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# Effects of Biological Control and a Ryegrass Rotation on Rhizoctonia Disease of Potato

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# EFFECTS OF BIOLOGICAL CONTROL AND A RYEGRASS ROTATION ON RHIZOCTONIA DISEASE OF POTATO

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# EFECTS OF BIOLOGICAL CONTROL AND A RYEGRASS ROTATION ON RHIZOCTONIA DISEASE OF POTATO

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An Abstract of the Thesis Presented
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*Rhizoctonia solani* is an important pathogen of potato capable of reducing tuber yield and quality. Integrated, sustainable methods including biocontrol and effective crop rotations are necessary for control of this pathogen. Twenty-eight potential biocontrol organisms were tested for efficacy against *R. solani* on potato in a series of greenhouse trials. Organisms tested consisted of field isolates of *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Penicillium* sp., *Trichoderma* sp., and *Rhizoctonia zeae*; known biocontrol isolates including *Laetisaria arvalis, Verticillium biguttatum, Cladorrhinum foecundissimum,* and *Stilbella aciculosa*; and commercial products of *Bacillus subtilis* (Kodiak), *Trichoderma virens* (SoilGard), and *Trichoderma harzianum* (RootShield). Different formulations and rates of several fungal isolates and the efficacy of combinations of effective antagonists were also investigated. None of the treatments, including a chemical control (azoxystrobin), effectively controlled stem canker and black

scurf in all trials. However, *B. subtilis* GB03, *R. zeae* LRNE17E, *S. aciculosa* 112-B, and the chemical control were most effective in reducing stem canker severity (40 to 49 % reduction) relative to the infested controls over all trials. *L. arvalis* ZH-1, *R. zeae* LRNE17E, and the chemical control reduced black scurf 54 to 60 % relative to the infested control. Other treatments also significantly reduced stem canker and black scurf, however they were slightly less effective. Different rates of biocontrol organisms provided varying disease reductions with higher rates usually providing the best control. One combination of biocontrol organisms, *B. subtilis* and *T. virens*, demonstrated somewhat better control of stem canker than each organism alone, suggesting that this approach may provide improved biocontrol efficacy. Greenhouse trials were also conducted to evaluate the effects of selected rotation crops including barley, two ryegrass varieties (common and "Lemtal"), clover, potato, and combinations of barley with ryegrass or clover, on populations of *R. solani* and Rhizoctonia disease. Potato and clover preceding potato resulted in higher disease severity of stem canker than most other rotations, whereas Lemtal ryegrass reduced stem canker severity. In addition, all ryegrass treatments resulted in substantially higher populations of *R. zeae*. Field trials evaluating selected biocontrol treatments in combination with different rotations were conducted at two locations in Maine. Potatoes were treated with *Laetisaria arvalis, Trichoderma virens*, or *Bacillus subtilis* and planted as subplots following rotation crops of barley and ryegrass, barley and clover, or potato. Treatment effects on shoot emergence, disease levels, tuber yield, soil microbial communities, and fatty acid methyl ester (FAME) profiles were determined. The barley/ryegrass rotation significantly reduced incidence and severity of stem canker and increased tuber yield at one location.

Efficacy of the biocontrol treatments varied by rotation and location, with *L. arvalis* and *T. virens* reducing black scurf severity and incidence in some rotations. *L. arvalis* and *T. virens* also increased some aspects of tuber yield at one location. General fungal and bacterial populations and fatty acid profiles demonstrated distinct differences in microbial community characteristics among rotation crops and biocontrol treatments. Significant crop by biocontrol interactions were observed indicating that biocontrol can be enhanced within beneficial rotations, leading to greater reductions of Rhizoctonia disease of potato.

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# Chapter 1

# BACKGROUND

## Literature Review

The soil fungus *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) causes Rhizoctonia disease of potato (*Solanum tuberosum* L.), which consists of stem and stolon canker and black scurf of tubers. This disease can have severe economic impacts on potato production because it reduces both tuber yield and quality, has a wide host range, and is found everywhere that potatoes are grown (Carling and Sumner, 1992). *R. solani* grows saprophytically in soil or can be carried on tubers as sclerotia, the long-term survival structures of the fungus. The pathogen is attracted by exudates released by the sprouting potato tuber, and if conditions are appropriate, begins to cause disease. Symptoms of Rhizoctonia disease of potato include necrosis and girdling of young sprouts, stolon lesions, aerial tubers, and stem and stolon canker. These symptoms result in small or malformed tubers, low tuber production, and in severe cases, death of the plant. Stem canker alone can result in up to a 10% yield loss (Parry, 1990). Another problem associated with *R. solani* is the formation of sclerotia on tubers, a condition commonly known as black scurf. Black scurf leads to a decrease in tuber quality and is a serious seed certification problem since the tuberborne sclerotia can be a source of inoculum (Frank and Leach, 1980).

*Rhizoctonia* is a very large and diverse genus (Carling and Sumner, 1992). Isolates within this genus belong to one of three teleomorphs, *Thanetephorus, Ceratobasidium*, or *Waitea*. *R. solani* is the asexual state of the basidiomycete *Thanetephorus cucumerus*.

The sexual state is rarely seen in nature, but it can easily be induced in the lab (Carling and Sumner, 1992). *Ceratobasidium* encompasses the binucleate *Rhizoctonia* anamorphs and *Waitea* is the teleomorphic form of *R. zeae, R. oryzae, and R. circinata* (Carling and Sumner, 1992; Sneh *et al.,* 1991*). R. solani* is a pathogen of many crops throughout the world. Some *Rhizoctonia* species, however, are not pathogenic (Papavizas and Lewis, 1986), and are even known to have beneficial plant associations (Sneh *et al.*, 1991).

*R. solani* can be subdivided into anastomosis groups (AG), which consist of fungal strains that are able to fuse their hyphae, or anastomose, with each other. AG's also vary in other traits, including host range and pathogenicity. Currently, *R. solani* can be categorized among thirteen different anastomosis groups (Carling and Sumner, 1992; Carling *et al.*, 1999; Carling *et al.*, 2002), many of which contain further subdivisions based on other traits, including sclerotial form, DNA base sequence homology, and cultural characteristics (Sneh *et al.,* 1991). The most common anastomosis group isolated from potatoes in Maine is AG-3 (Bandy *et al.*, 1988). This group is also the most virulent pathogen on potatoes (Carling and Leiner, 1986; Bains and Bisht, 1995). Other anastomosis groups that have been associated with potato diseases are AG-5, AG-4, AG 2-1, AG-8, and AG-9 (Bandy *et al.*, 1988; Carling and Leiner 1986; Carling and Sumner, 1992; Bains and Bisht, 1995).

A moist, cool environment is most conducive to Rhizoctonia disease of potato (Baker and Martinson, 1970; Frank, 1981), although the fungus can survive a temperature range of 5 to 45°C (Baker and Martinson, 1970). Carling and Leiner (1990) determined that AG-3 killed the most sprouts at 10°C, but caused the most damage to roots at 15.5°C. Richards (1921) reported that the most severe disease on potatoes caused by *Rhizoctonia*

occurred between 12.2 to 18.2°C. However, reduced plant vigor at these lower temperatures led to slower emergence and prolonged exposure to the pathogen, which could account for increased disease (Richards, 1921).

Control of *R. solani* is accomplished mainly by cultural practices, including planting clean seed, crop rotation with barley or oats, promoting rapid emergence of sprouts, and removing tubers from the field as soon as possible (Secor and Gudmestad, 1999). The use of resistant potato varieties is not an option for control of stem or stolon canker because *R. solani* can cause disease symptoms of varying degree on all commercial potato varieties (Leach and Garber, 1970). Chemical fungicides are often used if disease pressure is high (Parry, 1990). Fludioxonil and a combination of thiophanate methyl and mancozeb can be used as seed treatments and pentachloronitrobenzene (PCNB) or azoxystrobin can be applied in-furrow for control of both soilborne and tuberborne inoculum. However, none of these controls are completely effective or practical, and Rhizoctonia disease is a persistent problem in most potato fields.

Although chemical control may be somewhat effective in the short term, the long-term effects are less advantageous. Pesticides can kill beneficial microorganisms along with the pathogens (Cook and Baker, 1983), which can decrease the quality and productivity of the soil, ultimately affecting crop health and productivity. In addition, pathogens may develop resistance to the chemicals used for their control (Dekker, 1976). Pesticides also represent additional inputs and expenses for the grower, and may negatively impact the environment and human health through runoff, leaching, persistence, and residues. Low input sustainable alternatives are needed for the control of plant pathogens. Biological and cultural controls are solutions that work within the ecology of the microbial

communities, and are a balanced, potentially long lasting, environmentally sound means of control (Larkin *et al.*, 1998).

Biological control is defined as "the reduction of the amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man" (Cook and Baker, 1983). The effects of biocontrol can be long lasting, whereas pesticides need to be reapplied (Cook and Baker, 1983). Other advantages of biocontrol include unlikely pathogen resistance and less environmental impact (Larkin *et al.*, 1998).

Biocontrol organisms suppress disease by one or more different mechanisms of action including antibiosis, predation, competition, induced resistance, or hypovirulence. Antibiosis occurs when an antagonist produces a substance that inhibits another organism. Morris *et al.* (1995) observed that *Verticillium biguttatum* produces two antifungal metabolites, which suggests that antibiosis plays a role in its inhibition of *R. solani*. *V. biguttatum* is also a well-known mycoparasite of *R. solani* (Velvis and Jager, 1983; van den Boogert and Deacon, 1994). Parasitism, or predation, involves the destruction of a pathogen by an organism that is feeding on or within it (Larkin *et al.*, 1998). Competition occurs when an organism is able to suppress the disease or activity of a pathogen because it is better suited to that particular environment. The competitor is able to utilize resources more efficiently or colonize plant parts more effectively than the pathogen, which reduces the populations or disease-causing activity of the pathogen (Larkin *et al.*, 1998). Induced resistance involves an organism inducing a defense response in the plant that protects it from a pathogen (Cook and Baker, 1983). Nandakumar *et al.* (2001) determined that *Pseudomonas fluorescens* could induce systemic resistance in rice against *R. solani*.

Hypovirulence occurs when a less virulent or non-virulent isolate of the same genus or species competes with a pathogen or colonizes the host, which protects the infection site (Sneh, 1991). Hypovirulence can also involve the transmission of a mycovirus by hyphal anatomosis that leads to reduced virulence (Cook and Baker, 1983). Jian *et al.* (1997) showed that virulence of *R. solani* AG-3 could be reduced by infection with a dsRNA virus.

Several organisms have been observed to reduce inoculum of *R. solani* or Rhizoctonia disease on potato or its close relatives, tomato and eggplant. *Laetisaria arvalis* Burdsall is an effective biocontrol fungus on several crops, including potato in Maine (Lewis and Papavizas, 1992; Murdoch and Leach, 1993). *Verticillium biguttatum* Gams is a mycoparasite of *R. solani* that inhibits the formation of sclerotia on tubers and prevents sprout infection from soilborne inoculum (Jager and Velvis, 1984). *V. biguttatum* has been used successfully to control *R. solani* on potato in repeated field experiments in the Netherlands (Velvis and Jager, 1983; Jager and Velvis 1985, 1986). *Bacillus subtilis* (Ehrenberg) Cohn strain RB14 suppressed *R. solani* on tomato (Asaka and Shoda, 1996) by producing two antibiotics, iturin A and surfactin (Hiroaka *et al.*, 1992) and it also suppressed Rhizoctonia root rot of wheat (Ryder *et al.*, 1999). *Trichoderma harzianum* Rifae and *Trichoderma (Gliocladium) virens* Miller, Giddens, and Foster, the two most studied biocontrol organisms (Papavizas, 1992), reduced soilborne and tuberborne *R. solani* (Beagle-Ristaino and Papavizas, 1985). *T. virens* and *T. harzianum* also protected eggplant from *R. solani* (Lewis *et al.*, 1995a; Lewis and Larkin, 1997; Lewis *et al.*, 1998). *Paenibacillus polymyxa* Prazmowski has been isolated from the barley rhizosphere and showed in vitro antagonism toward *R. solani* (Nielsen and Sørensen, 1997) by producing

cell wall-degrading enzymes. *P. polymyxa* has also been isolated from local potato fields (Larkin, unpublished). *Pseudomonas fluorescens* Micula can be an antagonist of *R. solani* (Nielsen and Sørensen, 1997; Kloepper, 1991; Bagnasco *et al.*, 1998). It reduced damping-off of sugar beet caused by *R. solani* and inhibited both mycelial growth and sclerotia formation of the pathogen (Thrane *et al.*, 2001). *Cladorrhinum foecundissimum* Saccardo and Marchal reduced damping-off of eggplant caused by *R. solani* (Lewis *et al.*, 1995b; Lewis and Larkin, 1998). *Stilbella aciculosa* (Ellis and Everh.) Seifert is a fungus that may control *R. solani* by producing a toxic metabolite that interferes with pathogen hyphae and saprophytic growth (Lewis and Papavizas, 1993). The efficacy of many of these organisms as biocontrol agents in the *R. solani*-potato pathosystem in Maine is yet to be determined.

Although biocontrol is an alternative to pesticides, results are not immediate and often are not as effective as with chemical control. Biocontrol cannot be used simply to replace current agricultural practices. Instead, biological control should be optimized by making it a component of integrated pest management (IPM) within the existing agronomic system (Larkin *et al.*, 1998; Lumsden and Papavizas, 1988). The integration of biocontrol with traditional control methods may include using combinations of effective antagonists (Larkin *et al.*, 1998; Lumsden and Papavizas, 1988) or combining biocontrol with effective cultural practices, such as green sprouting (Larkin, 2003b) or crop rotations (van Bruggen *et al.*, 1996).

Crop rotations can be an effective option for sustainable disease control. Rotation crops and cover crops used as green manures have been studied extensively for suppression of *R. solani* (van Bruggen *et al.*, 1996). Crop rotation has been found to reduce

Rhizoctonia disease of potato (Honeycutt *et al.*, 1996; Larkin and Honeycutt, 2002; Davis *et al.*, 1991; Specht and Leach, 1987; Frank and Murphy, 1977; Johnston et al., 1994), especially in three-year rotations, but two-year rotations were also successful (Emmond and Ledingham, 1972). Canola, barley, and sweet corn rotations reduced Rhizoctonia disease of potato, while potato crops preceded by soybean or clover demonstrated increased disease levels (Larkin and Honeycutt, 2002; Larkin, 2004). Although many accounts of suppression of Rhizoctonia disease by crop rotations and residues have been reported, the mechanisms of suppression are rarely addressed. Rotation with a different crop may reduce disease by allowing enough time for inoculum levels to decline, directly antagonizing the pathogen, creating an environment that is conducive to microbial antagonists, or increasing soil microbial activity which would lead to competition with the pathogen (Larkin, 2004; Huber and Sumner, 1996).

In Maine, a two-year rotation with oats (*Avena sativa* L.) or barley (*Hordeum vulgare* L.) is the standard cropping system. These rotation crops are often planted with red clover (*Trifolium pratense* L.), which is used as a cover crop to reduce erosion and supply organic matter. Studies have indicated that clover and other legumes used as rotation crops can increase *R. solani* on subsequent susceptible crops (Rickerl *et al.*, 1992; Dabney *et al.*, 1996). Anecdotal reports from growers in Maine suggest that a variety ("Lemtal") of annual ryegrass (*Lolium multiflorum* Lam.) used in place of clover may reduce Rhizoctonia disease of potato. Although some growers that have adopted this rotation have seen positive results, no scientific studies have been conducted to determine its effectiveness. Field studies are needed to determine if Lemtal ryegrass rotations result in a significant reduction in Rhizoctonia disease, and whether Lemtal ryegrass provides

any better control than other ryegrass varieties. Annual ryegrass alone as a rotation with potato has suppressed Rhizoctonia disease (Specht and Leach, 1987; Johnston *et al.*, 1994), however, reductions were comparable to those observed with several other rotation crops and the mechanism(s) involved was not addressed.

## Objectives

The objectives of this research were to:

1. Evaluate the efficacy of numerous biological control organisms on suppression of Rhizoctonia disease of potato and assess combinations of biocontrol organisms for disease reduction,
2. Determine the effects of select rotation crops, including ryegrass, barley, and clover, on populations of *R. solani* and Rhizoctonia disease of potato, and
3. Evaluate the effects of integrated control by select rotations and microbial antagonists on populations of *R. solani*, soil microbial communities, and Rhizoctonia disease of potato.

## Chapter 2

# EFFICACY OF SEVERAL POTENTIAL BIOCONTROL ORGANISMS AGAINST *RHIZOCTONIA SOLANI* ON POTATO


## Chapter Abstract

Twenty-eight potential biocontrol organisms were tested for efficacy against *Rhizoctonia solani* on potato in a series of greenhouse trials. Organisms tested consisted of field isolates of *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Penicillium* sp., *Trichoderma* sp., and *Rhizoctonia zeae*; known biocontrol isolates including *Laetisaria arvalis, Verticillium biguttatum, Cladorrhinum foecundissimum,* and *Stilbella aciculosa*; and commercial products of *Bacillus subtilis* (Kodiak), *Trichoderma virens* (SoilGard), and *Trichoderma harzianum* (RootShield). Different formulations and rates of several fungal isolates and the efficacy of combinations of effective antagonists were also investigated. None of the treatments, including a chemical control (azoxystrobin), effectively controlled stem canker and black scurf in all trials. However, *B. subtilis* GB03, *R. zeae* LRNE17E, *S. aciculosa* 112-B, and the chemical control were most effective in reducing stem canker severity (40 to 49 % reduction) relative to the infested controls over all trials. *L. arvalis* ZH-1, *R. zeae* LRNE17E, and the chemical control reduced black scurf 54 to 60 % relative to the infested control. Other treatments also significantly reduced stem canker and black scurf, however they were slightly less effective. Different rates of biocontrol organisms provided varying disease reductions with higher rates usually providing the best control. One combination of biocontrol organisms, *B. subtilis* and *T. virens*, demonstrated somewhat better control of stem canker

than each organism alone, suggesting that this approach may provide improved biocontrol efficacy.

## Introduction

*Rhizoctonia solani* Kühn is an important fungal pathogen (Baker, 1970) that causes both stem canker and black scurf of potato (*Solanum tuberosum* L.), which lead to tuber yield reductions and losses in tuber quality. Currently, Rhizoctonia disease is managed by cultural practices, such as crop rotation with grains, and methods that minimize prolonged contact of the plant or tubers with the pathogen, such as planting in warmer, drier conditions to promote sprout emergence and promptly removing tubers from the field (Secor and Gudmestad, 1999). Chemical fungicides are often used when losses from *R. solani* are great (Parry, 1990). However, current cultural and chemical controls are not completely effective and Rhizoctonia disease remains a persistent problem.

Biological control of Rhizoctonia diseases has been demonstrated in some cases and represents an additional strategy that may provide effective and sustainable management. Biocontrol can be an effective means of control in many instances where chemical control is not available or practical (Lumsden and Papavizas, 1988). Several microbial antagonists, some of which are available in commercial formulations, have shown potential for control of *R. solani* on potato or other host crops. *Trichoderma harzianum* Rifai and *Trichoderma (Gliocladium) virens* Miller, Giddens, and Foster have successfully suppressed *R. solani* in several pathosystems (Beagle-Ristaino and Papavizas, 1985; Lewis *et al.*, 1995a; Lewis and Larkin, 1997; Lewis *et al.*, 1998).

*Verticillium biguttatum* Gams (Velvis and Jager, 1983; Jager and Velvis, 1984,1985, 1986), *Bacillus subtilis* (Ehrenberg) Cohn (Asaka and Shoda, 1996), *Pseudomonas fluorescens* Migula (Kloepper, 1991; Nielsen and Sørensen, 1997; Bagnasco *et al.*, 1998), *Stilbella aciculosa* (Ellis and Everh.) Seifert (Lewis and Papavizas, 1993), *Cladorrhinum foecundissimum* Saccardo and Marchal (Lewis *et al.*, 1995b; Lewis and Larkin, 1998), *Paenibacillus polymyxa* Prazmowski (Nielsen and Sørensen, 1997), and *Laetisaria arvalis* Burdsall (Lewis and Papavizas, 1992; Murdoch and Leach, 1993) have all shown some potential for disease control under the conditions studied. However, many of these organisms have not been studied for suppression of Rhizoctonia disease of potato and most have only been evaluated under limited conditions and have not been compared with each other in the same pathosystem.

Although the efficacy of many biocontrol organisms has been demonstrated under certain conditions, disease control is not always consistent (Larkin *et al.*, 1998). One way to improve biocontrol is to use multiple antagonists in effective combinations (Lumsden and Papavizas, 1988; Larkin *et al.*, 1998). The benefits of this approach include multiple mechanisms of action, synergistic effects, and wider ecological ranges of activity (Larkin *et al.*, 1998).

