activity toward the incidence of Rhizoctonia root rot. Comparisons were made using a natural field soil versus a pasteurized field soil.

Root disease score was determined to be significant for the inoculated versus control treatments in all three planting dates ($P < 0.05$). Root disease score was also significantly different in pasteurized inoculated versus natural inoculated soil ($P < 0.05$). Sampling date was significantly different only in the barley planted 14 days after glyphosate application ($P < 0.05$). The root disease score decreased significantly over these six sampling dates. The lowest score was in planting date 14 at sample date 8 (Table 12).

Visual symptoms of spear tipped roots and stunted plants was readily observed. Isolation of *R. solani* from representative diseased roots on 2% WA plus Rifampicin was only achieved at 3, 4, and 5 weeks after planting. The presence of *R. solani* was confirmed for sampling dates 6, 7 and 8 by use of the ELISA test (Table 13).

Shoot weight progressively increased over all sampling dates in all treatments. Significance was determined in inoculated versus control soil treatments ($P < 0.05$) (Table 14). No significant difference in shoot weight was determined in natural versus pasteurized soil treatments ($P = 0.83$).

Significant difference in sampling date ($P < 0.05$) occurred during weeks 7 and 8 in all three planting dates (Table 14). The amount of

Table 12. Influence of timing of seeding barley, after spraying volunteer winter wheat with glyphosate, planted into natural and pasteurized soils in relation to plant age, on the incidence of Rhizoctonia root rot in a controlled environment.

| Planting Date* | Sample Date** | Root Disease Rating*** | | | |
|---|---|---|---|---|---|
| | | Soil Treatment | | | |
| | | Natural | | Pasteurized | |
| | | Inoculated | Control | Inoculated | Control |
| 1 | 3 | 2.45 | 0.15 | 2.65 | 0.00 |
| | 4 | 2.05 | 0.00 | 2.75 | 0.00 |
| | 5 | 1.73 | 0.00 | 3.50 | 0.00 |
| | 6 | 1.65 | 0.10 | 2.75 | 0.00 |
| | 7 | 1.75 | 0.00 | 2.35 | 0.00 |
| | 8 | 2.20 | 0.00 | 2.39 | 0.00 |
| | LSD = 0.37 | | | | |
| 7 | 3 | 1.95 | 0.33 | 2.95 | 0.56 |
| | 4 | 1.50 | 0.15 | 2.70 | 0.00 |
| | 5 | 1.90 | 0.30 | 2.70 | 0.00 |
| | 6 | 1.70 | 0.10 | 2.35 | 0.00 |
| | 7 | 2.05 | 0.00 | 2.30 | 0.00 |
| | 8 | 2.00 | 0.15 | 2.15 | 0.00 |
| | LSD = 0.39 | | | | |
| 14 | 3 | 1.95 | 0.15 | 3.25 | 0.00 |
| | 4 | ND | ND | ND | ND |
| | 5 | 1.50 | 0.30 | 2.40 | 0.10 |
| | 6 | ND | ND | 2.40 | 0.00 |
| | 7 | ND | ND | 2.16 | 0.00 |
| | 8 | ND | 0.00 | 1.40 | 0.00 |
| | LSD = 0.36 | | | | |

* Planting Date = days after spraying volunteer with glyphosate and planting of Clark barley.

** Sample Date = weeks after planting barley.

*** % pinched off roots- 0 = 0%, 1 = 1-24%, 2 = 25-49%, 3 = 50-75%, 4 = 76-100%

ND = no data due to loss of plants by environmental stress.

Table 13. ELISA assays of *Rhizoctonia solani* on barley grown in a controlled environment.

| Weeks After Planting | Absorbance @ 410 nm* | |
|---|---|---|
| | Inoculated | Control |
| 6 | 1.49 | 0.37 |
| 7 | 1.07 | 0.38 |
| 8 | 1.52 | 0.35 |

Value > 0.60 = positive reading. Positive control = 0.82. Negative control = 0.29.
* Values are an average of five root samples x two replications.

shoot material harvested was greatest in the barley planted 14 days after glyphosate application at sample date 8. Shoot weight increased approximately 30% in the natural and pasteurized control plots and approximately 50% in the pasteurized inoculated treatment. This is in comparison to sample date 8 in planting dates 1 and 7 (Table 14). No comparisons could be made for the natural inoculated treatment due to a loss of plant material by environmental stress.

## Alternate Weed Host Analysis

Downy brome, jointed goatgrass, green foxtail, and wild oat sampled before glyphosate application, tested positive for the presence of *R. solani*. This was confirmed by use of the ELISA test (Table 15). Visual analysis of the roots revealed the spear tipping associated with Rhizoctonia root rot.