The objective of this study was to determine the efficacy of several biocontrol organisms in the control of Rhizoctonia disease of potato in the greenhouse. These organisms included: field isolates of *Trichoderma* sp., *Paenibacillus polymyxa*, and *Pseudomonas fluorescens*; known biocontrol isolates of *L. arvalis, V. biguttatum, C. foecundissimum,* and *S. aciculosa*; and commercially available products consisting of the organisms *B. subtilis, T. virens*, and *T. harzianum*. In addition, the role of organisms

associated with potato cropping systems in Maine and their potential biocontrol properties were investigated, including a *Penicillium* sp. that is associated with potato roots (Larkin, 2003a) and *Rhizoctonia zeae*, which is associated with ryegrass rotations. The efficacy of several combinations of effective antagonists was also investigated.

## Materials and Methods

### Inoculum preparation

An isolate of *R. solani* (RS31B) recovered from diseased potato roots from a field in Maine was used as the source of pathogen inoculum in all experiments. This isolate caused considerable stem canker and black scurf in preliminary greenhouse trials (Appendix A). Fatty acid methyl ester (FAME) analysis confirmed that this isolate belonged to AG-3 (Larkin, unpublished). Inoculum was prepared by transferring four plugs of potato dextrose agar (PDA, Difco Laboratories, Inc., Sparks, MD) containing 7- to 14-day-old cultures of *R. solani* to petri dishes filled with 20 to 30 g of sterile organic cracked wheat. The grain was prepared by adding 3 ml deionized water per 5 g of organic cracked wheat (Associated Buyers, Barrington, NH) and autoclaving for 60 min on two consecutive days (Papavizas and Lewis, 1986). The inoculated wheat was incubated at room temperature (21 –25°C) for 8 days then air dried for 48 hours. Viability of inoculum was confirmed by plating on PDA. Inoculum was kept in a paper bag at 4 to 5°C for no more than 1 month until needed.

### Soil and seed preparation

Field soil from Newport, ME, an unnamed variant of a Bangor silt loam (coarse-loamy, mixed, frigid, Typic Haplorthod), was used for all experiments. The soil was

sieved through a 6 x 6 mm screen and combined with sterile sand at a 3:1 (w/w) soil to sand ratio. Pathogen inoculum was incorporated at a rate of 4 g per kg of soil mix and incubated in the dark at room temperature for 24 hours. This rate provided approximately 25 propagules of *R. solani* per gram of soil.

Potato variety 'Shepody' was used in all experiments. Shepody is susceptible to soilborne diseases, such as Rhizoctonia disease and common scab (*Streptomyces scabiei*). Seedpieces were prepared by washing in warm water, removing visible sclerotia, and treating for 2 min in 2% formaldehyde in water to prevent contamination by seedborne inoculum (Bandy *et al.*, 1988). Seed was green-sprouted in the greenhouse for approximately 2 weeks and cut to approximately 3 to 6 cm in length containing at least one viable sprout per piece. The seedpieces were removed from the greenhouse and allowed to suberize at room temperature for 5 to 7 days prior to planting.

Approximately 1,200 g of pathogen-infested soil was added to each 15-cm pot and three seedpieces were arranged in the pot 2 to 3 cm below the soil surface. In addition, control treatments with no pathogen inoculum (a noninfested control, a wheat control, and a *Penicillium* sp. control) were prepared. Four grams of sterile cracked wheat per kilogram of soil mix was added to the wheat control to determine the effect, if any, of the cracked wheat on Rhizoctonia disease. The *Penicillium* sp. control was used to determine the effect of this fungus, which is associated with potato roots in the field (Larkin, 2003a), on the potato plant.

Azoxystrobin (Quadris, Syngenta Crop Protection, Greensboro, NC), a broad-spectrum protectant fungicide, was used for chemical control for comparison purposes. It

was prepared by applying 1 ml of a 0.1% aqueous solution in-furrow per seed piece, the manufacturer's recommended rate.

**Biocontrol treatments**

The biocontrol treatments, consisting of different formulations, concentrations, and combinations, were applied in several screening trials. All field isolates were acquired from potato fields in Newport or Presque Isle, ME.

Bacterial antagonists included *Bacillus subtilis* GBO3, the active ingredient of Kodiak (Gustafson, Dallas, TX), five field isolates of *Paenibacillus polymyxa,* and three field isolates of *Pseudomonas fluorescens*. Treatments consisted of aqueous solutions prepared from 3-day-old cultures of the bacteria grown on tryptic soy agar (Difco Laboratories, Inc.). Solutions were adjusted to transmittance values of 50% +/- 2% at 590 nm and concentrations were determined by plating on tryptic soy agar. Suspensions contained concentrations of $10^7$ to $10^8$ cfus/ml for all bacterial preparations except *P. polymyxa* which contained $10^6$ cfus /ml. Thirty milliliters of each suspension were added to the field soil mix and incorporated prior to planting.

SoilGard (Olympic Horticultural Products, Mainland, PA), a commercial formulation of *Trichoderma virens* GL-21, was added at rates of 2.6 g per pot and 1.3 g per pot (0.2 and 0.1 % incorporation rate [w/w], respectively). The RootShield drench (BioWorks, Geneva, NY), containing *Trichoderma harzianum* T-22, was added to the soil as directed by the manufacturer at a rate of 240 ml of drench (0.59 g /L of water) per pot of soil.

Aqueous spore suspensions of the active ingredients of SoilGard and RootShield, several *Trichoderma* field isolates, *V. biguttatum* M73 (ATCC #90587), and three

*Penicillium* sp. field isolates were prepared from active, sporulating cultures growing on potato dextrose agar (PDA) (8-14 day old cultures for *Trichoderma* isolates and 14-21 days old for *Verticillium* and *Penicillium* isolates). Spore concentrations were adjusted to $10^7$ conidia per milliliter for *Trichoderma* isolates, and $10^6$ conidia per milliliter for *V. bigutatum* and *Penicillium* sp. *T. virens* was also added at rates of $10^6$ conidia per milliliter and $10^5$ conidia per milliliter to determine if the biocontrol was also effective at lower doses. Thirty milliliters of each suspension were added to each pot and incorporated prior to planting.

*R. zeae* field isolates RZLRNE17E and RZRF27I, *R. zeae* 504 (obtained from Roseann Leiner, Fairbanks, AK), *L. arvalis* LA-1 (ATCC #52088), and *L. arvalis* ZH-1 (ATCC #62715) were also screened. These non-spore-forming fungal treatments were prepared as described above for the pathogen inoculum except the incubation time was decreased to 5 days. Three grams of infested wheat was added to soil near the seedpiece to simulate an in-furrow application. One and a half grams of *L. arvalis* ZH-1 inoculum were also added as a separate treatment to determine the most effective rate.

*C. foecundissimum* C91 (ATCC #62373), *S. aciculosa* 112B-1 (ATCC #90820), and an additional *L. arvalis* LA-1 treatment were prepared by adding a petri dish of potato dextrose agar covered with the culture to 150 ml sterile water. The solution was mixed vigorously on a stir plate for 30 min and 30 ml was added to each pot and incorporated prior to planting.

Plants were grown in the greenhouse for 3 to 4 weeks until the noninfested and wheat controls had fully emerged. Plants were harvested, washed, and rated for stem canker on a scale of 0 to 5 as follows: 0 = no disease symptoms; 1 = brown discoloration

of stems; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % stems covered by cankers; 4 = 75 % coverage by stem cankers; and 5 = stem completely nipped off or death of the plant. When two or more shoots emerged from one seed piece the average rating of all shoots was recorded. After harvest, newly formed sclerotia were visible on the seed pieces, therefore, black scurf was also assessed on a scale of 0 to 5 as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1 % of the skin-covered tuber surface; 2 = 2 % to 5 % covered; 3 = 5 % to10 % covered; 4 = 10 % to15 % covered; and 5 = > 15% tuber covered by sclerotia. Differences in shoot size among treatments were also detected at harvest, so shoots were either weighed or rated for size. In the first trial, weight measurements were taken for roots and shoots of each plant. In subsequent trials (trials 2 through 4), ratings of 0 to 3 (0 = no growth, 1 = shoot height < 2 cm, 2 = shoot height of 2 to 5 cm, and 3 = shoot height > 5 cm) were used.

**Statistical analysis**

All trials used a randomized complete block design. There were three replications of each treatment in the first experiment and four replications per treatment in trials 2 through 4. Each replicate consisted of one pot containing three potato seedpieces. Data from each trial was analyzed separately by analysis of variance using SAS (ver. 7, SAS Institute, Cary, NC). In addition, data representing percent disease suppression for each treatment were combined for multiple trials and analyzed to compare overall efficacy rates. Mean separation was accomplished using Fisher's least significant difference (LSD) at $P = 0.05$.

## Results

### Efficacy of biocontrol organisms

In the first trial of biocontrol organisms, twenty-six different isolates and three controls were tested. Treatments that reduced the incidence (50 % to 89 %) and severity of stem canker (35 % to 63 %) relative to the infested control included the chemical control, *T. virens* GL-21 (spore suspension), *Trichoderma* TW, *Trichoderma* T2, *T. harzianum* T-22 (spore suspension), *R. zeae* RF27I, *P. fluorescens* 3, *P. polymyxa* 2, *P. polymyxa* 3, and *P. polymyxa* 5 (Table 2.1). The *Trichoderma* TY treatment also reduced stem canker severity (39 %). Treatments of *L. arvalis* ZH-1, *Penicillium* YG 1, *Penicillium* YG 2, *Penicillium* YG 3, *R. zeae* 504, all *Trichoderma* isolates except *Trichoderma* TY, *V. biguttatum* M73, and the chemical control all reduced black scurf severity (46 % to 100 %) relative to the infested control. Treatments that provided some control for both stem canker and black scurf in trial 1 included *T. harzianum* T-22 (spore suspension), *T. virens* GL-21 (spore suspension), *Trichoderma* T2, and *Trichoderma* TW. Based on the results of this first trial, the number of biocontrol organisms were reduced by selecting no more than two isolates per species to be used in subsequent tests. Several new organisms were also added to later trials.

In subsequent trials, all isolates tested reduced incidence or severity of stem canker in at least one of the three tests, except *R. zeae* RF27I and *Trichoderma* T2 (Table 2.2). However, no treatment, including the chemical control, reduced both incidence and severity of stem canker in all tests. The *B. subtilis* treatment reduced stem canker severity in all three tests, but incidence was reduced in only one test. The *P. polymyxa* 3 treatment reduced severity in two of three trials and incidence in one trial.

**Table 2.1.** Effect of potential biocontrol organisms on stem canker and black scurf in trial 1.

| Treatment | Stem canker | | | Black scurf | |
|---|---|---|---|---|---|
| | Incidence[a] (%) | Severity[b] | Reduction[c] (%) | Severity[d] | Reduction[e] (%) |
| *Bacillus subtilis* GBO3 | 61.1 | 1.88 | 30.0 | 3.00 | 18.0 |
| *Laetisaria arvalis* ZH-1 | 55.6 | 1.89 | 29.4 | 1.67* | 54.7* |
| *Laetisaria arvalis* LA-1 | 77.8 | 2.67 | 0.1 | 3.33 | 9.0 |
| *Paenibacillus polymyxa* 1[+f] | 72.2 | 2.13 | 20.6 | 3.67 | 0.0 |
| *Paenibacillus polymyxa* 2[+] | 44.4*[g] | 1.56* | 42.0* | 2.67 | 27.7 |
| *Paenibacillus polymyxa* 3[+] | 44.4* | 1.56* | 42.0* | 3.33 | 9.0 |
| *Paenibacillus polymyxa* 4[+] | 66.7 | 1.89 | 29.4 | 3.00 | 18.0 |
| *Paenibacillus polymyxa* 5[+] | 50.0* | 1.75* | 34.8* | 3.00 | 18.3 |
| *Penicillium* sp. YG 1[+] | 72.2 | 2.25 | 15.8 | 1.00* | 72.7* |
| *Penicillium* sp. YG 2[+] | 77.8 | 1.88 | 29.9 | 1.00* | 73.0* |
| *Penicillium* sp. YG 3[+] | 100.0 | 2.60 | 2.6 | 1.33* | 64.0* |
| *Pseudomonas fluorescens* 1[+] | 77.8 | 2.13 | 20.6 | 3.00 | 18.3 |
| *Pseudomonas fluorescens* 2[+] | 77.8 | 2.00 | 25.1 | 2.33 | 36.7 |
| *Pseudomonas fluorescens* 3[+] | 33.3* | 1.67* | 37.8* | 3.33 | 18.3 |
| *Rhizoctonia circinata* W616 | 100.0 | 2.75 | -2.9 | 2.33 | 36.7 |
| *Rhizoctonia circinata* W630 | 88.9 | 2.33 | 12.8 | 2.33 | 36.3 |
| *Rhizoctonia zeae* LRNE17E[+] | 55.5 | 1.88 | 30.0 | 2.33 | 36.3 |
| *Rhizoctonia zeae* RF27I [+] | 50.0* | 1.50* | 44.0* | 3.00 | 18.0 |
| *Rhizoctonia zeae* RZ504 | 88.9 | 2.11 | 21.0 | 1.67* | 54.7* |
| *Trichoderma harzianum* T-22 | 27.8* | 1.29* | 52.1* | 2.00* | 46.0* |
| *Trichoderma* sp. T2[+] | 33.3* | 1.50* | 44.1* | 0.00* | 100.0* |
| *Trichoderma* sp. TN2RA[+] | 66.7 | 1.89 | 29.3 | 1.33* | 64.0* |
| *Trichoderma* sp. TW[+] | 38.9* | 1.57* | 41.4* | 1.33* | 64.0* |
| *Trichoderma* sp. TY[+] | 66.7 | 1.63* | 39.3* | 2.33 | 36.7 |
| *Trichoderma virens* GL-21 | 33.3* | 1.33* | 50.3* | 0.67* | 82.0* |
| *Verticillium biguttatum* M73 | 66.7 | 2.33 | 12.8 | 0.67* | 82.0* |
| infested control | 100.0 | 2.67 | 0.0 | 3.67 | 0.0 |
| noninfested control | 0.0* | 0.22* | 91.8* | 0.00* | 100.0* |
| chemical control (azoxystrobin) | 11.1* | 1.00* | 62.9* | 2.00* | 46.0* |
| LSD ($P$=0.05) | 46.2 | 0.92 | 34.4 | 1.36 | 37.2 |

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Percent reduction in stem canker severity relative to the infested control.
[d]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1 % of the skin-covered tuber surface; 2 = 2 % to 5 % covered; 3 = 5 % to 10 % covered; 4 = 10 % to 15 % covered; and 5 = > 15 % tuber covered by sclerotia.
[e]Percent reduction in black scurf severity relative to the infested control.
[f]Isolate names followed by a plus (+) sign are soil isolates isolated from a potato field in Maine.
[g]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P$ = 0.05).

Treatments of *R. zeae* LRNE17E and *S. aciculosa* reduced incidence and severity of stem canker in two trials. The *Penicillium* YG 2 treatment reduced severity of stem canker in two trials and the *L. arvalis* ZH-1 treatment reduced incidence in two trials.

Most isolates tested, with the exception of *Penicillium* YG 3, *R. zeae* RF27I, *Trichoderma* T2, and *T. harzianum* (spores suspension), also reduced the incidence and severity of black scurf in at least one of three subsequent trials (Table 2.3). None of the treatments reduced incidence and severity of black scurf across all tests. The *R. zeae* LRNE17E and *L. arvalis* ZH-1 treatments reduced severity in all trials and the *R. zeae* LRNE17E treatment reduced incidence in one of three trials. The chemical control, *Penicillium* YG 2, *T. virens* GL-21 (spore suspension), *P. polymyxa* 2, and *P. polymyxa* 3 treatments reduced black scurf severity in two of three trials. The chemical control and the *Penicillium* YG 2 treatment reduced incidence in two of three and one of three trials, respectively.

When averaged over all trials, *B. subtilis*, *R. zeae LRNE17E*, *S. aciculosa*, and the chemical control treatments were most successful at reducing stem canker severity with reductions of 40 to 49 % relative to the infested control (Figure 2.1A). *P. fluorescens* 3, *P. polymyxa* 3, *P. polymyxa* 2, *C. foecundissimum, L. arvalis* ZH-1, *Penicillium* YG 2, *Penicillium* YG 4, *T. virens* (spore suspension and formulation), *T. harzianum* (spore suspension), and *Trichoderma* T2 also reduced stem canker severity (29 % to 39 %). Treatments that reduced black scurf severity most effectively across all trials (54 % to 60 %) included *L. arvalis* ZH-1, *R. zeae* LRNE17E, and the chemical control (Figure 2.1 B). Other treatments that significantly reduced black scurf (38 % to 44 %) included *B.*

**Table 2.2.** Incidence and severity of stem canker as affected by biocontrol treatments for trials 2, 3, and 4.

| Treatment | Trial2 | | Trial 3 | | Trial 4 | |
|---|---|---|---|---|---|---|
| | Incidence[a] (%) | Severity[b] | Incidence (%) | Severity | Incidence (%) | Severity |
| *Bacillus subtilis* GBO3 | 33.3*[c] | 1.64* | 50.0 | 1.64* | 54.2 | 1.73* |
| *Cladorrhinum foecundissiumum* C91 | 66.7 | 2.36 | 50.0 | 1.92* | - | - |
| *Laetisaria arvalis* ZH-1 | 41.7* | 2.25 | 33.3* | 1.25* | 66.7 | 2.08 |
| *Laetisaria arvalis* LA-1 | -[d] | - | 41.7* | 1.67* | - | - |
| *Paenibacillus polymyxa* 2[+e] | 83.3 | 3.58 | 33.3* | 1.17* | 58.4 | 1.92 |
| *Paenibacillus polymyxa* 3[+] | 75.0 | 2.92 | 33.4* | 1.36* | 50.0 | 1.75* |
| *Penicillium* sp. YG 2[+] | 66.7 | 1.82* | 66.7 | 2.17 | 66.7 | 1.75* |
| *Penicillium* sp. YG 3[+] | - | - | 33.3* | 1.09* | 83.4 | 2.18 |
| *Pseudomonas fluorescens* 2[+] | 66.7 | 2.92 | 61.1 | 1.88* | - | - |
| *Pseudomonas fluorescens* 3[+] | 75.0 | 2.58 | 20.8 | 1.11* | 41.7 | 1.92 |
| *Rhizoctonia zeae* LRNE17E[+] | 33.3* | 1.10* | 16.7* | 0.83* | 66.7 | 1.92 |
| *Rhizoctonia zeae* RF27I[+] | - | - | - | - | 91.7 | 2.75 |
| *Stilbella aciculosa* 112B-1 | 37.5* | 1.82* | 58.4 | 1.55* | - | - |
| *Trichoderma harzianum* T-22 | - | - | - | - | 50.0 | 1.75* |
| *Trichoderma harzianum* T-22 (form.) | - | - | 54.2 | 1.73* | 70.8 | 2.38 |
| *Trichoderma* sp. T2[+] | - | - | - | - | 50.0 | 1.92 |
| *Trichoderma virens* GL-21 | 50.0* | 2.42 | 62.5 | 2.00 | 66.7 | 2.00 |
| *Trichoderma virens* GL-21 (form.) | 58.4* | 2.33 | 16.7* | 1.18* | 70.9 | 1.82 |
| *Verticillium biguttatum* M73 | 66.7 | 3.00 | 58.3 | 1.73* | 50.0 | 1.83 |
| infested control | 100.0 | 3.45 | 91.7 | 2.83 | 83.4 | 2.50 |
| noninfested control | 8.3* | 0.33* | 0.0* | 0.00* | 29.2* | 1.00* |
| chemical control (azoxystrobin) | 33.3* | 1.67* | 33.4* | 0.91* | 66.7 | 2.27 |
| LSD ($P$=0.05) | 41.6 | 1.42 | 42.7 | 0.85 | 42.6 | 0.74 |

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by cankers; and 5=stem completely nipped off or death of the plant).
[c]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P$ = 0.05).
[d]Treatment was not tested in this trial.
[e]Isolate names followed by a plus (+) sign are soil isolates isolated from a potato field in Maine.

**Table 2.3.** Black scurf incidence and severity for trials 2, 3, and 4.

| Treatment | Trial2 | | Trial 3 | | Trial 4 | |
|---|---|---|---|---|---|---|
| | Incidence (%) | Severity[a] | Incidence (%) | Severity | Incidence (%) | Severity |
| *Bacillus subtilis* GBO3 | 50.0 | 0.58*[b] | 79.2 | 1.91 | 54.2* | 1.18 |
| *Cladorrhinum foecundissiumum* C91 | 66.7 | 0.83* | 100.0 | 2.33 | - | - |
| *Laetisaria arvalis* ZH-1 | 50.0 | 0.58* | 75.0 | 1.00* | 75.0 | 0.83* |
| *Laetisaria arvalis* LA-1 | -[c] | - | 83.3 | 1.50* | - | - |
| *Paenibacillus polymyxa* 2[+d] | 75.0 | 0.75* | 91.7 | 1.50* | 75.0 | 1.67 |
| *Paenibacillus polymyxa* 3[+] | 66.7 | 0.92* | 100.0 | 1.55* | 91.7 | 1.42 |
| *Penicillium* sp. YG 2[+] | 58.3 | 0.67* | 100.0 | 2.42 | 50.0* | 0.67* |
| *Penicillium* sp. YG 3[+] | - | - | 100.0 | 2.27 | 66.7 | 1.45 |
| *Pseudomonas fluorescens* 2[+] | 75.0 | 0.92* | 88.9 | 2.00 | - | - |
| *Pseudomonas fluorescens* 3[+] | 66.7 | 0.75* | 100.0 | 1.44* | 58.4 | 1.25 |
| *Rhizoctonia zeae* LRNE17E[+] | 75.0 | 0.75* | 91.7 | 1.25* | 33.3* | 0.50* |
| *Rhizoctonia zeae* RF27I[+] | - | - | - | - | 100.0 | 1.75 |
| *Stilbella aciculosa* 112B-1 | 75.0 | 1.08* | 100.0 | 2.27 | - | - |
| *Trichoderma harzianum* T-22 | - | - | - | - | 91.7 | 1.42 |
| *Trichoderma harzianum* T-22 (form.) | - | - | 100.0 | 1.73* | 79.2 | 1.38 |
| *Trichoderma* sp. T2[+] | - | - | - | - | 100.0 | 2.08 |
| *Trichoderma virens* GL-21 | 75.0 | 1.08* | 87.5 | 2.09 | 66.7 | 0.92* |
| *Trichoderma virens* GL-21 (form.) | 33.3* | 0.33* | 100.0 | 1.91 | 70.9 | 1.55 |
| *Verticillium biguttatum* M73 | 58.3 | 0.75* | 87.5 | 1.82 | 75.0 | 1.42 |
| infested control | 91.7 | 1.75 | 100.0 | 2.42 | 100.0 | 1.83 |
| noninfested control | 16.7* | 0.17* | 0.0* | 0.00* | 8.3* | 0.09* |
| chemical control (azoxystrobin) | 33.3* | 0.42* | 75.0* | 1.00* | 83.4 | 1.36 |
| LSD ($P$=0.05) | 42.3 | 0.56 | 23.5 | 0.67 | 42.4 | 0.76 |

[a]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1 % of the skin-covered tuber surface; 2 = 2 % to 5 % covered; 3 = 5 % to 10 % covered; 4 = 10 % to 15 % covered; and 5 = > 15 % tuber covered by sclerotia.

[b]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P$ = 0.05).

[c]Treatment was not tested in this trial.

[d] Isolate names followed by a plus (+) sign are soil isolates isolated from a potato field in Maine.

*subtilis, P. fluorescens* 3, *Penicillium* YG 2, *V. biguttatum*, and *T. virens* (spore suspension and formulation).

Several treatments showed some control of both aspects of Rhizoctonia disease when reductions in stem canker severity and black scurf severity were averaged across all trials. The most notable treatment was *R. zeae* LRNE17E, which reduced stem canker 49 % and black scurf 58 %. Other treatments that controlled both stem canker and black scurf included *B. subtilis*, *P. fluorescens* 3, *L. arvalis* ZH-1, *Penicillium* YG 2, *R. zeae* LRNE17E, *T. virens* (spore suspension and formulation) and the chemical control (Figure 2.1).

The size of potato shoots also varied among treatments. Several treatments reduced plant size when compared to the noninfested control (Figure 2.2). As expected, the infested control was one of these treatments. However, *P. polymyxa* 2, *P. fluorescens* 2, *C. foecundissimum*, *V. biguttatum*, and *T. harzianum* (spore suspension) treatments also reduced shoot size. Some treatments tested in trial 1 also reduced shoot size. These included *P. fluorescens* 1, *Penicillium* YG 1, *Trichoderma* TN2RA, and *Trichoderma* TW. Whether these treatments reduced shoot size due to delayed emergence, deleterious effects on the plants themselves, or because the treatments did not effectively suppress *R. solani* could not be determined from these tests. However, none of the treatments reduced plant size relative to the infested control.

The addition of sterile wheat or *Penicillium* YG 2 to noninfested soil did not affect disease levels or plant size compared to the noninfested control (Table 2.4). Although not significant, the *Penicillium* YG 2 treatment tended to have slightly higher incidence and severity of both stem canker and black scurf than the other noninfested

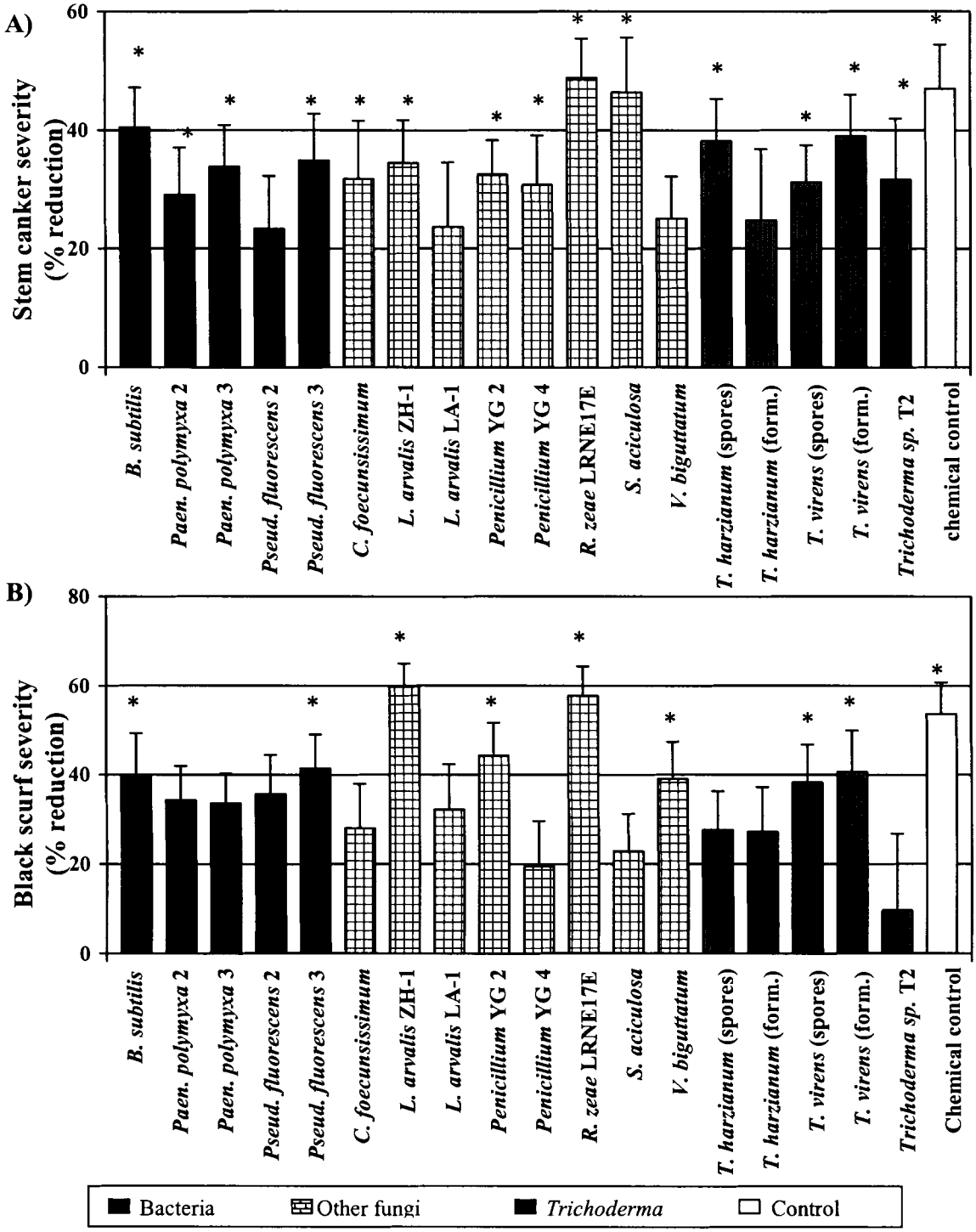



**Figure 2.1.** Reductions in A) stem canker severity and B) black scurf severity by select biocontrol treatments and a chemical control relative to the infested control. *Pseud.* = *Pseudomonas* and *Paen.* = *Paenibacillus*. *Indicates a significant reduction relative to the infested control. LSD = 28.5 (stem canker) and 36.8 (black scurf). Disease severity in the infested control averaged 2.86 and 2.13 (0 to 5 scale) for stem canker and black scurf, respectively.

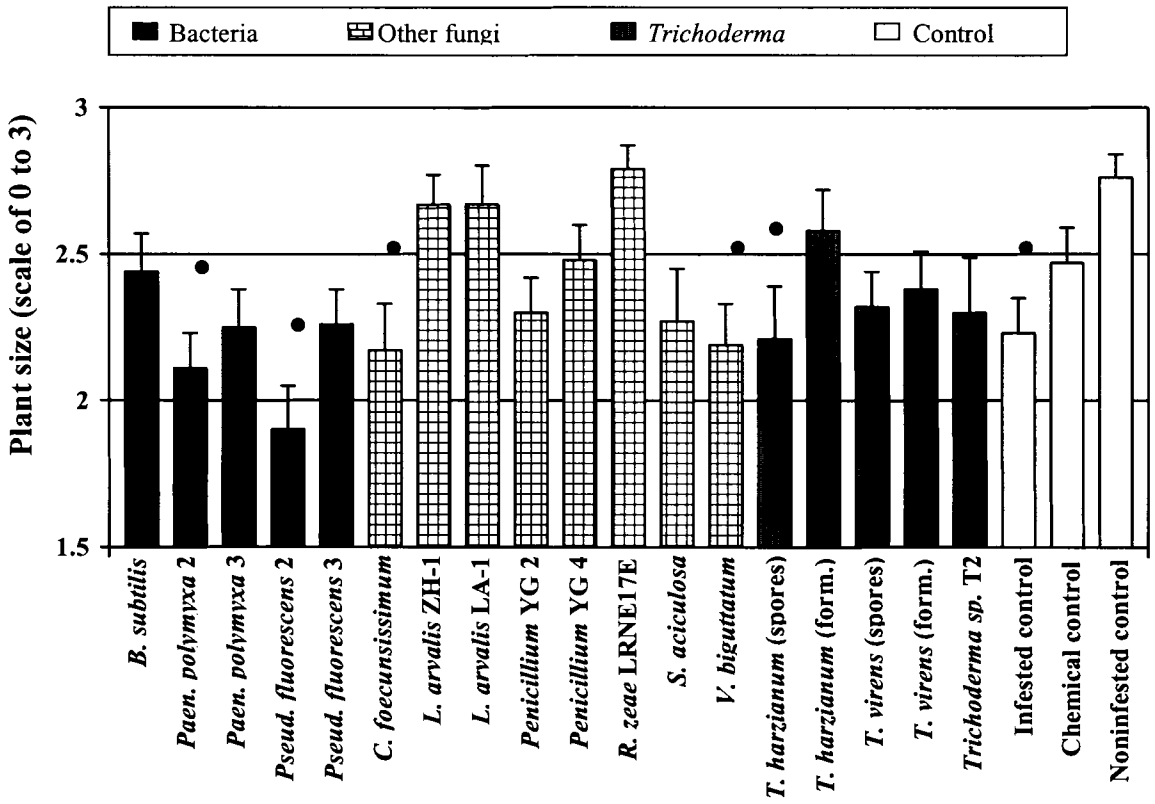



**Figure 2.2.** Shoot size as affected by select biocontrol treatments. Pseud = Pseudomonas and Paen. = Paenibacillus. LSD = 0.53. • Significantly reduced plant size relative to the noninfested control.

**Table 2.4.** Comparison of noninfested control, wheat control, and *Penicillium* sp. control for stem canker, black scurf, and shoot size.

| Treatment | Stem canker | | Black scurf | | Shoot size[d] |
|---|---|---|---|---|---|
| | Incidence[a] (%) | Severity[b] | Incidence (%) | Severity[c] | |
| noninfested control | 10.0 | 0.40 | 8.3 | 0.08 | 2.76 |
| wheat control | 15.6 | 0.63 | 5.6 | 0.06 | 2.79 |
| *Penicillium* YG 2 control | 16.7 | 0.74 | 16.7 | 0.26 | 2.70 |
| LSD ($P$=0.05) | 36.1 | 0.84 | 31.0 | 0.82 | 0.53 |

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1 % of the skin-covered tuber surface; 2 = 2 % to 5 % covered; 3 = 5 % to 10 % covered; 4 = 10 % to 15 % covered; and 5 = > 15 % tuber covered by sclerotia.
[d]Plant size was rated on a scale of 0 to 3 as follows: 0 = no growth; 1 = shoot height < 2 cm; 2 = shoot height of 2 to 5 cm; and 3 = shoot height > 5 cm.

controls. Perhaps the presence of this fungus influences populations or activity of native *Rhizoctonia* in the soil in some way.

**Comparisons of biocontrol formulations and rates**

Two different formulations of *L. arvalis* LA-1 were evaluated in one trial. The formulations, a blended culture of the fungus on PDA and the fungus grown on cracked wheat, were similar, with neither formulation reducing stem canker or black scurf. Reductions in stem canker severity were 0.1 % and –2.8 % for the cracked wheat and PDA formulation, respectively. Reductions in black scurf severity were 9.0 % for the cracked wheat and 18.3 % for the PDA formulation.

The initial 3 g per pot rate of *L. arvalis* ZH-1 was compared with a reduced rate of 1.5 g per pot in one trial. The reduced rate treatment resulted in higher incidence (66.7 %) and less reduction in severity (22.2 %) of stem canker than the initial rate (41.7 %

incidence and 34.3 % reduction in severity). The initial rate treatment significantly lowered the incidence of stem canker, whereas the reduced rate did not. Both treatments performed similarly for control of black scurf. Incidence was 50 % for the initial rate treatment and 58 % for the reduced rate treatment. The reduction in black scurf severity was 66.8 % for both treatments.

Different formulations, as a spore suspension and the commercial product, of *T. virens* GL-21 and *T. harzianum* T-22 were also evaluated. When averaged over all trials, the spore suspension treatment of *T. harzianum* T-22 reduced stem canker, whereas the commercial product treatment did not (Figure 2.1A). Neither treatment reduced black scurf (Figure 2.1B). However, the commercial product treatment did reduce stem canker severity (Table 2.1 and Table 2.2) and black scurf severity (Table 2.3) in 1 of 2 trials, and incidence and severity of black scurf were also reduced in one of two tests for the spore suspension. When reductions were averaged across all trials for both formulations of *T. virens* GL-21, both the spore suspension and commercial product significantly reduced both stem canker (Figure 2.1A) and black scurf (Figure 2.1B). In individual trials, the commercial product reduced stem canker severity in two of three trials and incidence in one trial, whereas the spore suspension treatment reduced incidence of stem canker in only one of three trials (Table 2.2). However, the spore suspension treatment reduced black scurf severity in two of three trials and the commercial product treatment reduced incidence and severity in one of three trials (Table 2.3).

In one trial, different rates of the spore suspension and commercial product of *T. virens* GL-21 were compared. All treatments reduced the incidence of stem canker relative to the infested control and had similar incidence values (Table 2.5). For stem

canker severity, neither the commercial formulation nor spore suspension reduced severity. Only the commercial formulation treatment at the higher rate reduced incidence of black scurf. However, all treatments, with the exception of the spore suspension at the lowest rate, reduced black scurf severity. The higher rate of the commercial formulation and the $10^7$ rate of the spore suspension were most effective in reducing black scurf.

**Biocontrol combinations**

Some combinations of biocontrol organisms effectively reduced Rhizoctonia disease in one trial. The combination of *B. subtilis* GBO3 / *T. virens* GL-21 was the only mixed treatment to reduce the incidence of stem canker (Table 2.6). This combination and the *L. arvalis* ZH-1 / *T. virens* GL-21 combination reduced stem canker severity. *B. subtilis* GBO3 alone reduced stem canker severity (30.9 %), but the *B. subtilis* GBO3 / *T. virens* GL-21 combination was slightly more effective (46.7 %). The combined treatments of *Penicillium* YG 2 / *R. zeae* LRNE17E, *Penicillium* YG 2 / *L. arvalis* ZH-1, and *L. arvalis* ZH-1 / *T. virens* GL-21 all reduced incidence of black scurf. As individual treatments, *Penicillium* YG 2 and *R. zeae* LRNE17E also had lower incidence of black scurf. *R. zeae* LRNE17E was more effective alone (33.3 %) at reducing black scurf incidence than in combination with *Penicillium* YG 2. All combinations that reduced black scurf incidence also reduced black scurf severity. The *L. arvalis* ZH-1 / *V. biguttatum* M73 combination also reduced black scurf severity. *Penicillium* YG 2 (63.8 %), *R. zeae* LRNE17E (72.8 %), and *L. arvalis* ZH-1 (54.9 %) also reduced black scurf severity alone. The *Penicillium* YG 2 / *L. arvalis* ZH-1 combination tended to be most effective at reducing black scurf, and was slightly more effective than either organism alone. However, only one combination, *L. arvalis* ZH-1 / *T. virens* GL-21 controlled both stem canker and

**Table 2.5.** Comparison of *Trichoderma virens* GL-21 rates and formulations on stem canker and black scurf of potato.

| Treatment | Stem canker | | | Black scurf | | |
|---|---|---|---|---|---|---|
| | Incidence[a] (%) | Severity[b] | Reduction[c] (%) | Incidence (%) | Severity[d] | Reduction[e] (%) |
| Spore suspension (conidia / pot) | | | | | | |
| *T. virens GL-21* ($3 \times 10^8$) | 50.0*[f] | 2.42 | 29.6 | 75.0 | 1.08* | 38.3* |
| *T. virens GL-21* ($3 \times 10^7$) | 54.2* | 2.09 | 39.0 | 50.0 | 0.67* | 62.0* |
| *T. virens GL-21* ($3 \times 10^6$) | 58.4* | 2.92 | 12.4 | 75.0 | 1.25 | 28.8 |
| Commercial formulation | | | | | | |
| *T. virens* GL-21 (2.6 g / pot) | 58.4* | 2.33 | 31.8 | 33.3* | 0.33* | 81.0* |
| *T. virens* GL-21 (1.3 g / pot) | 54.2* | 2.64 | 23.1 | 66.7 | 0.83* | 52.5* |
| infested control | 100.0 | 3.45 | 0.0 | 91.7 | 1.75 | 0.3 |
| noninfested control | 8.3* | 0.33* | 90.3* | 16.7* | 0.17* | 90.5* |
| chemical control | 33.3* | 1.67* | 51.3* | 33.3* | 0.42* | 76.3* |
| LSD ($P$=0.05) | 41.6 | 1.42 | 46.8 | 42.3 | 0.56 | 32.1 |

[a]Incidence of plants with obvious lesions (severity rating of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Percent reduction in stem canker severity relative to the infested control.
[d]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1 % of the skin-covered tuber surface; 2 = 2 % to 5 % covered; 3 = 5 % to 10% covered; 4 = 10 % to 15 % covered; and 5 = > 15 % tuber covered by sclerotia.
[e]Percent reduction in black scurf severity relative to the infested control.
[f]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P$ = 0.05).