Table 14. Influence of timing of seeding barley, after spraying volunteer winter wheat with glyphosate, planted into natural and pasteurized soils inoculated with *Rhizoctonia solani* AG 8, on plant shoot weight in relation to plant age.

Dry Shoot Weight (grams)

| Planting Date* | Sample Date** | Soil Treatment | | | |
|---|---|---|---|---|---|
| | | Natural | | Pasteurized | |
| | | Inoculated | Control | Inoculated | Control |
| 1 | 3 | 0.0136 | 0.0195 | 0.0277 | 0.0278 |
| | 4 | 0.0403 | 0.0583 | 0.0394 | 0.0643 |
| | 5 | 0.0499 | 0.0863 | 0.0413 | 0.0760 |
| | 6 | 0.0714 | 0.1055 | 0.0526 | 0.1003 |
| | 7 | 0.0692 | 0.1212 | 0.0592 | 0.1337 |
| | 8 | 0.1083 | 0.1824 | 0.1217 | 0.1879 |
| | LSD = 0.017 | | | | |
| 7 | 3 | 0.0136 | 0.0236 | 0.0123 | 0.0297 |
| | 4 | 0.0468 | 0.0570 | 0.0329 | 0.0702 |
| | 5 | 0.0508 | 0.0824 | 0.0399 | 0.0839 |
| | 6 | 0.0795 | 0.0998 | 0.0641 | 0.1197 |
| | 7 | 0.0893 | 0.1426 | 0.0917 | 0.1406 |
| | 8 | 0.1169 | 0.1872 | 0.1196 | 0.2143 |
| | LSD = 0.018 | | | | |
| 14 | 3 | 0.0375 | 0.0485 | 0.0166 | 0.0321 |
| | 4 | ND | ND | ND | ND |
| | 5 | 0.0504 | 0.0524 | 0.0546 | 0.0997 |
| | 6 | ND | ND | 0.1032 | 0.1307 |
| | 7 | ND | ND | 0.1191 | 0.1922 |
| | 8 | ND | 0.2425 | 0.1811 | 0.2623 |
| | LSD = 0.028 | | | | |

* Planting Date = days after spraying volunteer with glyphosate and planting of Clark barley.
** Sample Date = weeks after planting barley.
ND = No data due to plant loss by environmental stress.

Table 15. ELISA assays of *Rhizoctonia solani* on grassy weeds or barley where barley is planted into infected weed residue one day after glyphosate application.

WEED HOST STUDY*

Weed Root Test

| Treatment | Downy Brome | Jointed Goatgrass | Green Foxtail | Wild Oat |
|---|---|---|---|---|
| Inoculated | 1.16 | 1.36 | 1.39 | 1.56 |
| Control | 0.24 | 0.22 | 0.20 | 0.18 |

Barley Root Test

| Treatment | Downy Brome | Jointed Goatgrass | Green Foxtail | Wild Oat |
|---|---|---|---|---|
| Inoculated | 0.87 | 0.90 | 0.54 | 0.98 |
| Control | 0.20 | 0.28 | 0.24 | 0.18 |

Value > 0.30 = positive reading. Positive control = 1.44. Negative control = 0.10.
* Values are an average of 8 plant root samples x 2 replications.

The barley plants removed from each weed treatment exhibited spear tipped roots and plant stunting. Symptoms were easily identified on the majority of plant samples. ELISA testing confirmed the presence *R. solani* on representative barley root samples from each of the four weed treatments (Table 15).

# DISCUSSION

Isolation of *R. solani* AG 8 from Montana soils was a major success of this research project. It is the first report of this root rooting organism in the state. Symptoms of bare patches and plant stunting associated with Rhizoctonia root rot had been observed in cereal production fields prior to the initiation of this research project, but isolation of *Rhizoctonia* from plant samples had always been negative.

The difficulty in isolating *Rhizoctonia* from mature plants has been shown in both field and controlled environment experiments. This supports similar observations in Europe and the U.S.(14,31,41). Isolation from plants that are greater than five weeks old was negative in all of the experiments where it was attempted. This may be the reason Rhizoctonia root rot has not been previously identified in Montana.

Other root rotting organisms such as *Pythium spp.*, *Cochliobolis sativus*, and *Fusarium spp.* were usually recovered by isolations from the root system. These fungi were usually determined as the cause of root decay. These three organisms were isolated from barley or wheat roots in all of the experimental research plots. They appear to be ubiquitous in Montana. They may also be secondary root rotting pathogens that enter the roots after *Rhizoctonia* has initially attacked the plant, which has been suggested by previous research in Europe and the U.S.(12,14,30,61).