**Table 2.6.** Efficacy of biocontrol combinations against Rhizoctonia disease of potato.

| Treatments | Stem canker | | | Black scurf | | |
|---|---|---|---|---|---|---|
| | Incidence[a] (%) | Severity[b] | Reduction[c] (%) | Incidence (%) | Severity[d] | Reduction[e] (%) |
| *L. arvalis* ZH-1 / *B. subtilis* GBO3 | 83.4 | 2.50 | 0.0 | 66.7 | 1.17 | 36.7 |
| *Penicillium* YG2 / *R. zeae* LRNE17E | 75.0 | 2.17 | 13.3 | 41.7* | 0.50* | 72.9* |
| *Penicillium* YG2 / *L. arvalis* ZH-1 | 87.5 | 2.45 | 1.8 | 25.0* | 0.27* | 85.3* |
| *B. subtilis* GBO3 / *T. virens* GL-21 | 33.3*[f] | 1.33* | 46.7* | 75.0 | 1.17 | 36.6 |
| *R. zeae* LRNE17E / *T. virens* GL-21 | 91.7 | 2.45 | 1.8 | 100.0 | 2.00 | -8.6 |
| *B. subtilis* GBO3 / *V. biguttatum* M73 | 58.3 | 2.08 | 16.7 | 75.0 | 1.17 | 36.7 |
| *L. arvalis* ZH-1 / *T. virens* GL-21 | 58.3 | 1.58* | 36.7* | 53.4* | 1.00* | 45.7* |
| *L. arvalis* ZH-1 / *V. biguttatum* M73 | 83.4 | 2.58 | -3.3 | 58.3 | 0.67* | 63.9* |
| infested control | 83.4 | 2.50 | 0.0 | 100.0 | 1.83 | 0.0 |
| noninfested control | 29.2* | 1.00* | 60.0* | 8.3* | 0.09* | 95.1* |
| chemical control | 66.7 | 2.27 | 9.1 | 83.4 | 1.36 | 25.9 |
| LSD ($P$=0.05) | 42.6 | 0.74 | 29.5 | 42.4 | 0.76 | 41.1 |

[a]Incidence of plants with obvious lesions (severity of 2 or higher).
[b]Severity ratings for stem canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by cankers; and 5 = stem completely nipped off or death of the plant).
[c]Percent reduction in stem canker severity relative to the infested control.
[d]Severity ratings were as follows: 0 = no visible sclerotia; 1 = sclerotia covering 1 % of the skin-covered tuber surface; 2 = 2 % to 5 % covered; 3 = 5 % to 10 % covered; 4 = 10 % to 15 % covered; and 5 = > 15 % tuber covered by sclerotia.
[e]Percent reduction in black scurf severity relative to the infested control.
[f]Values followed by an asterisk are significantly different from the infested control according to Fisher's LSD ($P$ = 0.05).

black scurf in this test. Thus, little improvement in controlling both aspects of Rhizoctonia disease was observed with the combinations in this test.

## Discussion

Biological control of *R. solani* on various crops has much potential for disease management, though there are several problems with practical implementation (Cook and Baker, 1983; Velvis and Jager, 1983; Murdoch and Leach, 1993; Lewis *et al.*, 1995a,1995b; Lewis and Larkin, 1998). Numerous organisms demonstrated biocontrol capability against *R. solani* in the present study, but also much variability in control from one trial to the next. Efficacy of organisms against stem canker or black scurf, two important components of Rhizoctonia disease that reduce tuber yield and quality, was not consistent among trials. Although most organisms tested significantly reduced stem canker or black scurf in at least one greenhouse trial, no treatment reduced both incidence and severity of either stem canker or black scurf in all trials. Treatments also varied in their abilities to suppress both disease components. The most consistently effective treatments for control of stem canker were not necessarily the most effective for control of black scurf. Over all trials, *B. subtilis* GB03, *R. zeae* LRNE17E, *S. aciculosa* 112-B, and the chemical control were most effective at reducing stem canker severity (40 to 49 %) and *L. arvalis* ZH-1, *R. zeae* LRNE 17E, and the chemical control were most effective at reducing black scurf severity (54 to 60 %). Some biocontrol treatments reduced disease relative to the infested control as well as, or better than, the chemical control.

*B. subtilis* GB03 was among the most consistent and effective biocontrol treatments for control of stem canker. The commercial product of this isolate also

reduced incidence and severity of stem canker (Larkin, 2001; Larkin 2002a) and scurf severity (Larkin, 2002a) on potato caused by *R. solani* in field studies. In the present study, results were also consistent with previous studies on *L. arvalis*, which can reduce population levels of *R. solani* (Allen *et al.*, 1985; Larsen *et al.*, 1985; Murdoch and Leach, 1993), as well as stem canker, stolon canker, and black scurf of potato (Murdoch and Leach, 1993).

*V. bigutattum* has been studied extensively for suppression of *R. solani* on potato (Velvis and Jager, 1983; Jager and Velvis, 1984, 1985; Jager *et al.* 1991, Wicks *et al.*, 1995) and has been previously reported to suppress *R. solani* from both tuberborne (Velvis and Jager, 1983) and soilborne (Jager and Velvis, 1984) inoculum sources. Although most success with *V. biguttatum* has been with control of black scurf, it has also successfully reduced stem canker (Jager and Velvis, 1984). Previous studies demonstrated that *V. biguttatum* was more effective against *R.solani* than the biocontrol organisms *Gliocladium* and *Trichoderma* (Velvis and Jager, 1983). In the present study, treatment with *V. biguttatum* did not reduce stem canker. It was somewhat effective at reducing black scurf, although reductions were less than with some other more effective organisms. The efficacy of *V. biguttatum* varies based on the type of soil used with it being least effective in sandy soils due to high population levels of *R. solani* (Jager and Velvis, 1985). The soil used in the present study was a sandy soil, which may account for the low success of *V. biguttatum* demonstrated.

*S. aciculosa* reduced damping off caused by *R. solani* on cotton, sugarbeet, and radish (Lewis and Papavizas, 1993). Nielsen and Sorensen (1997) determined that strains of *Paenibacillus polymyxa* inhibited *R. solani* in vitro and produced cell wall-degrading

enzymes. *S. aciculosa* and three of five strains of *P. polymyxa* tested in the present study reduced stem canker. This is the first report to suggest that *S. aciculosa* and *P. polymyxa* can reduce stem canker on potato caused by *R. solani*.

The present study also details the first account of *R. zeae* and a potato root associated *Penicillium* sp. as potential biocontrols of *R. solani*. *R. zeae* is a known pathogen of turfgrasses (Martin and Lucas, 1983; Burpee and Martin, 1992). However, isolates tested in the present study did not cause disease on barley or ryegrass (Appendix C). Some isolates of *R. zeae*, including those tested in the present study, have produced stem lesions on potato plants, although to a much lesser degree than *R. solani* (Carling and Leiner, 1990; Appendix C). Surprisingly, *R. zeae* LRNE17E was the most effective treatment for reducing both stem canker and black scurf relative to the infested control. The mechanism of action was not determined, but perhaps it is competition. *R. zeae* may colonize roots more effectively and out compete *R. solani*, and as a weak pathogen it may prevent *R. solani* from causing more severe disease. *R. zeae* demonstrates potential as a biocontrol organism but additional isolates need to be screened to find an isolate that is an effective antagonist, but does not cause damage or disease to important crops.

*Penicillium* sp. are frequently found when soil cropped to potato or potato roots are plated on culture media (Larkin, 2003a). Isolates of this organism demonstrated some control of both stem canker and black scurf. This fungus may serve a role in protection of the roots and newly formed tubers from root pathogens such as *R. solani*. It did not, however, demonstrate in vitro antagonism towards *R. solani* (Appendix D).

A treatment that suppresses both stem canker and black scurf would be best as a biocontrol strategy. Several treatments demonstrated potential to suppress both aspects in

the present study. Also, combinations of organisms that provide enhanced control of both disease symptoms would be beneficial. Some combinations provided better control of stem canker, but none suppressed black scurf better than individual biocontrol organisms.

The formation of black scurf on the seed pieces was assessed in the present study after differences in severity were detected among the various treatments upon sprout harvest. Minimizing tuberborne inoculum is crucial in suppression of Rhizoctonia disease (Frank and Leach, 1980). Although the reduction of black scurf on daughter tubers would be a major goal of disease control, the assessments of the formation of black scurf on the seed pieces was useful, because it demonstrated whether or not potential antagonists were able to inhibit sclerotia formation. However, control of black scurf on the seed tuber may or may not accurately predict ability to reduce scurf on progeny tubers, because some organisms may not persist long enough to provide protection of developing tubers. Nevertheless, this method provides a relatively simple, effective way to assess black scurf formation on tubers while screening biocontrol or chemical treatments for suppression of stem canker on potato shoots.

Different formulations and rates of *L. arvalis, T. virens*, and *T. harzianum* were also compared for efficacy. There were no significant differences between rates or formulations of *T. virens* across all trials for black scurf. However, as expected, the formulation tended to be more consistent within the different trials for both stem canker and black scurf. Both the initial rate (3 g per pot) and reduced rate (1.5 g per pot) *of L. arvalis* ZH-1 reduced black scurf, but only the initial rate reduced stem canker. Although the spore suspension of *T. harzianum* reduced stem canker, surprisingly the commercial

product was not very effective in the present study. In field studies with the same type of soil as the soil used in the present experiments, the commercial product did not control black scurf or stem canker (Larkin, 2001; Larkin, 2002a). The commercial formulation may not have been successful because of the specific soil type used in these experiments or perhaps it is not the best formulation for use with potato.

Although none of the biocontrol treatments provided control for stem canker and black scurf over all trials, many showed potential as effective antagonists against Rhizoctonia disease of potato. Additional studies on the mechanism(s) of action of newly discovered antagonists are necessary to fully understand the potential beneficial role they may serve in a sustainable potato cropping system. Variability seen with biocontrol may be best addressed by using combinations of successful antagonists or by applying beneficial organisms within effective crop rotations. These integrated management practices may potentially increase the consistency and efficacy of biocontrol.

## Chapter 3

# EFFECTS OF A RYEGRASS ROTATION AND BIOLOGICAL CONTROL ON RHIZOCTONIA DISEASE OF POTATO AND SOIL MICROBIAL COMMUNITIES


### Chapter Abstract

*Rhizoctonia solani* is an important pathogen of potato capable of reducing tuber yield and quality. Integrated, sustainable methods including biocontrol and effective crop rotations are necessary for management of this pathogen. Greenhouse trials were conducted to evaluate the effects of selected rotation crops including barley, two ryegrass varieties (common and "Lemtal"), clover, potato, and combinations of barley with ryegrass or clover, on populations of *R. solani* and Rhizoctonia disease. Potato and clover preceding potato resulted in higher disease severity of stem canker than most other rotations, whereas Lemtal ryegrass reduced stem canker severity. In addition, all ryegrass treatments resulted in substantially higher populations of *R. zeae*. Field trials evaluating selected biocontrol treatments in combination with different rotations were conducted at two locations in Maine. Potatoes were treated with *Laetisaria arvalis, Trichoderma virens*, or *Bacillus subtilis* and planted as subplots following rotation crops of barley and ryegrass, barley and clover, or potato. Treatment effects on shoot emergence, disease levels, tuber yield, soil microbial communities, and fatty acid methyl ester (FAME) profiles were determined. The barley/ryegrass rotation significantly reduced incidence and severity of stem canker and increased tuber yield at one location. Efficacy of the biocontrol treatments varied by rotation and location, with *L. arvalis* and

*T. virens* reducing black scurf severity and incidence in some rotations. *L. arvalis* and *T. virens* also increased some aspects of tuber yield at one location. General fungal and bacterial populations and fatty acid profiles demonstrated distinct differences in microbial community characteristics among rotation crops and biocontrol treatments. Significant crop by biocontrol interactions were observed indicating that biocontrol can be enhanced within beneficial rotations, leading to greater reductions of Rhizoctonia disease of potato.

## Introduction

*Rhizoctonia solani* Kühn is a persistent pathogen of potato (*Solanum tuberosum* L.) that causes stem and stolon canker. *R. solani* is also responsible for black scurf, which is the formation of sclerotia, the long-term survival structures of the pathogen, on newly formed tubers. These symptoms result in reduced tuber quality and yield. Current cultural and chemical controls are not always practical or effective and Rhizoctonia disease of potato continues to be a serious problem wherever potatoes are grown (Frank, 1981; Bandy *et al.,* 1988; Johnson and Leach, 2003).

Effective, alternative disease control options that are consistent with sustainable production practices are needed. The use of improved crop rotations and biocontrol organisms represents an integrated approach that may effectively reduce Rhizoctonia disease and contribute to agricultural sustainability and environmental quality.
The effect of crop rotations and organic amendments on Rhizoctonia disease of potato is an area that has been widely studied (van Bruggen *et al.*, 1996). Crop rotations can suppress disease by allowing inoculum levels to decline in the absence of the host, directly inhibiting the pathogen by producing a toxic compound, increasing specific

antagonists that inhibit the pathogen, or by increasing general microbial populations that compete with the pathogen (Larkin, 2004; Huber and Sumner, 1996).

Presently, barley (*Hordeum vulgare* L.) or oats (*Avena sativa* L.) underseeded with a cover crop of red clover (*Trifolium pratense* L.) in a 2-year rotation with potato is the preferred management practice on commercial farms in Maine. Barley rotations can be effective in suppressing Rhizoctonia diseases (Larkin and Honeycutt, 2002; Larkin, 2004), however, more suitable cover crops may be available. Legume rotations have been implicated in decreased yields of subsequent crops mainly as a result of increases in disease by *R. solani* (Rickerl *et al.*, 1992; Dabney *et al.*, 1996). Recent observations in the field have suggested that ryegrass (*Lolium multiflorum* Lam.), in particular the "Lemtal" variety, may more effectively suppress black scurf when used as a cover crop with barley; however, this observation has not been scientifically validated.

Another potentially sustainable method of disease suppression is the addition of microbial antagonists. Many biocontrol organisms have shown suppressive activity towards *R. solani* (Beagle-Ristaino and Papavizas, 1985; Jager and Velvis, 1984; Asaka and Shoda, 1996; Murdoch and Leach, 1993; Lewis and Papavizas, 1992), but results were not always consistent, especially when compared to chemical controls. One strategy that may enhance biocontrol would be to use these beneficial organisms within effective crop rotations (van Bruggen *et al.*, 1996).

One way that crop rotations, cover crop amendments, and biological control treatments suppress disease levels is by altering the structure of soil microbial communities (Huber and Sumner, 1996; Henis *et al.*, 1967; Davey and Papavizas, 1960). These changes may include increases in total microbial populations, population changes

within specific groups of organisms, or the changes in the functional characteristics of soil microbial communities. Soil dilution plating is one method used to monitor soil microbial communities, but it has been estimated that less than 5 % of the soil microbes are accounted for by this method (Bakken, 1985), and, in general, actively growing organisms cannot be distinguished from dormant propagules. Whole soil fatty acid methyl ester (FAME) analysis is a relatively quick and simple technique that generates fatty acid profiles used to compare and evaluate soil microbial communities (Cavigelli *et al.*, 1995). This technique can effectively show changes in community structure associated with different cropping systems (Ritchie *et al.*, 2000; Larkin, 2003a; Larkin, 2004; Zelles *et al.*, 1994) and changes in community structure associated with the addition of microbes (Siciliano and Germida, 1998). This method accounts for all microbes present in the soil and inactive propagules, such as spores, are not as influential in the community profile.

The purpose of this research was to determine if the use of a specific crop rotation, barley and ryegrass, and selected biocontrol organisms, could effectively reduce Rhizoctonia disease of potato and whether the combination of both strategies resulted in further improvement of disease control. Biocontrol organisms studied were *T. virens* GL-21, *L. arvalis* ZH-1, and *B. subtilis* GBO3. Another goal of this research was to determine the effects of these rotations and biocontrol organisms on soil microbial communities.

## Materials and Methods

### Inoculum preparation

Inoculum of *Rhizoctonia solani* RS31B, a virulent isolate of AG-3 recovered from a potato plant in Maine (Appendix A), was used for all experiments. Inoculum was prepared as previously described (Papavizas and Lewis, 1986) by growing it for two weeks on organic cracked wheat (3 ml DI water per 5 g wheat) that had been autoclaved for 60 min on two consecutive days (Papavizas and Lewis, 1986). The infested wheat was dried at room temperature for 48 hours, passed through a 2.0 mm sieve, and stored in a paper bag at 4 to 5°C until needed.

### Greenhouse rotation trials

Crop treatments tested in the greenhouse experiments included two varieties of annual ryegrass ("Lemtal" and common), barley ("Robust"), red clover ("Cinnamon"), barley and Lemtal ryegrass together, barley and red clover together, and potato. Potato variety "Shepody" was used for all experiments. Shepody is susceptible to soilborne diseases, such as Rhizoctonia disease and common scab (*Streptomyces scabiei*). Field soil from Newport, ME, an unnamed variant of a Bangor silt loam (coarse-loamy, mixed, frigid, Typic Haplorthod) was used for these experiments. The soil was sieved through a 6 x 6 mm screen and combined with sterile sand at a 3:1 w/w soil to sand ratio. Pathogen inoculum was incorporated with the soil mix at a rate of 4 g per kilogram and incubated for 24 hours at room temperature.

Two kilograms of pathogen-infested soil mix was added to each 25 cm x 17 cm x 8 cm flat and seed was added in the following amounts: annual ryegrass, 0.67 g; barley, 4 g; clover with Nitro-Fix clover/alfalfa inoculant (Trace Chemicals LLC), 0.5 g; and

potato, 4 cut seedpieces treated for 2 min in 2% formaldehyde (Bandy *et al.*, 1988) and green-sprouted for two weeks. Theses seeding rates were chosen because they resulted in dense growth of each crop. Rates used in the greenhouse studies were approximately seven times those used in commercial fields.

Crops were grown in the greenhouse for approximately one month and the treatments containing barley, ryegrass, or clover were cut to soil level and replanted to keep crop growth active. All crops were grown for approximately one more month so that the length of active crop growth was comparable to that of field crops in Maine. Crop biomass from all treatments was incorporated into the soil by hand and, approximately one month later, four potato seedpieces treated as described above were planted and grown for approximately 3 weeks.

Soils were sampled after infestation with *R. solani*, after two months of crop growth, and one month after crop incorporation to determine the effects of crop growth and incorporation on populations of *R. solani*. Six cores (8 cm deep x 2 cm diameter) were taken and combined for each potting tray. Samples were plated on five plates of semi-selective media (Flowers, 1976) amended with neomycin sulfate (700 mg/L), pyroxychlor (90 mg/L), and benomyl (2mg/L) using the soil pellet sampler method (Henis *et al.*, 1978). Plates were incubated at room temperature for 24 hours and *Rhizoctonia*-like mycelia emanating from the soil pellets were enumerated by observation at 10x to 20x magnification. The total number of *R. solani* propagules per gram of soil was calculated from the percent of pellets colonized. This number was subjected to the multiple colonization correction formula (Sneh *et al.*, 1966). Due to the high numbers of propagules for most samples and variability in propagule size, the amount of mycelia

emerging from each pellet was also evaluated on a scale of 0 to 5 (0 = no mycelia; 1 = 1 to 3 emerging mycelia; 2 = more than 3 emerging mycelia but less than 30 % of pellet surrounded; 3 = 30 % to 70 % of pellet surrounded; 4 = 75 % to 100 % of pellet surrounded; and 5 = 100 % of pellet surrounded in very dense growth). The size ratings for all pellets on a plate were averaged and this number was termed inoculum potential, which corresponded to the activity of each propagule. This measurement is relevant because smaller, less active propagules would be more subject to suppression (Henis *et al.*, 1978)

After 3 weeks of growth, total plant emergence was noted and all potato plants were harvested, washed, and rated for stem canker on a scale of 0 to 5 (0 = no disease symptoms; 1 = brown discoloration of stems; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 to 75 % coverage by cankers; 4 = > 75 % coverage by stem cankers; and 5 = stem completely nipped off or death of the plant). Differences in potato shoot size were detected, so shoots were also rated for size on a scale of 0 to 3 (0 = no growth; 1 = shoot height < 2 cm; 2 = shoot height of 2 to 5 cm; and 3 = shoot height > 3 cm).

The experimental design was a randomized complete block with 4 replications and the experiment was repeated once.

**Field set-up and design**

The field experiment was conducted over the 2001 to 2002 field seasons on USDA research plots in Newport (location 1) and Presque Isle (location 2), Maine. The Newport site is located in central Maine and the Presque Isle site is in northern Maine approximately 300 km northeast of the Newport site. Soil at the Newport site was

described earlier. Soil at the Presque Isle site is a Caribou sandy loam, a fine-loamy, mixed frigid, Typic Haplorthod. The Newport site had previously been planted to millet, ryegrass, or was fallow the previous 5 years, with potatoes grown within the past 10 years. The Presque Isle site had been in sod the previous 3 to 9 years, but also had potatoes grown within the past ten years. All procedures were conducted similarly at the two locations except that planting, harvesting, and sampling was approximately one week later for location 2 than location 1. The experimental design was a randomized complete block split-plot with four replications. Three rotation crop treatments, barley underseeded with "Lemtal" ryegrass, barley underseeded with red clover, and potato were planted in early June 2001 and followed the next spring by potato planted in early June 2002. The main plots (18 m x 3.6 m) contained the rotation crops and the subplots (4.5 m x 3.6 m) consisted of three different biocontrol treatments applied to the potato crop and a control plot with no biocontrol amendment. Prior to planting the rotation crops, *R. solani* inoculum was added to all field plots at a rate of 64 kg/ha and incorporated into the soil approximately 15 cm by disk or harrow.

Barley was planted at a rate of 135 kg/ha with 45 kg N/ha using a 10-10-10 fertilizer. The cover crops, clover and ryegrass, were planted with the barley at rates of 17 kg/ha and 23 kg/ha, respectively. Nitro-Fix clover/alfalfa inoculant (Trace Chemicals LLC, Pekin Illinois) was added to the clover seed prior to planting. The potatoes were hand planted with 35 cm spacing within rows and 0.9 m spacing between rows and fertilized at a rate of 169 kg N/ha. Imidacloprid (Provado, Bayer Crop Science, Research Triangle Park, NC) and chlorothalonil (Bravo, Syngenta Crop Protection, Greensboro, NC) were applied to potato plots at recommended rates as needed for control of Colorado

Potato Beetle and late blight (*Phytophthora infestans*), respectively. Crops were managed and cultivated using recommended production practices.

The barley was harvested in early fall and the cover crops were incorporated by chisel plow the following spring one month prior to potato planting (Van Bruggen *et al.*, 1996). Biocontrol treatments, including *Bacillus subtilis* GBO3 (active ingredient of Kodiak, Gustafson, Dallas, TX), *Trichoderma virens* GL-21 (SoilGard, Olympic Horticultural Products, Mainland, PA), or *Laetisaria arvalis* ZH-1 (ATCC #62715) were added as in-furrow amendments to the potato crop planted in 2002. No organisms were added to the control plot. Potato plots were cultivated and maintained as described for 2001. In early October 2002, six linear meters of tubers were harvested by hand from the middle two rows of each plot.

**Biocontrol treatment preparation**

*B. subtilis* inoculum was prepared by adding deionized water to plates of the isolate grown on 0.1 % tryptic soy agar (Difco Laboratories, Inc., Sparks, MD). The slurry was transferred to 4 L of trypticase soy broth (Difco Laboratories, Inc.) and incubated on a platform shaker for 3 days at 28°C. The broth was added to 6 L of deionized water and the preparation was added in-furrow at a rate of approximately $10^4$ cfus/cm$^3$ soil ($10^9$ cfus/linear meter). *T. virens* was added in-furrow as the SoilGard formulation (Olympic Horticultural Products) as directed by the manufacturer at a rate of 0.1 % (g/cm$^3$). Four plugs of *L. arvalis* cultures actively growing on potato dextrose agar (Difco Laboratories, Inc.) were added to petri dishes of sterile cracked wheat prepared as mentioned earlier and grown at 26°C for 5 days. The inoculum was dried at room

temperature (21 to 25°C) for 24 hours and added at a 0.1 % ($g/cm^3$) incorporation rate. All treatments were used in the field immediately after preparation.

**Disease and yield assessments**

Shoot emergence was determined by counting the number of plants visible in the middle two rows of each plot over several weeks in late June and early July of 2002. Stem and stolon cankers were assessed in mid-August of 2002. Two plants were harvested from the first and fourth rows of every plot and the stems and stolons of the plants were rinsed with water to remove soil so that cankers would be visible. Stems and stolons were rated on a scale of 0 to 5 (0 = no disease symptoms; 1 = brown discoloration of stems or stolons; 2 = cankers covering < 25% of the stem or stolon circumference; 3 = 25 to 75 % coverage by cankers; 4 = 75 % coverage by stem or stolon cankers; and 5 = stem or stolons completely nipped off or death of the plant). When two or more shoots or stolons emerged from one seed piece the average rating of all shoots or stolons was recorded.

After harvest, tubers were washed and graded into three categories based on size, and total and marketable yield were determined. The categories consisted of small (< 4.7 cm), medium (4.7-5.6 cm), and large (> 5.6 cm) tuber sizes. Marketable yield included the medium and large size classes. In addition, the percentage of misshapen tubers (by weight) was determined. Thirty tubers from the marketable yield category in each plot were arbitrarily chosen and rated for black scurf and common scab, if present, based on the percent of each tuber covered by sclerotia or lesions.

**Soil microbial populations**

Soil samples were taken prior to and after the addition of *R. solani* inoculum in May 2001, in late-June 2001 after emergence of the crops, in mid-August 2001, and in mid-October 2001 after all crops had been harvested. In 2002, soil samples were taken in May prior incorporation of the cover crops, in June immediately prior to planting, in mid-August, and in mid-October immediately after harvest. In 2001 and the two samplings prior to planting in 2002, 10 cores (15 cm deep by 2 cm diameter) were taken from each plot and combined. The remaining samples from 2002 contained 8 cores from each plot with 3 cores each from the center two rows of potatoes and two cores from between the rows. Samples were sieved (3.35 mm mesh) and kept at 5°C until processed.

Inoculum levels of *R. solani* were determined for all soil samples taken in 2001 and the two samples taken prior to planting in 2002 by the soil pellet sampler method (Henis *et al.*, 1978) as described previously. *Rhizoctonia*-like mycelia were transferred to PDA for visual identification. Populations of culturable bacteria and fungi were determined for soil samples taken in mid-August 2002 by soil dilution plating to observe effects of the previous rotation crop and biocontrol organisms on the soil microflora. Dilutions were plated on 0.1 % tryptic soy agar (Difco Laboratories, Inc.) for the enumeration of general bacteria and amended potato dextrose agar (Difco Laboratories, Inc) modified with tergitol (1 ml/L) and chlortetracycline (50 mg/L) to determine general fungal populations (Larkin, 1993). Three 10 g subsamples were taken from each composite soil sample per plot and used for soil dilution population estimates. Bacterial plates were incubated for 3 days at 28°C and fungal plates were incubated for 7 days at room temperature. Populations of *Trichoderma* sp. and a dominant *Penicillium* sp.

associated with potato roots in Maine (Larkin, 2003a) were identified by colony morphology and were also enumerated on amended potato dextrose agar used for general fungal counts.

Whole soil fatty acid methyl ester (MIDI-FAME) analysis was used to evaluate changes and differences in soil microbial communities. This analysis was performed on the initial soil samples of 2001 to obtain base level fatty acid data. The two sets of samples taken prior to planting in 2002 were analyzed to observe changes in the microbial communities prior to and after incorporation of crop residues. The mid-August soil samples from 2002 were also assayed to determine effects of both previous rotation crops and biocontrol organisms on soil microflora. Depending on soil moisture levels, either 4 or 4.5 g of soil was used to approximate 3 g soil dry weight. Fatty acids were extracted from three soil subsamples per plot according to a modification of the Microbial Identification System (MIS, MIDI, Inc, Newark, DE) as previously described by Larkin (2003a). Fatty acid composition was determined by gas chromatography using an automated procedure by MIDI on an HP 6890 gas chromatograph (Hewlett-Packard, Wilmington, DE). Fatty acids were identified by software developed for the MIS. Only fatty acids that accounted for at least 0.25 % of the total fatty acid content were used for analysis (Larkin, 2003a). In addition, dicarboxylic acids and those with a chain length greater than 20 carbons were not included in the analyses. With these criteria, analyses consisted of 43-45 unique fatty acids. Fatty acids were also categorized by structural classes, including saturated straight chain, monounsaturated, polyunsaturated, branched, and hydroxy fatty acid classes. These classes and select individual fatty acids were used as indicators (biomarkers) for particular microorganism groups (Larkin, 2003a).

## Statistical analysis

All significant treatment effects were detected using analysis of variance and means were separated using Fisher's least significant difference at $P = 0.05$. Fatty acid data was analyzed by principal components analysis (PCA). Principal components were subjected to multivariate analysis of variance and one-way ANOVA was run on individual principal components and key fatty acid groups. SAS (ver. 7, SAS Institute, Cary, NC) general linear models procedures were used to carry out statistical analyses.

# Results

## Greenhouse rotation trials

The effects of different rotation crops on soil populations of *R. solani* and Rhizoctonia disease of potato were examined under controlled conditions in two greenhouse experiments. Initial populations of *R. solani*, after infestation, were approximately 250 propagules per 10 g soil in both experiments. Populations were substantially lower after two months of crop growth and even lower after crop residue incorporation in both experiments (Fig. 3.1). In experiment 1, the potato rotation resulted in higher populations of *R. solani* than all other rotations prior to residue incorporation. After residue incorporation, populations were higher in both clover and potato than all other rotations. In experiment 2, there were no differences among crops at either sampling time.

In addition to *R. solani*, *R. zeae* was also detected in soil from most rotations. In both experiments, populations of *R. zeae* were much greater after residue incorporation in rotations containing ryegrass (barley/ryegrass, Lemtal ryegrass, and common ryegrass)

than the other rotations (amounts ranging from ~ 4 to 100 times greater) (Fig. 3.2). Although no data is available for populations of *R. zeae* prior to residue incorporation in experiment 1 since this fungus was not yet detected, *R. zeae* was most likely present in low numbers. In experiment 2, although there were some differences in population levels of *R. zeae* among rotations prior to residue incorporation, differences were much more pronounced after incorporation (Fig. 3.2B).

Stem canker severity of potato shoots also varied based on the previous rotation crop. The clover and potato rotations had higher disease levels than barley, barley/clover, and Lemtal ryegrass rotations in experiment 1 (Fig. 3.3A) and higher disease levels than the barley/ryegrass, Lemtal ryegrass and common ryegrass rotations in experiment 2 (Fig. 3.3B). When averaged over both experiments, the potato and clover rotations resulted in higher disease severity (1.47 for potato and 1.52 for clover) than all other rotations (0.90 to 1.10). There were no differences in incidence of discolored stems in experiment 1 (Table 3.1). However, the potato rotation had a higher incidence than the Lemtal ryegrass rotation in experiment 2. The clover rotation had a higher incidence of stem canker than the barley rotation in experiment 1. There were no differences in incidence of stem canker in experiment 2.

There were significant differences in potato shoot size as determined by preceding rotation crop in both experiments. In experiment 1, barley/clover, both ryegrass treatments, and barley/ryegrass rotations yielded larger shoots than the clover rotation (Table 3.1). Barley/clover and both ryegrass rotations also yielded larger shoots than the potato rotation. In experiment 2, the barley/clover, barley/ryegrass, Lemtal ryegrass, and potato rotations all yielded larger shoots than the clover rotation.

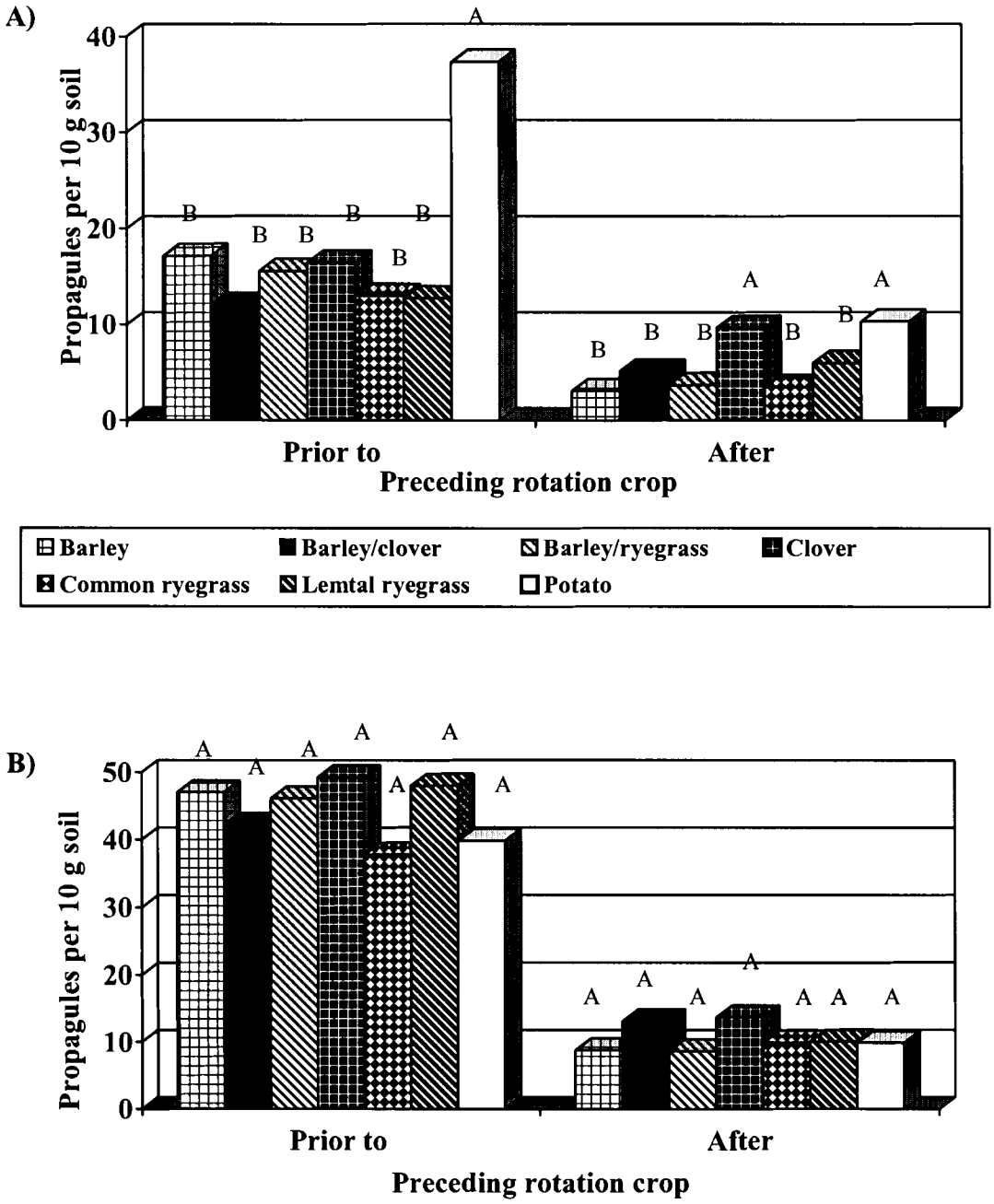



**Figure 3.1.** Populations of *R. solani* prior to and after incorporation of rotation crop residues in greenhouse experiments A) 1 and B) 2.

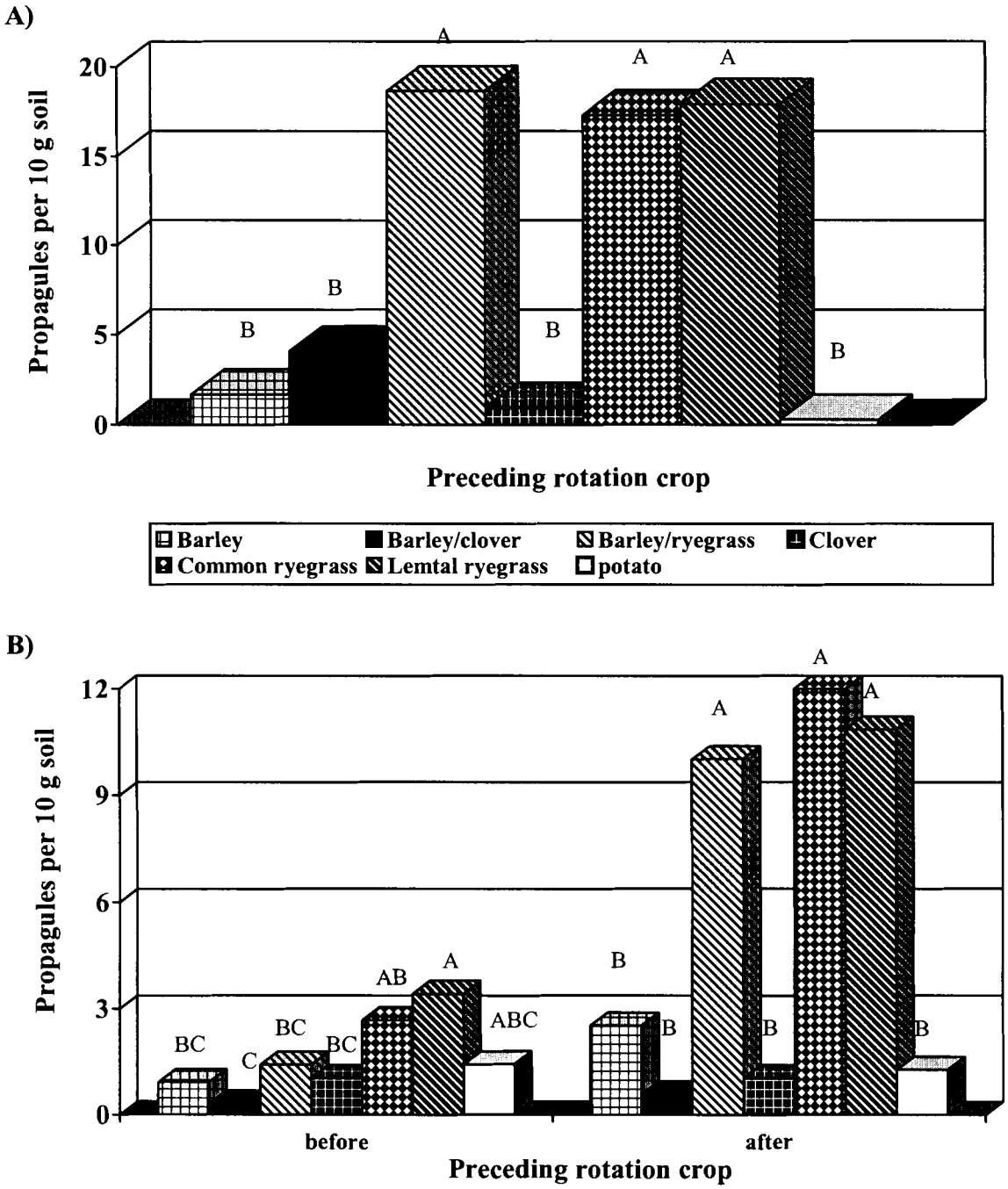
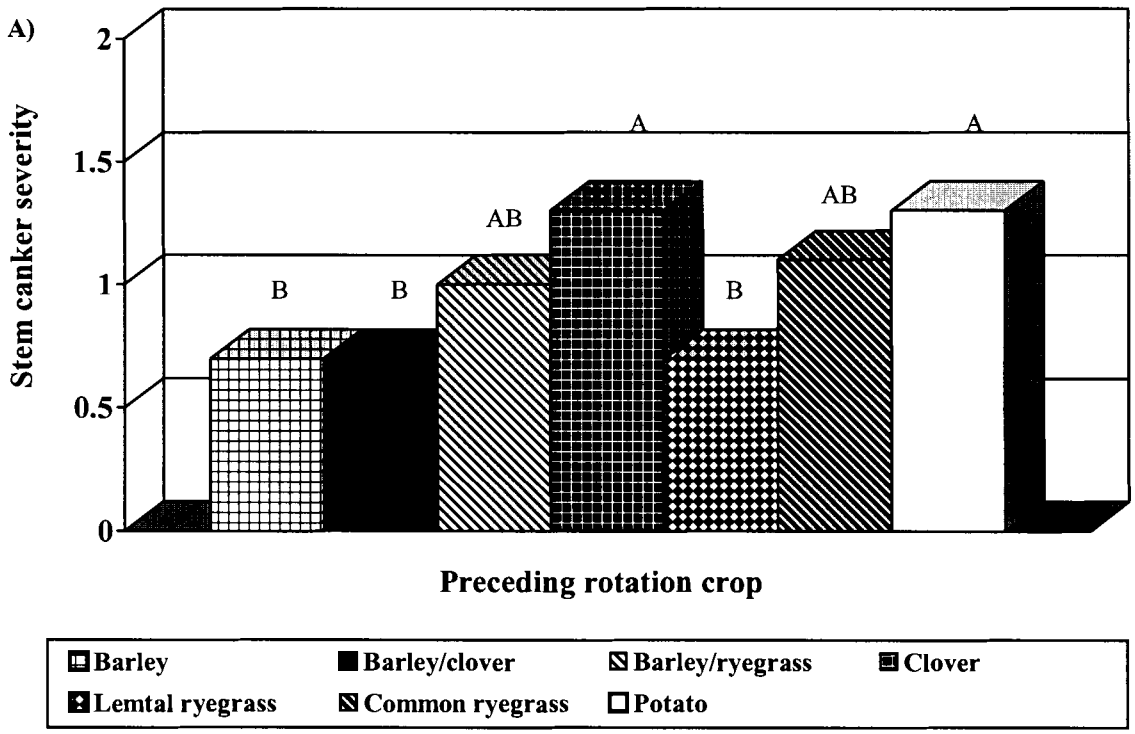



**Figure 3.2.** Populations of *R. zeae* in A) experiment 1 after incorporation of rotation crop residues and B) experiment 2 both prior to and after incorporation of residues.