Anastomosis testing for identification of *R. solani* in laboratory testing proved to be difficult. It is a procedure that requires much

expertise. Other methods to classify *Rhizoctonia* into specific anastomosis groups have been previously described (1,11,21,34,56,57,58). These methods need to be simplified or new procedures investigated to permit rapid and accurate anastomosis testing methods.

Plant stunting in a field situation is not usually noticeable early in the growing season. As plants mature and develop shoots, patches of stunted plants become more noticeable. This is when growers become concerned and submit plant samples to plant pathologists to determine the cause of the symptoms.

ELISA can be an accurate diagnostic tool in the detection of *R. solani* from mature plants. It can be used successfully in diagnostic clinics when large amounts of plant samples need to be tested. The ELISA test kits are also available for a few other fungi and these kits have also been shown to be an accurate diagnostic tool. One setback for its use is the high cost of the test kit and the time period within which an opened kit must be used, i.e., once the reagents have been exposed to air, they must be used within two weeks to insure accurate diagnosis. The cost to test one plant sample is approximately six dollars.

Previous research has shown that weeds can act as secondary hosts for many *Rhizoctonia spp.* (15,17,40,43,48). Results of this research prove that common grassy weeds in Montana cereal production areas can harbor *R. solani* and subsequently serve as sources of inoculum to infect barley roots. Downy brome, jointed goatgrass, green foxtail, and wild oat all exhibited symptoms of spear tipped roots caused by Rhizoctonia root rot. *R. solani* subsequently infected barley after it was planted into these infected weedy grasses.

The role of weeds as a 'green bridge' of infection by *R. solani* has been determined through this research. This supports similar research conducted in the Pacific Northwest (51). Controlling weeds by chemical or mechanical tillage when they germinate is essential to reduce the build up of *R. solani* inoculum in the soil. Allowing the weeds to establish before planting may result in a high incidence of Rhizoctonia root rot. The incidence of this disease is dependent on the amount of available inoculum in the soil as determined in Australia (25,43).

Volunteer winter wheat can also act as a green bridge for Rhizoctonia root rot to barley in continuous crop management systems. Although presence of spear tipped roots was not seen nor isolation of the pathogen successful from the winter wheat sampled during the spring of 1992, root discoloration was noticeable. This could be due to the effect of secondary root rotting fungi.

Winter wheat may not be as susceptible to *R. solani* due to warmer soil temperatures at planting compared to cooler soil temperatures when spring crops are planted. *R. solani* prefers temperatures below 15°C. As the soil temperature cools in the fall, *R. solani* may then begin growth and colonize winter wheat roots. By this time plants have begun the dormancy process and roots may be less susceptible to pathogen attack. When they begin active growth in the spring, the pathogen is near immature root tissues and can cause some rotting, thus predisposing the plant to colonization by other soilborne fungi. These then may override the effect of *R. solani*.

Spring cereal crops are more susceptible to attack by *R. solani*, especially if seeding is performed within two weeks of spraying weeds or

volunteer grain with glyphosate. *Rhizoctonia* infected weeds or volunteer that have been sprayed may provide a nutrient source for the pathogen or other soilborne fungi. This situation has been described in previous research in the Pacific Northwest (51). This can increase the amount of inoculum in the soil, thus making the newly seeded crop more susceptible.

Planting two weeks after application of glyphosate reduced the root disease rating as plants matured. This was shown in the controlled environment experiment. When plants were eight weeks old, shoot weight was 50% greater in the inoculated treatments planted 14 days after glyphosate application versus inoculated treatments planted one or seven days before spraying. Shoot weight was 30% greater in the control plots of the same comparison.

Volunteer wheat may not only serve as a green bridge for *R. solani*, but it possibly competes for water and nutrients early in plant development if crops are planted within two weeks of glyphosate application. Volunteer senescence following glyphosate application is not complete until two weeks after spraying. The significance of volunteer was seen in the 1992 field experiment. Yield was 25% greater in control plots with no volunteer versus the average of control plots with some amount of volunteer. The interaction of glyphosate application and seeding date did not hold true for the downy brome experiment of 1991. *R. solani* was isolated from plants in plots where no glyphosate was applied. In this situation the downy brome was very dense. This shows that transfer of *R. solani* to newly seeded crops can occur if they are planted into fields heavily infested with weeds. This is not likely

to occur in a standard cropping system. Downy brome was shown to be a secondary host to *R. solani* after one year of fallow at this location.