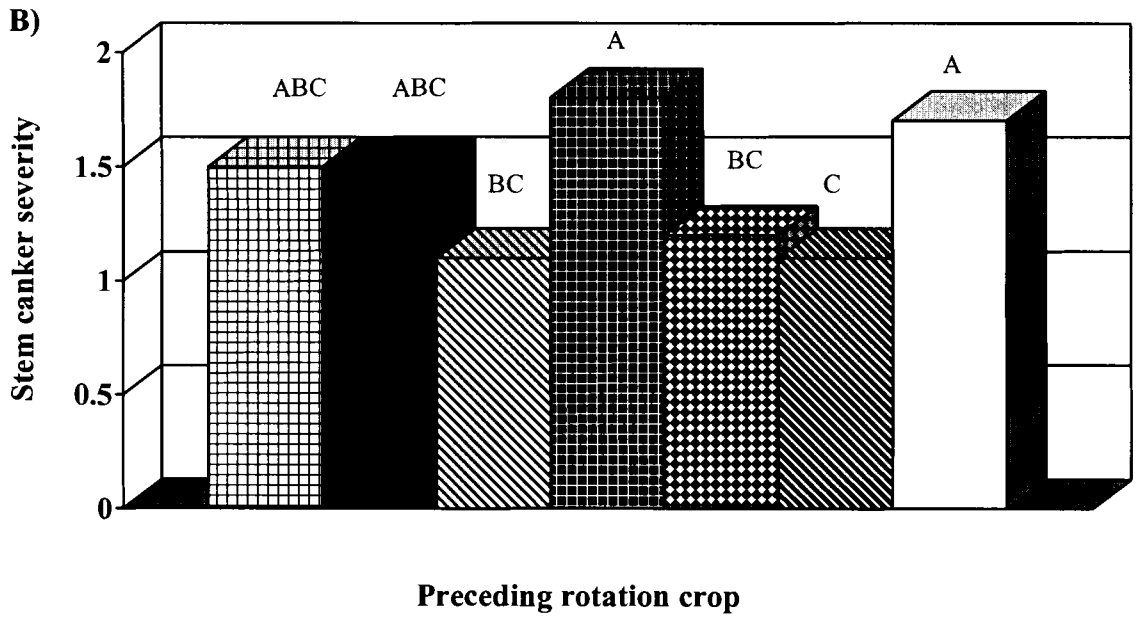




**Figure 3.3.** Stem canker severity ratings of potato shoots based on preceding rotation crop for greenhouse experiments A) 1 and B) 2.

**Table 3.1.** Effects of preceding rotation crops on stem canker and size of potato shoots in greenhouse experiments.

| | Discolored stem incidence[a] (%) | Stem canker incidence[b] (%) | Shoot size[c] |
|---|---|---|---|
| **Experiment 1[d]** | | | |
| Barley | 68.8 a | 0.0 b | 2.31 abc |
| Barley/clover | 62.5 a | 6.3 ab | 2.56 a |
| Barley/ryegrass | 87.5 a | 12.5 ab | 2.37 ab |
| Clover | 68.8 a | 37.5 a | 1.93 c |
| Common ryegrass | 56.3 a | 12.5 ab | 2.44 a |
| Lemtal ryegrass | 87.5 a | 18.8 ab | 2.44 a |
| potato | 93.8 a | 25.0 ab | 2.00 bc |
| **Experiment 2** | | | |
| Barley | 93.8 ab | 39.5 a | 2.33 ab |
| Barley/clover | 87.5 ab | 31.3 a | 2.62 a |
| Barley/ryegrass | 93.8 ab | 18.8 a | 2.73 a |
| Clover | 91.8 ab | 43.8 a | 2.15 b |
| Common ryegrass | 83.3 ab | 14.5 a | 2.38 ab |
| Lemtal ryegrass | 68.8 b | 33.3 a | 2.69 a |
| potato | 100.0 a | 37.5 a | 2.71 a |

[a]Discolored stems included those with a severity rating of 1 or greater. Severity ratings for stem and canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25% of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by stem or stolon cankers; and 5 = stem or stolons completely nipped off or death of the plant).
[b]Incidence of stem canker was represented by the percentage of plants showing obvious canker symptoms (severity of 2 or greater).
[c]Shoot size ratings were on a scale of 0 to 3 as follows: 0 = no growth; 1= shoot height < 2 cm; 2 = shoot height of 2 to 5 cm; and 3 = shoot height > 5 cm.
[d]Means within the same column for each experiment followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

**Field trial emergence and disease ratings**

Potato shoot emergence four weeks after planting was comparable among all rotations at both field locations, but overall emergence was lower at location 1 than location 2 (Table 3.2). There were, however, some differences due to biocontrol treatments at both locations. *L. arvalis* treatments resulted in greater emergence than *T. virens* at location 1, whereas *B. subtilis* and the control treatments resulted in greater emergence than *L. arvalis* treatments at location 2.

Differences in stem canker were detected among rotation crops at location 1 (Table 3.3). Incidence of stem canker was significantly lower in both barley rotations than in continuous potato and stem canker severity was significantly lower in the barley/ryegrass rotation than the potato rotation (Table 3.2). However, no differences in stem canker were detected among rotation crops at location 2. There were no biocontrol effects or rotation by biocontrol interactions for stem canker at either location. There were no treatment differences at either location for incidence and severity of stolon canker. However, incidence and severity values were greater at location 2 than location 1 (Table 3.2).

Incidence and severity of black scurf was uniformly low at location 2 (severity 0.2 to 0.3 % tuber surface covered with sclerotia, incidence 15 to 21 %) with no differences detected among rotation crops or biocontrol treatments. Disease levels were higher at location 1 than location 2. At location 1, there were significant rotation crop by biocontrol interactions for black scurf incidence and severity. Although within the potato rotation there were no differences among biocontrol treatments, within the barley/ryegrass rotation, black scurf severity was lower with *L. arvalis* treatments than

**Table 3.2.** Rhizoctonia disease ratings by preceding rotation crop or biocontrol treatment.

| | Shoot emergence[a] (%) | Stem canker | | Stolon canker | |
|---|---|---|---|---|---|
| | | Severity[b] | Incidence[c] (%) | Severity[b] | Incidence[c] (%) |
| **Location 1**[d] | | | | | |
| Preceding rotation crop (mainplots) | | | | | |
| Barley/ryegrass | 75.4 a | 1.3 b | 25.0 b | 1.3 a | 26.6 a |
| Barley/clover | 79.3 a | 1.5 ab | 39.1 b | 1.2 a | 23.4 a |
| Potato | 84.2 a | 1.7 a | 59.4 a | 1.4 a | 37.5 a |
| Biocontrol treatment (subplots) | | | | | |
| *Laetisaria arvalis* | 83.9 a | 1.5 a | 45.8 a | 1.3 a | 25.0 a |
| *Trichoderma virens* | 76.7 b | 1.6 a | 45.8 a | 1.4 a | 37.5 a |
| *Bacillus subtilis* | 77.6 ab | 1.5 a | 37.5 a | 1.3 a | 25.0 a |
| Control | 80.4 ab | 1.5 a | 35.4 a | 1.3 a | 29.2 a |
| **Location 2** | | | | | |
| Preceding rotation crop (mainplots) | | | | | |
| Barley/ryegrass | 93.6 a | 1.6 a | 53.1 a | 1.6 a | 53.1 a |
| Barley/clover | 92.7 a | 1.7 a | 56.3 a | 1.6 a | 50.0 a |
| Potato | 96.0 a | 1.6 a | 54.7 a | 1.6 a | 48.4 a |
| Biocontrol treatment (subplots) | | | | | |
| *Laetisaria arvalis* | 90.6 b | 1.6 a | 54.2 a | 1.7 a | 56.3 a |
| *Trichoderma virens* | 92.4 ab | 1.7 a | 56.3 a | 1.5 a | 43.8 a |
| *Bacillus subtilis* | 97.0 a | 1.7 a | 54.2 a | 1.6 a | 54.2 a |
| Control | 96.4 a | 1.6 a | 54.2 a | 1.6 a | 47.9 a |

[a]Emergence values were taken approximately four weeks after planting.
[b]Severity ratings for stem and stolon canker were on a scale of 0 to 5 (0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25% of the stem or stolon circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by stem or stolon cankers; and 5 = stem or stolons completely nipped off or death of the plant).
[c]Incidence of stem and stolon canker was represented by the percentage of plants showing obvious canker symptoms (severity of 2 or greater).
[d]Means within the same column for each location and biocontrol treatment or preceding rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

in the control and black scurf incidence was lower with *L. arvalis* than with *T. virens* treatments (Table 3.4). The incidence of tubers with black scurf coverage of 2 % or greater was also significantly reduced by the *L. arvalis* and *B. subtilis* treatments. Within the barley/clover rotation, black scurf severity and the incidence of tubers with black scurf coverage of 2 % or greater was lower with *T. virens* than all other treatments. *T. virens* treatments also reduced black scurf incidence compared to the control and *L. arvalis* treatments.

After tubers were harvested and washed, substantial amounts of common scab caused by *Streptomyces scabiei* were detected on tubers and rated based on the proportion of each tuber surface covered by scab lesions. There were no significant rotation or biocontrol treatment effects or interactions for scab at location 1, which had high incidences, but low scab severity across all samples (Table 3.5). Scab severity was much higher at location 2, although incidence of scab was only slightly higher than location 1. At location 2, the barley/clover rotation resulted in tubers with greater scab severity than the potato rotation. However, there were no biocontrol effects or rotation by biocontrol interactions.

**Tuber yield**

Preceding rotation crops significantly affected tuber yield at location 1, but not at location 2. There were no rotation crop by biocontrol interactions at either location. At location 1, the barley/ryegrass rotation resulted in significantly higher total, marketable, and large size class yield than the potato rotation (Table 3.6). The barley/ryegrass rotation also produced more large size class tubers than the barley/clover rotation. The

**Table 3.3.** Analysis of variance table for incidence of stem canker, stolon canker, and black scurf.

| | df | *P* value | | |
|---|---|---|---|---|
| | | Stem Canker | Stolon Canker | Black Scurf |
| **Location 1** | | | | |
| Block | 3 | 0.002 | 0.003 | <0.001 |
| Rotation crop (A) | 2 | 0.01 | 0.38 | 0.73 |
| Error A | 6 | | | |
| Biocontrol treatment (B) | 3 | 0.64 | 0.42 | 0.64 |
| Crop * biocontrol | 6 | 0.19 | 0.48 | 0.01 |
| Error B | 27 | | | |
| **Location 2** | | | | |
| Block | 3 | <0.001 | 0.003 | <0.001 |
| Rotation crop (A) | 2 | 0.96 | 0.39 | 0.43 |
| Error A | 6 | | | |
| Biocontrol treatment (B) | 3 | 0.99 | 0.95 | 0.22 |
| Crop * biocontrol | 6 | 0.27 | 0.62 | 0.50 |
| Error B | 27 | | | |

**Table 3.4.** Black scurf ratings for biocontrol treatments as affected by preceding rotation crop at location 1.

| **Rotation crop**[a] **Biocontrol treatment** | **Severity**[b] | **Incidence (%)** | **Incidence ≥ 2**[c] **(%)** |
|---|---|---|---|
| Barley/ryegrass | | | |
| *Laetisaria arvalis* | 1.7 b | 64.9 b | 43.3 b |
| *Trichoderma virens* | 2.2 ab | 87.3 a | 55.8 ab |
| *Bacillus subtilis* | 2.0 ab | 84.3 ab | 43.4 b |
| control | 2.8 a | 85.8 ab | 71.8 a |
| Barley/clover | | | |
| *Laetisaria arvalis* | 1.8 a | 74.1a | 49.9 a |
| *Trichoderma virens* | 1.0 b | 52.4 b | 25.8 b |
| *Bacillus subtilis* | 1.8 a | 70.8 ab | 45.8 a |
| Control | 1.6 a | 83.4 a | 49.1 a |
| Potato | | | |
| *Laetisaria arvalis* | 1.8 a | 75.8 a | 60.1 a |
| *Trichoderma virens* | 1.8 a | 79.0 a | 56.6 a |
| *Bacillus subtilis* | 2.0 a | 75.1 a | 57.5 a |
| Control | 1.5 a | 64.9 a | 47.5 a |

[a]Means within the same column for each preceding rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).
[b]Black scurf severity was based on the percent of the tuber surface covered by sclerotia.
[c]Incidence of tubers with severity rating of 2 % coverage or greater.

**Table 3.5.** Common scab disease ratings of tubers by preceding rotation crop or biocontrol treatment.

| | Severity[a] | Incidence (%) | Incidence > 10[b] (%) |
|---|---|---|---|
| **Location 1**[c] | | | |
| Preceding rotation crop (mainplots) | | | |
| Barley/ryegrass | 2.1 a | 91.3 a | 0.0 a |
| Barley/clover | 2.0 a | 85.8 a | 0.2 a |
| Potato | 2.5 a | 95.6 a | 2.7 a |
| Biocontrol treatment (subplots) | | | |
| *Laetisaria arvalis* | 2.3 a | 90.3 a | 0.0 a |
| *Trichoderma virens* | 2.0 a | 90.6 a | 3.6 a |
| *Bacillus subtilis* | 2.3 a | 89.3 a | 0.0 a |
| Control | 2.3 a | 93.3 a | 0.3 a |
| **Location 2** | | | |
| Preceding rotation crop (mainplots) | | | |
| Barley/ryegrass | 26.8 ab | 99.3 a | 78.3 a |
| Barley/clover | 30.9 a | 99.8 a | 87.0 a |
| Potato | 22.5 b | 99.6 a | 74.1 a |
| Biocontrol treatment (subplots) | | | |
| *Laetisaria arvalis* | 27.3 a | 99.7 a | 81.1 a |
| *Trichoderma virens* | 25.9 a | 99.1 a | 76.9 a |
| *Bacillus subtilis* | 28.7 a | 99.4 a | 82.0 a |
| Control | 25.1 a | 100.0 a | 79.3 a |

[a]Severity based on percent of tuber surface covered by scab.
[b]Incidence of tubers with severity rating greater than 10 %.
[c]Means within the same column for each location and biocontrol treatment or preceding rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

potato rotation produced more medium size class tubers than the barley/clover rotation. The barley/ryegrass rotation also resulted in a greater percentage of tubers that were marketable or in the large size class than the potato rotation at location 1 (Fig. 3.4).

Biocontrol treatments significantly affected some aspects of tuber yield at location 2, but not at location 1 (Table 3.6). At location 2, *L. arvalis* and *T. virens* treatments resulted in a greater percentage of tubers in the large and marketable size classes than the control (Fig. 3.5), although total and marketable yield size classes were not statistically different among trials (Table 3.6). *L. arvalis* and *T. virens* treatments also resulted in fewer tubers in the small and medium size classes than the control.

**Table 3.6.** Tuber yield by size class for preceding rotation crop or biocontrol treatment.

| | Yield (Mg/ha) | | | | | |
|---|---|---|---|---|---|---|
| | **Small[a]** | **Medium[b]** | **Large[c]** | **Total** | **Marketable[d]** | **Misshapen** |
| **Location 1[e]** | | | | | | |
| Preceding rotation crop (mainplots) | | | | | | |
| Barley/ryegrass | 0.87 a | 2.30 ab | 14.10 a | 17.27 a | 16.40 a | 1.31 a |
| Barley/clover | 0.92 a | 2.03 b | 12.42 b | 15.36 ab | 14.45 ab | 1.58 a |
| Potato | 1.54 a | 3.05 a | 10.14 c | 14.73 b | 13.19 b | 1.62 a |
| Biocontrol treatment (subplots) | | | | | | |
| *Laetisaria arvalis* | 1.05 a | 2.64 a | 12.21 a | 15.90 a | 14.85 a | 1.45 a |
| *Trichoderma virens* | 1.19 a | 2.17 a | 12.08 a | 15.44 a | 14.25 a | 1.31 a |
| *Bacillus subtilis* | 1.01 a | 2.60 a | 12.42 a | 16.02 a | 15.01 a | 1.52 a |
| Control | 1.19 a | 2.44 a | 12.16 a | 15.79 a | 14.60 a | 1.74 a |
| **Location 2** | | | | | | |
| Preceding rotation crop (mainplots) | | | | | | |
| Barley/ryegrass | 1.72 a | 4.32 a | 10.64 a | 16.68 a | 14.96 a | 0.75 a |
| Barley/clover | 1.87 a | 4.27 a | 12.36 a | 18.51 a | 16.63 a | 0.80 a |
| Potato | 1.74 a | 5.50 a | 12.31 a | 19.55 a | 17.81 a | 0.78 a |
| Biocontrol treatment (subplots) | | | | | | |
| *Laetisaria arvalis* | 1.39 b | 4.09 b | 12.93 a | 18.42 a | 17.02 a | 0.66 a |
| *Trichoderma virens* | 1.46 b | 3.90 b | 12.29 a | 17.66 a | 16.20 a | 0.87 a |
| *Bacillus subtilis* | 1.92 ab | 4.98 ab | 12.33 a | 19.23 a | 17.31 a | 0.90 a |
| Control | 2.34 a | 5.82 a | 9.53 a | 17.68 a | 15.34 a | 0.67 a |

[a]Small size class consisted of tubers less than 47 mm in diameter.
[b]Medium size class consisted of tubers 47 mm to 56 mm in diameter.
[c]Large size class consisted of tubers greater than 56 mm in diameter.
[d]Marketable class consisted of tubers in the medium and large size classes combined.
[e]Means within the same column for each location and biocontrol treatment or preceding rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

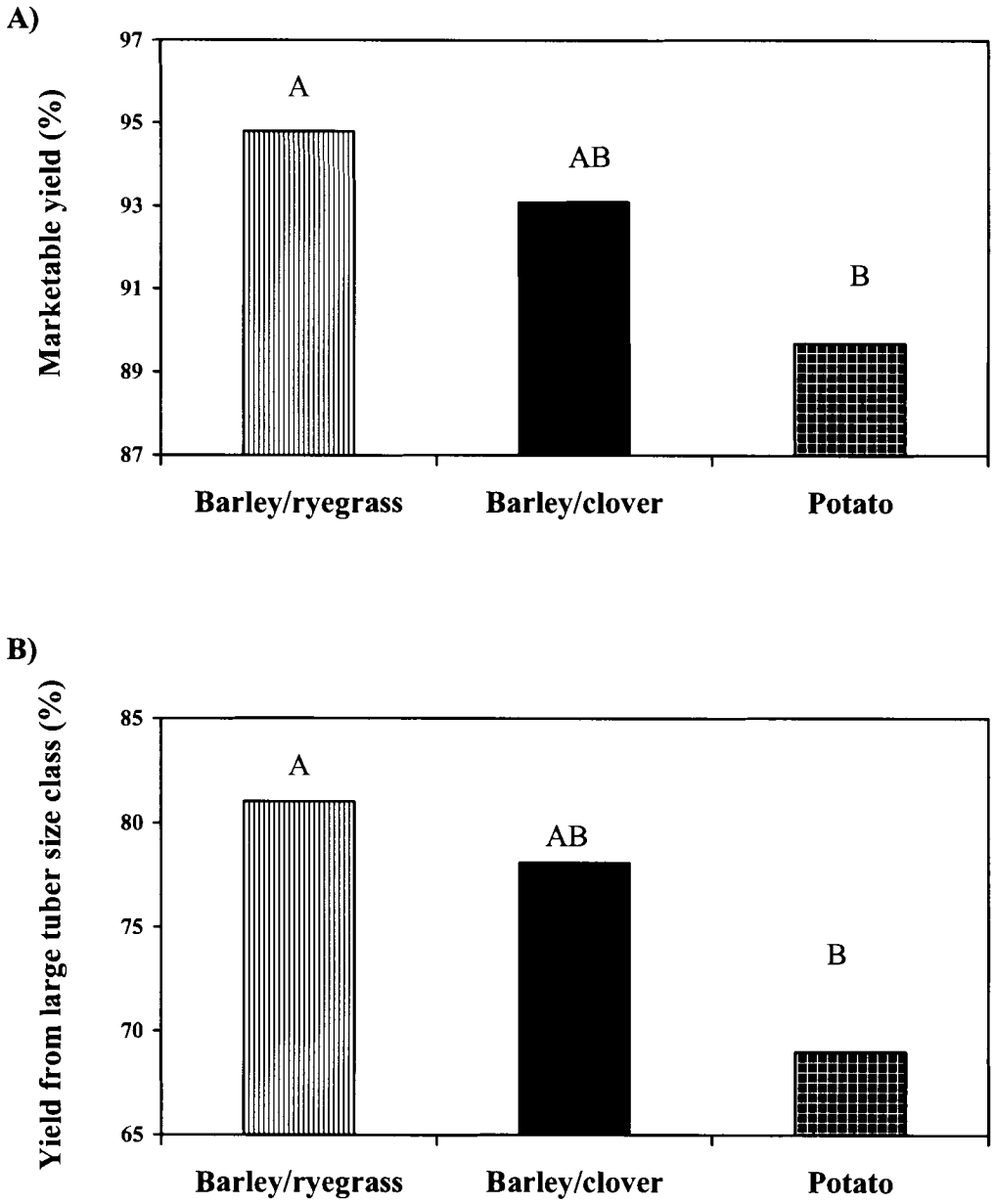



**Figure 3.4.** Percent yield by preceding rotation crop at location 1 for A) marketable tubers and B) large size class tubers.

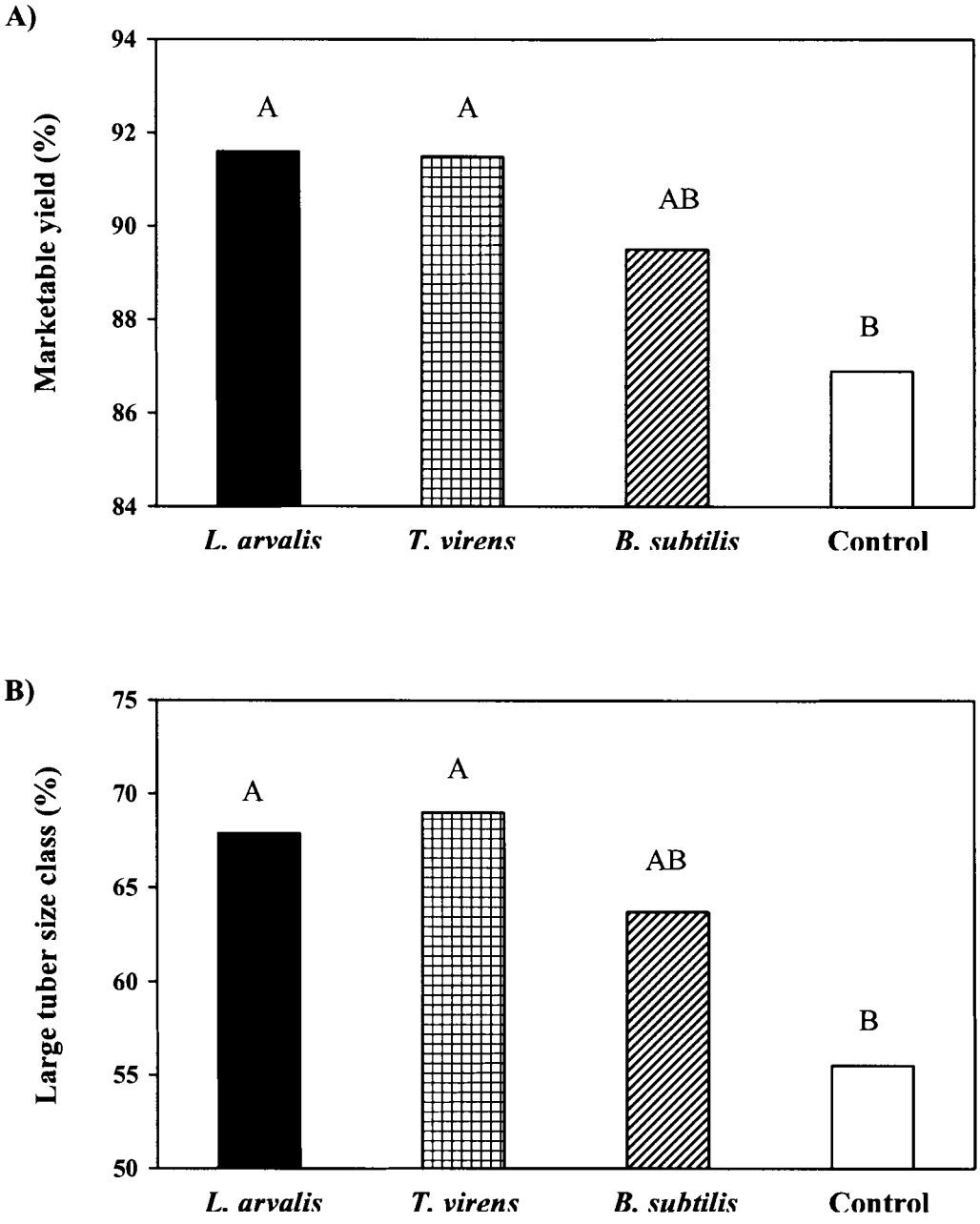



**Figure 3.5.** Percent yield by biocontrol treatment at location 2 for A) marketable tubers and B) large size class tubers.

**Soil microbial populations**

Soil populations of *R. solani* were generally too low to detect any differences (0 to 0.5 propagules per 10 g soil) among treatments. At location 1, another *Rhizoctonia* sp. was frequently isolated (0 to 20 propagules per 10 g soil) in May 2002 sampling times (both prior to and after incorporation of rotation crop residues). This organism was identified as *R. zeae* based on the size, shape, and color of mycelia and sclerotia (Sneh *et al.*, 1991). Fatty acid analysis and comparison of fatty acid profiles with similar known isolates were also used to identify this organism as *R. zeae*. These known isolates included *R. zeae, R. oryzae,* and *R. circinata* (obtained from Dr. Roseanne Leiner, University of Alaska, Fairbanks). *R. zeae* was not significantly associated with a particular rotation crop in the field experiment, although it was highly associated with rotations containing barley or ryegrass in greenhouse experiments. The soil used in the greenhouse experiments was collected from location 1 of the field experiment. *R. zeae* was not isolated from location 2 soil.

At location 2, bacterial populations associated with the barley/clover rotation were higher than those with the barley/ryegrass rotation (Table 3.7). There were no significant differences among preceding rotation crops or biocontrol treatments for populations of culturable bacteria at location 1. However, there was a significant rotation crop by biocontrol effect. Within the barley/ryegrass rotation, populations associated with *L. arvalis* treatments were higher than *B. subtilis* or control treatments (Table 3.8). Within the potato and barley/clover rotations, there were no observed differences in populations of bacteria among biocontrol treatments.

**Table 3.7.** Populations of culturable microbes in August 2002 by preceding rotation crop or biocontrol treatment at location 2.

| | Colony forming units (cfu) per gram of soil | | | |
|---|---|---|---|---|
| | **Bacteria**[a] **(x $10^7$)** | **Fungi**[b] **(x $10^4$)** | ***Trichoderma* sp.**[c] **(x $10^4$)** | ***Penicillium* YG**[c] **(x $10^4$)** |
| Preceding rotation crop (mainplots)[d] | | | | |
| Barley/ryegrass | 3.9 b | 41.6 a | 2.5 a | 5.2 a |
| Barley/clover | 6.2 a | 38.8 a | 2.3 a | 3.6 a |
| Potato | 4.6 ab | 40.9 a | 1.8 a | 7.3 a |
| Biocontrol treatment (subplots) | | | | |
| *Laetisaria arvalis* | 4.9 a | 50.3 a | 2.7 a | 5.7 a |
| *Trichoderma virens* | 4.1 a | 34.3 b | 2.6 a | 3.5 b |
| *Bacillus subtilis* | 5.0 a | 39.8 b | 2.2 ab | 6.3 a |
| Control | 5.1 a | 37.4 b | 1.4 b | 6.2 a |

[a]Bacteria were enumerated on 0.1 % TSA after 72 hr incubation at 28°C.
[b]Fungi were enumerated on PDA with antibiotics after incubation for 7 days at room temperature.
[c]*Trichoderma* sp. and *Penicillium* YG were enumerated based on colony morphology on PDA with antibiotics after incubation for 7 days at room temperature.
[d]Means within the same column for each biocontrol treatment or preceding rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

There were differences in total populations of fungi among biocontrol treatments at location 2 and there was a rotation crop by biocontrol interaction at location 1. At location 2, *L. arvalis* treatments resulted in higher fungal populations than all other biocontrol treatments (Table 3.7). At location 1, within the barley/ryegrass rotation, the control had higher fungal populations than all biocontrols (Table 3.8). Within the potato rotation, populations of general fungi associated with the *L. arvalis* treatment were significantly higher than populations in the *T. virens* treatment. There were no differences among biocontrol treatments within the barley/clover rotation.

There were no significant rotation effects for populations of *Trichoderma* sp. at location 2. However, there were significant biocontrol effects. Over all rotations, *L.*

**Table 3.8.** Microbial populations associated with biocontrol treatments as affected by preceding rotation crop in August 2002 at location 1.

| Rotation crop[a] biocontrol | Colony forming units (cfu) per gram of soil | | | |
|---|---|---|---|---|
| | Bacteria[b] ($x 10^7$) | Fungi[c] ($x 10^4$) | *Trichoderma* sp.[d] ($x 10^4$) | *Penicillium* YG[d] ($x 10^4$) |
| Barley/ryegrass | | | | |
| *Laetisaria arvalis* | 7.8 a | 26.3 b | 5.0 a | 3.9 b |
| *Trichoderma virens* | 5.5 ab | 33.1 b | 2.6 b | 12.1 ab |
| *Bacillus subtilis* | 4.0 b | 29.9 b | 4.2 ab | 5.5 b |
| control | 5.2 b | 55.9 a | 4.0 ab | 22.3 a |
| Barley/clover | | | | |
| *Laetisaria arvalis* | 5.7 a | 40.4 a | 4.4 a | 10.2 ab |
| *Trichoderma virens* | 5.7 a | 41.5 a | 5.7 a | 14.8 a |
| *Bacillus subtilis* | 5.2 a | 34.0 a | 2.2 a | 11.8 ab |
| Control | 5.6 a | 31.6 a | 4.0 a | 7.4 b |
| Potato | | | | |
| *Laetisaria arvalis* | 3.9 a | 50.3 a | 3.1 a | 23.7 a |
| *Trichoderma virens* | 4.9 a | 33.7 b | 2.6 a | 12.8 b |
| *Bacillus subtilis* | 4.8 a | 42.7 ab | 2.3 a | 21.8 ab |
| Control | 5.5 a | 39.8 ab | 2.0 a | 18.8 ab |

[a]Means within the same column for each rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).
[b]Bacteria were enumerated on 0.1 % TSA after 72 hr incubation at 28°C.
[c]Fungi were enumerated on PDA with antibiotics after incubation for 7 days at room temperature.
[d]*Trichoderma* sp. and *Penicillium* YG were enumerated based on colony morphology on PDA with antibiotics after incubation for 7 days at room temperature.

*arvalis* and *T. virens* treatments were associated with greater populations of *Trichoderma* sp. than the control. Within the barley/ryegrass rotation at location 1, *L. arvalis* treatments were associated with higher populations than *T. virens* treatments.

Populations of a particular *Penicillium* sp. associated with potato roots were not significantly different among preceding crop rotations at either location, although they tended to be nominally higher in the potato rotations. There were no differences among biocontrol treatments at location 1, although there was a significant rotation by biocontrol interaction. Within the barley/ryegrass rotation, the control treatment had higher populations of *Penicillium* than *L. arvalis* and *B. subtilis* treatments (Table 3.8). Within the barley/clover rotation, *T. virens* treatments resulted in higher populations of *Penicillium* than the control treatment. Within the potato rotation, *L. arvalis* treatments resulted in higher populations than those with *T. virens*. At location 2, there was a significant biocontrol effect, but no interactions. Populations were lower with *T. virens* treatments than with all other treatments (Table 3.7).

**FAME profiles**

Fatty acid profiles revealed distinct differences in soil microbial communities associated with preceding rotation crops both prior to and after incorporation of crop residues at both locations, as indicated by principal components (PC) analysis. At location 1, FAME profiles from continuous potato were distinct from the barley rotations both prior to and after crop incorporation, whereas both barley rotations had similar profiles (Fig. 3.6). PC 1 and PC 2 accounted for 29 % and 16 % of the variability, respectively. The fatty acids that contributed most to PC 1 at location 1 were 12:0, 13:0 anteiso, 18:2 ω6c, 17:1 ω3c, and C:16 N alcohol. Those that contributed most to PC 2

included 16:1 ω5c, C16 N alcohol, 18:3 ω6c, 16:1 ω7c, and 18:2 ω6c. The primary effect of crop incorporation appeared to be a decrease in PC 1 and an increase in PC 2, with similar effects on all profiles. At location 2, profiles associated with the three rotations were more distinct prior to crop incorporation than after (Fig. 3.7). The effect of crop incorporation on profiles was different depending on rotation. The barley/ryegrass communities changed the most after incorporation (decreased PC 1 and PC 2) and the potato profile changed the least (no change in PC 1, decreased PC 2). At location 2, PC 1 and PC 2 accounted for 38 % and 17 % of the total variability, respectively. PC 1 was represented mostly by 18:0 2OH, C:20 N alcohol, 18:1 ω9c, 16:0, and 17:1 ω7c and PC 2 by C20 N alcohol, 18:0 2OH, 16:1 ω7c, 16:0 3OH, and 18:1 ω9c.

In August 2002, FAME profiles were influenced by both rotation crop and biocontrol treatments at location 1, with biocontrol treatments producing somewhat different effects depending on the rotation crop (Fig. 3.8). For example, *T. virens* treatments resulted in greater PC 1 values than the control within the barley/ryegrass rotation, but in the potato rotation, *T. virens* treatments resulted in greater PC 2 values and comparable PC 1 values as the control. At location 1, PC 1 and PC 2 accounted for 58 % and 11 % of the total variability, respectively. Variability within PC 1 was determined primarily by 12:0, 16:0, 13:0 anteiso, 18:1 ω9c, and 18:2 ω6c. The fatty acids that accounted for the most variability in PC 2 were 16:1 ω5c, 16:1 ω7c, 18:2 ω6c, 16:0, and 14:0. Also at location 1, preceding rotation crops resulted in differences in FAME profiles as indicated by PC 2 and PC 3 values (Fig. 3.9). FAME profiles associated with the potato rotation were distinctly different from both barley rotation profiles, with lower

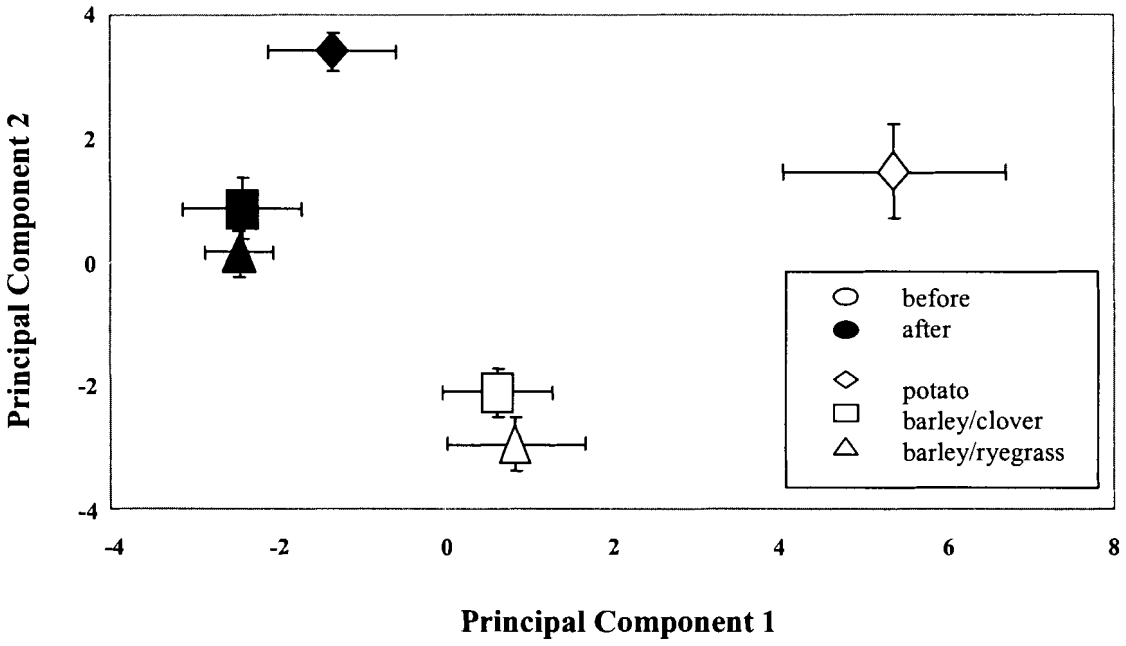



**Figure 3.6.** Principal components of soil microbial communities associated with rotation crops prior to and one month after incorporation of cover crop residues in spring 2002 at location 1.

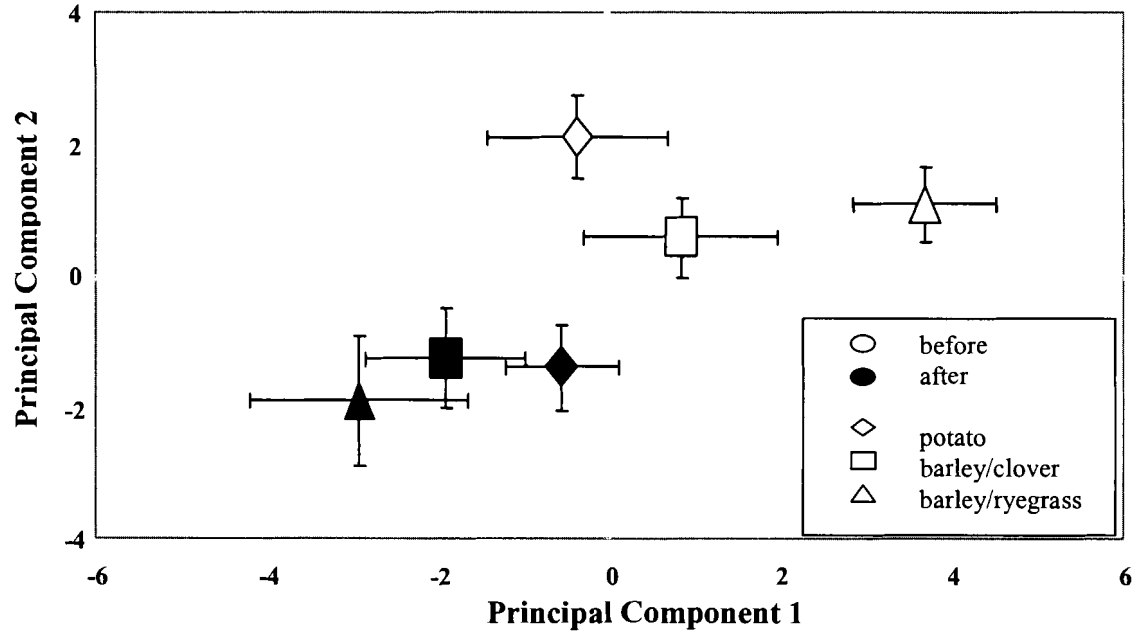



**Figure 3.7.** Principal components of soil microbial communities associated with rotation crops prior to and one month after incorporation of cover crop residues in spring 2002 at location 2.

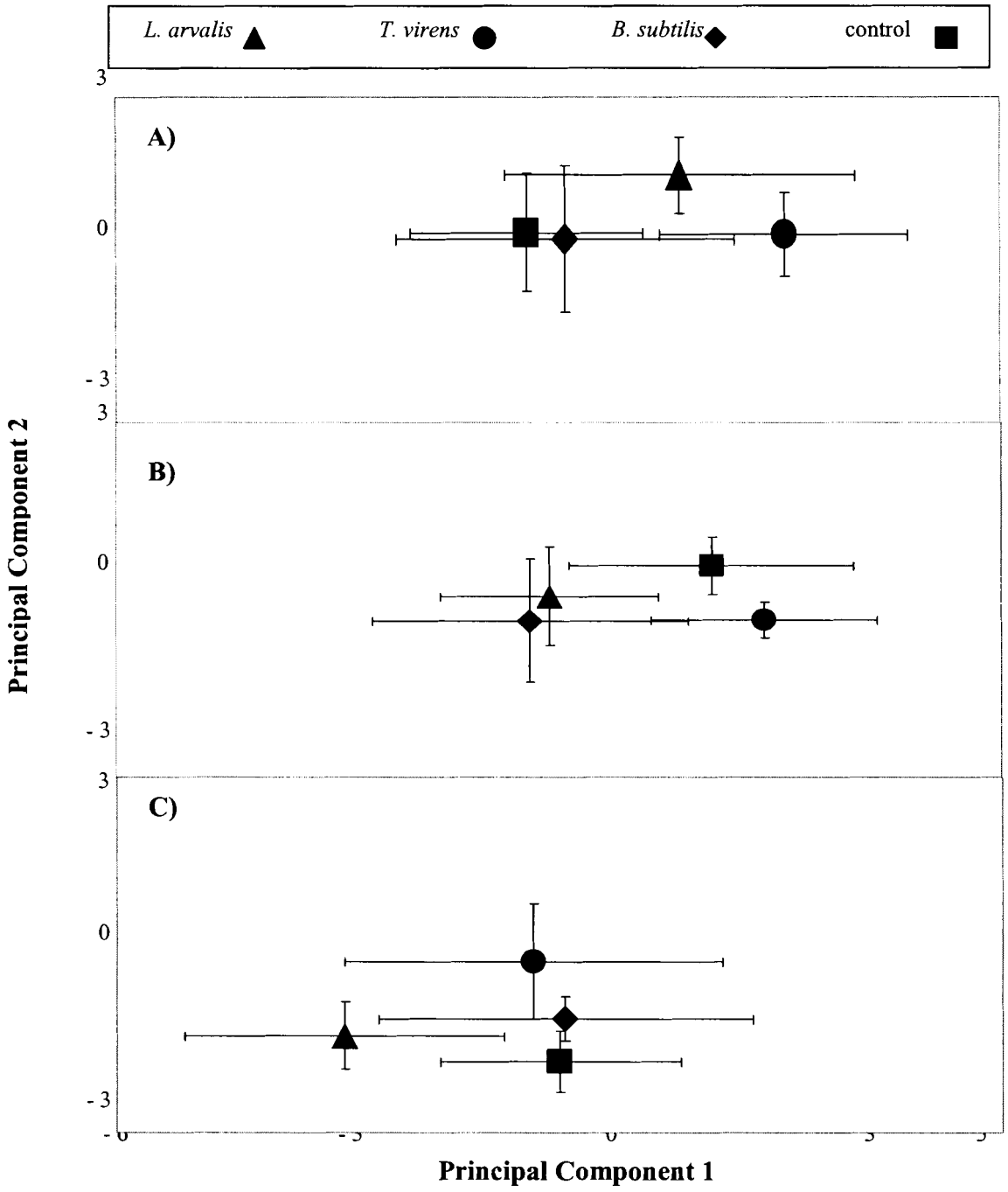



**Figure 3.8.** Principal components of fatty acid profiles from soils sampled in August 2002 at location 1 as affected by biocontrol treatments and each previous rotation crop: A) barley/ryegrass, B) barley/clover, and C) potato.