Rhizoctonia root rot can significantly reduce yield if the pathogen is present in the soil. Yield was reduced by 23% in the Post Farm inoculum study of 1991 and by 9% in the 1992 field plots. Spring barley yields have been reduced as much as 50% in Oregon field experiments involving Rhizoctonia root rot (51). The difference in the amount of yield reduction between the two field trials may be due to the different methods used for infesting the soil. *Rhizoctonia* inoculum was banded with the barley seed in 1991, but incorporated perpendicular to the barley in 1992. Therefore, there was more available inoculum at the root zone in the 1991 experiment.

The potential of soil microorganisms affecting the incidence of Rhizoctonia root rot was not fully investigated in this research project. However, the Basamid fumigated plots did show a decline in root disease rating over the three week sampling period while the non-fumigated plots held a relatively constant root score. The root disease score declined by more than 200% over the three week sampling period. The root disease score was always higher in the early sampling dates in the fumigated treatments versus the natural soil.

In the controlled environment study where pasteurized soil was used, root disease score also showed a decline over all sampling dates. This was especially true for the barley planted 14 days after glyphosate application. The root score declined more that 200% over the six week sampling period. Root disease rating remained relatively constant in the

natural inoculated soil. The root disease score was higher in the early sampling date in the pasteurized soil versus the natural soil.

A hypothesis for this rapid decline in root disease score may be that there are fewer soilborne antagonistic organisms present in the fumigated soil when the *R. solani* inoculum was incorporated in the soil. This resulted in the higher root disease score compared to the natural soil. Bacterial antagonists can multiply rapidly when there is no competition by soilborne fungi. As the sampling date progressed in time, these antagonists were able to increase their numbers significantly, resulting in a decline in root disease. This has been suggested in previous research by Specht (55).

The natural soil had probably developed a balance between soil microorganisms and populations of antagonists which may suppress *R. solani*. This may explain why the root disease score remained constant.

Root disease rating varied in all experimental trials. This again may relate to the amount of available inoculum near the root zone of the newly seeded crop as suggested in previous research (25,43). Root scores were highest in inoculated experiments in 1991 and the greenhouse experiment in 1992. Lower scores occurred in the 1992 field plots. The lowest root disease rating was in the downy brome experiment in 1991 where no inoculum was incorporated into the soil and infection was dependent on any naturally occurring inoculum.

# CONCLUSIONS

Rhizoctonia root rot caused by *R. solani* AG 8 is present in Montana soils. It may be the primary pathogen causing bare patches and plant stunting in cereal production fields under reduced tillage management. Observations and testing for the pathogen need to be completed early in the growing season. Isolation of the organism is only accomplished from plants within five weeks after planting. Visual analysis of root systems for spear tipping during the early growth stage is a characteristic symptom caused by this organism.

ELISA testing is an accurate diagnostic tool in identifying the pathogen on plants greater than five weeks old. It can be a useful test in plant disease diagnostic clinics.

Rhizoctonia root rot can cause serious yield losses in Montana cropping systems utilizing reduced tillage management systems. Yield was reduced as much as 23% in inoculated field plots. Seeding within a two week period after glyphosate application can cause a higher incidence of disease if the pathogen is present in the soil. Also, planting into volunteer during this time period can also reduce yield due to competition by the volunteer for moisture, nutrients, and sunlight early in cereal crop development.

Downy brome, jointed goatgrass, green foxtail and wild oats can act as secondary hosts for *R. solani*. They have potential to provide a nutrient source for the pathogen. These weeds should be kept under

control to reduce the amount of inoculum in the soil. They should be controlled with a non-selective herbicide or by tillage at least two weeks before planting to reduce possibility of infection by Rhizoctonia root rot if it is present in the soil.

Montana cereal producers using reduced tillage management need to follow these few recommendations to reduce the potential of Rhizoctonia root rot. The pathogen may also predispose spring cereals to other root rooting pathogens which may consequently colonize the crop.

There is a possibility that there are native soilborne antagonists to *R. solani* present in the soil. A rapid decline in root disease ratings in pasteurized and fumigated soil versus natural soil supports this statement.

Future studies of Rhizoctonia root rot are necessary to determine how widespread the organism is throughout Montana. Its potential has been shown in the Montana environment and it can become or is already prevalent in areas using reduced tillage management systems.

Four common grassy weeds of Montana have been determined as secondary hosts of *R. solani*. Further research is necessary to identify other potential weed hosts for this pathogen.

Presently there are no known chemical control methods for Rhizoctonia root rot. Further investigation of soilborne antagonists as a biological control is necessary, especially in today's environmentally conscious world.