PC 2 and higher PC 3 values. Barley/clover and barley/ryegrass showed similar profile characteristics, but also showed some detectable differences. PC 3 accounted for 6 % of the total variability. Fatty acids that contributed most to PC 3 values included C20 N alcohol, 16:0, c16 N alcohol, 16:1 ω5c, and 18:3 ω6c. At location 2, there were no detectable differences in FAME profiles associated with the different rotation crops or biocontrol treatments in August 2002 due to high levels of variabilty among samples.

Differences in fatty acid profiles were also expressed as the proportion of extracted fatty acids in specific structural classes, which also showed differences among preceding rotation crops both prior to and after crop residue incorporation at both locations (Table 3.9). Overall, the proportion of straight chain saturated and branched

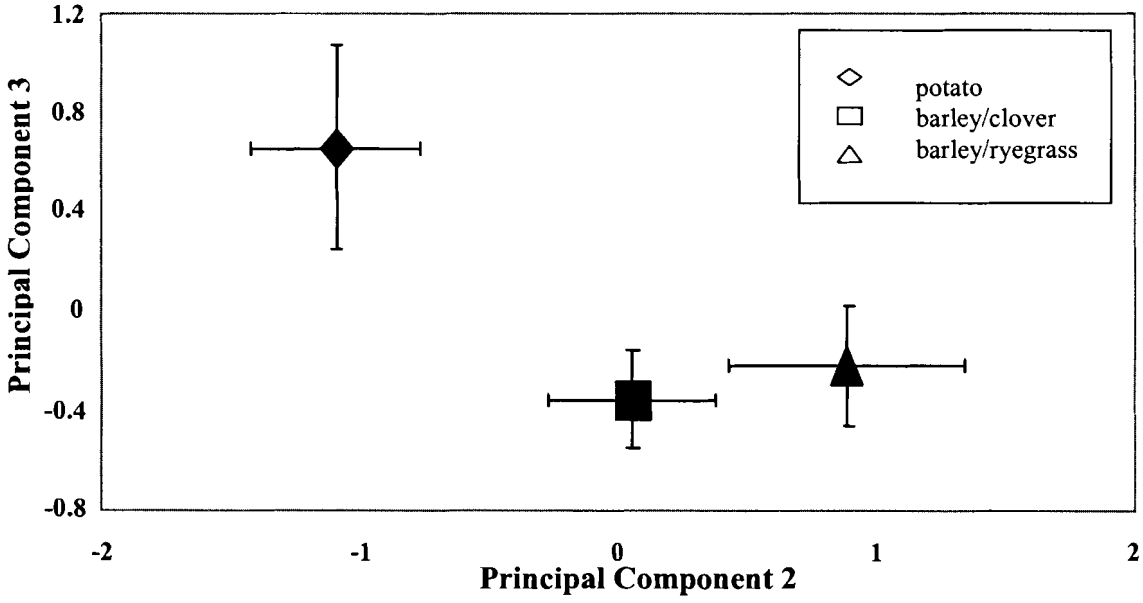



**Figure 3.9.** Principal components of soil microbial communities associated with rotation crops in August 2002 when all plots were planted to potato at location 1.

fatty acids tended to be greater prior to residue incorporation than after incorporation. The fungal to bacterial fatty acid ratio was greater after residue incorporation. The potato rotation resulted in higher levels of straight chain saturated and branched fatty acids and lower levels of polyunsaturated fatty acids and monounsaturated to saturated fatty acid ratios than the barley rotations both prior to and after residue incorporation. Prior to residue incorporation, continuous potato had a lower fungal to bacterial ratio and after residue incorporation, a lower proportion of monounsaturated fatty acids than the barley rotations at both locations.

There were also differences in the proportion of fatty acids in specific structural classes and ratios of those proportions in the August 2002 soil samples. There were significant rotation crop effects at location 2. Both potato and barley/clover rotations resulted in a greater proportion of hydroxyl fatty acids than barley/ryegrass at location 2 (Table 3.10). Also, the potato rotation resulted in a lower ratio of monounsaturated to saturated fatty acids than both barley rotations. There were also significant biocontrol effects at location 2. *T. virens* resulted in a greater proportion of polyunsaturated fatty acids than the control, whereas the control resulted in a greater proportion of branched fatty acids than *L. arvalis* and *T.virens* and a greater Gram (+) to Gram (–) ratio than *L. arvalis*. *L. arvalis* resulted in a greater proportion of hydroxyl group containing fatty acids than *B. subtilis*. At location 1, there were significant rotationcrop by biocontrol interactions for saturated and polyunsaturated fatty acid classes and the ratio of fungal to bacterial fatty acids. There were also significant differences among biocontrol treatments within at least one of the rotation crops for the remaining fatty acid classes and class

**Table 3.9.** Effect of rotation crops both prior to and after residue incorporation in spring 2002 on different fatty acid structural classes and class ratios.

| | Fatty acid classes (%)[a] | | | | | Class ratios[b] | | |
|---|---|---|---|---|---|---|---|---|
| | Sat | Mono | Poly | Branch | Hydrox | Mono/ sat | Gram+/ Gram- | Fungi/ bact |
| **Location 1**[c] | | | | | | | | |
| After growth | | | | | | | | |
| Barley/ryegrass | 32.5 b | 43.0 a | 10.6 a | 20.8 b | 11.0 b | 1.33 a | 1.94 a | 0.34 a |
| Barley/clover | 32.9 b | 41.4 a | 11.0 a | 20.0 b | 11.9 ab | 1.27 a | 1.71 a | 0.35 a |
| Potato | 38.0 a | 42.1 a | 8.5 b | 23.2 a | 13.3 a | 1.11 b | 1.76 a | 0.24 b |
| After incorporation | | | | | | | | |
| Barley/ryegrass | 30.7 ab | 41.9 a | 12.4 a | 18.5 ab | 9.4 a | 1.37 a | 1.99 a | 0.45 a |
| Barley/clover | 30.1 b | 39.2 b | 11.9 a | 18.2 b | 10.6 a | 1.31 a | 1.78 a | 0.42 a |
| Potato | 32.3 a | 38.5 b | 12.0 a | 19.6 a | 10.0 a | 1.20 b | 2.02 a | 0.41 a |
| **Location 2** | | | | | | | | |
| After growth | | | | | | | | |
| Barley/ryegrass | 20.0 a | 22.5 a | 6.5 ab | 8.8 b | 12.5 a | 1.14 a | 0.72 b | 0.31 a |
| Barley/clover | 20.0 a | 22.3 a | 7.3 a | 9.4 b | 12.3 a | 1.12 a | 0.84 b | 0.35 a |
| Potato | 20.5 a | 22.5 a | 5.1 b | 10.4 a | 10.6 a | 1.10 a | 1.04 a | 0.25 a |
| After incorporation | | | | | | | | |
| Barley/ryegrass | 18.6 a | 24.7 a | 7.5 a | 8.9 a | 12.8 a | 1.36 a | 0.83 a | 0.37 a |
| Barley/clover | 19.8 a | 23.1 ab | 7.1 a | 9.1 a | 11.1 a | 1.18 ab | 0.89 a | 0.36 ab |
| Potato | 19.8 a | 21.3 b | 6.0 b | 9.2 a | 12.3 a | 1.09 b | 0.81 a | 0.28 b |

[a]These classes are composed of the proportion of fatty acids belonging to each structural class, where Sat = straight chain fatty acids (9:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0), Mono = monounsaturated fatty acids (14:1 ω9c, 15:1 ω8c, 16:1 ω7c, 16:1 ω5c, 17:1 ω7c, 17:1 ω3c, 18:1 ω9c, 20:1 ω6c), Poly = polyunsaturated fatty acids (18:3 ω6c, 18:2 ω6c), Branch = saturated branched fatty acids (11:0 iso, 13:0 iso, 13:0 anteiso, 14:0 iso, 14:0 anteiso, 15:0 iso, 15:0 anteiso), Hydrox = fatty acids with hydroxyl groups (10:0 3OH, 12:0 2OH, 12:0 3OH, 15:0 iso 3OH, 16:0 2OH, 16:0 3OH, 18:0 2OH).

[b]Ratios are of structural class proportions that are used as biomarkers for specific groups of organisms. Mono/sat = the ratio of monounsaturated fatty acids to straight chain saturated fatty acids, Gram+/Gram- = the ratio of branched to hydroxy fatty acids, and Fungi/bact = the ratio of polyunsaturated fatty acids to branched and hydroxyl fatty acids.

[c]Means within the same column for each location and sampling time followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

**Table 3.10.** Effects of preceding rotation crops and biocontrol treatments on different fatty acid structural classes and class ratios in August 2002 at location 2.

| | Fatty acid classes (%)[a] | | | | | Class ratios[b] | | |
|---|---|---|---|---|---|---|---|---|
| | **Sat** | **Mono** | **Poly** | **Branch** | **Hydrox** | **Mono/ sat** | **Gram+/ Gram-** | **Fungi/ bact** |
| Preceding rotation crop | | | | | | | | |
| Barley/ryegrass | 29.7 a | 29.6 a | 24.1 a | 15.7 a | 13.8 b | 1.00 a | 1.18 a | 0.82 a |
| Barley/clover | 30.2 a | 29.4 a | 23.2 a | 16.0 a | 16.1 a | 0.98 a | 1.04 a | 0.73 a |
| Potato | 30.8 a | 28.7 a | 22.8 a | 15.5 a | 15.7 a | 0.93 b | 1.18 a | 0.74 a |
| Biocontrol treatment | | | | | | | | |
| *L. arvalis* | 30.7 a | 29.2 a | 23.5 ab | 15.5 b | 16.2 a | 0.96 a | 1.01 b | 0.75 a |
| *T.virens* | 30.1 a | 29.4 a | 23.9 a | 15.5 b | 15.3 ab | 0.98 a | 1.08 ab | 0.78 a |
| *B.subtilis* | 30.3 a | 29.2 a | 23.4 ab | 15.6 ab | 14.5 b | 0.97 a | 1.14 ab | 0.78 a |
| control | 30.0 a | 29.1 a | 22.9 b | 16.2 a | 14.8 ab | 0.98 a | 1.16 a | 0.74 a |

[a]These classes are composed of the proportion of fatty acids belonging to each structural class, where Sat = straight chain fatty acids (9:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0), Mono = monounsaturated fatty acids (14:1 ω9c, 15:1 ω8c, 16:1 ω7c, 16:1 ω5c, 17:1 ω7c, 17:1 ω3c, 18:1 ω9c, 20:1 ω6c), Poly = polyunsaturated fatty acids (18:3 ω6c, 18:2 ω6c, 20:4 ω6c, 20:5 ω3c), Branch = saturated branched fatty acids (11:0 iso, 13:0 iso, 13:0 anteiso, 14:0 iso, 14:0 anteiso, 15:0 iso, 15:0 anteiso), Hydrox = fatty acids with hydroxyl groups (10:0 3OH, 12:0 2OH, 12:0 3OH, 15:0 iso 3OH, 16:0 2OH, 16:0 3OH).

[b]Ratios are of structural class proportions that are used as biomarkers for specific groups of organisms. Mono/sat = the ratio of monounsaturated fatty acids to straight chain saturated fatty acids, Gram+/Gram- = the ratio of branched to hydroxy fatty acids, and Fungi/bact = the ratio of polyunsaturated fatty acids to branched and hydroxyl fatty acids.

[c]Means within the same column for each preceding rotation crop or biocontrol organism followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

ratios presented (Table 3.11). However, there were no consistent trends over classes or class ratios among any of the treatments

## Discussion

Ryegrass, specifically the variety "Lemtal", reduced severity of Rhizoctonia stem canker alone in the greenhouse and at one of two field locations when combined with barley. Reductions in severity were not detected at one of the locations, most likely because both the ryegrass and clover cover crops were not fully established the following spring due to severe weed pressure that substantially reduced cover crop stands the previous fall. An increase in tuber yield with the barley/ryegrass rotation also occurred at the same location where disease reductions were detected. Stem canker can result in severe losses in yield (Parry, 1990), and the reductions in stem canker by the barley/ryegrass rotation may have led to the increase in yield.

Clover rotations were not as beneficial as ryegrass rotations. In the greenhouse, a rotation with clover alone increased *R. solani* populations as much as or more than potato rotations. These increases in populations and disease levels by clover and other legumes have been seen in other studies (Larkin 2002; Larkin, 2004, Dabney *et al.*, 1996; Rickerl *et al.*, 1992; Johnston *et al.*, 1994). In fact, *R. solani* AG-3 produced stem lesions on clover in a controlled study (Appendix B), and leguminous crops, including clover, have shown increases in lesions caused by *R. solani* on subsequent susceptible crops (Dabney *et al.*, 1996).

The present study indicates that ryegrass is a more suitable cover crop than clover for control of Rhizoctonia disease of potato. Clover increases populations of *R. solani*

**Table 3.11.** Effect of biocontrol organisms as determined by preceding rotation crop on fatty acid classes and class ratios in August 2002 at location 1.

| | Fatty acid classes (%)[a] | | | | | Class ratios[b] | | |
|---|---|---|---|---|---|---|---|---|
| | **Sat** | **Mono** | **Poly** | **Branch** | **Hydroxy** | **Mono/ sat** | **Gram+/ Gram-** | **Fungi/ bact** |
| Barley/ryegrass | | | | | | | | |
| *L. arvalis* | 26.3 a | 26.9 a | 7.6 a | 14.5 a | 6.3 a | 1.04 a | 2.39 a | 0.37 ab |
| *T. virens* | 24.6 a | 26.8 a | 8.8 a | 13.1 b | 5.9 a | 1.11 a | 2.38 a | 0.47 a |
| *B. subtilis* | 26.7 a | 26.2 a | 6.9 a | 13.0 b | 6.3 a | 1.00 a | 2.24 a | 0.36 ab |
| control | 26.8 a | 26.7 a | 7.2 a | 13.6 ab | 7.3 a | 1.01 a | 2.02 a | 0.35 b |
| Barley/clover | | | | | | | | |
| *L arvalis* | 26.3 ab | 26.6 ab | 7.7 a | 14.0 a | 6.7 b | 1.02 b | 2.14 a | 0.38 a |
| *T. virens* | 24.5 b | 27.8 a | 7.8 a | 14.1 a | 6.2 b | 1.13 a | 2.32 a | 0.39 a |
| *B. subtilis* | 27.4 a | 25.9 b | 6.4 b | 13.8 a | 7.8 a | 0.98 b | 1.84 b | 0.30 b |
| control | 26.4 ab | 27.2 ab | 7.9 a | 13.9 a | 6.1 b | 1.04 ab | 2.38 a | 0.40 a |
| Potato | | | | | | | | |
| *L. arvalis* | 29.4 a | 24.9 a | 5.2 b | 14.5 ab | 7.8 a | 0.88 a | 1.96 a | 0.24 b |
| *T.virens* | 27.9 ab | 26.6 a | 6.2 ab | 14.2 b | 7.9 a | 0.98 a | 2.05 a | 0.29 ab |
| *B.subtilis* | 26.6 ab | 25.0 a | 6.9 a | 15.5 a | 6.8 a | 0.96 a | 2.38 a | 0.34 a |
| control | 25.6 b | 24.6 a | 6.7 ab | 14.6 ab | 7.4 a | 0.97 a | 2.09 a | 0.32 ab |

[a]These classes are composed of the proportion of fatty acids belonging to each structural class, where Saturated = straight chain fatty acids (9:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0), Monounsaturated = monounsaturated fatty acids (14:1 ω9c, 15:1 ω8c, 16:1 ω7c, 16:1 ω5c, 17:1 ω7c, 17:1 ω3c, 18:1 ω9c, 20:1 ω6c), Polyunsaturated = polyunsaturated fatty acids (18:3 ω6c, 18:2 ω6c, 20:4 ω6c, 20:5 ω3c), Branched = saturated branched fatty acids (11:0 iso, 13:0 iso, 13:0 anteiso, 14:0 iso, 14:0 anteiso, 15:0 iso, 15:0 anteiso), Hydroxy = fatty acids with hydroxyl groups (10:0 3OH, 12:0 2OH, 12:0 3OH, 15:0 iso 3OH, 16:0 2OH, 16:0 3OH).

[b]Ratios are of structural class proportions that are used as biomarkers for specific groups of organisms. Monounsat/sat = the ratio of monounsaturated fatty acids to straight chain saturated fatty acids, Gram+/Gram- = the ratio of branched to hydroxy fatty acids, and Fungi/bact = the ratio of polyunsaturated fatty acids to branched and hydroxyl fatty acids.

[c]Means within the same column for each preceding rotation crop followed by the same letter are not significantly different according to Fisher's LSD ($P = 0.05$).

canker severity and increased yield at one of the two locations. These beneficial effects were not seen with the barley/clover rotation. Although detrimental effects of clover are more obvious when grown alone than in combination with barley (Larkin, 2004), the barley/ryegrass combination should ultimately provide better disease control.

A possible mechanism of disease suppression by ryegrass is that it stimulates microbial activity (Johnston *et al.,* 1994). In addition, the ryegrass rotation could stimulate specific antagonists that reduce disease. In the present study, ryegrass rotations increased populations of *R. zeae* in the greenhouse. *R. zeae* has mostly been considered a pathogen of turfgrasses (Martin and Lucas, 1983; Burpee and Martin, 1992), although isolates recovered in Maine did not cause disease symptoms on either ryegrass or barley. However, these isolates did cause some lesions on potato, but to a much lesser extent than *R. solani* AG-3 (Appendix C; Carling and Leiner, 1990). *R. zeae* has shown potential biocontrol activity against *R. solani* and may compete with *R. solani* for colonization or infection of roots. Increases in *R. zeae* in the barley/ryegrass rotation were not detected in the field, and the increase in population levels observed in the greenhouse trials was evident in soil from only one of the two locations. *R. zeae* is isolated more frequently and is also more virulent under warmer temperature conditions (Martin and Lucas, 1983; Carling and Leiner 1990) which could explain why it was not detected in soil from the more northerly location that has a cooler climate. Additional tests need to be conducted to determine the role of *R. zeae* and if it is involved in stem canker suppression by ryegrass rotations.

The barley/ryegrass rotations did not reduce black scurf in the present study. However, Specht and Leach (1987) demonstrated that ryegrass rotations resulted in fewer

malformed and scurf infested tubers than five other rotations, although differences were not statistically significant. Differences in black scurf among rotations may not have been observed in the present study because severity levels were relatively low. Also, disease measurements were taken after only one complete rotation cycle and it may take more rotation cycles prior to differences can be detected.

Principal components analysis of fatty acid profiles and the proportions of fatty acid structural classes and biomarkers revealed distinct differences for all rotations, indicating that changes in soil microbial community characteristics occurred based on the preceding crop. In this study, values for the fatty acid structural classes are proportions of the total fatty acids. It is important to note that they represent relative abundance of particular fatty acids, not actual population levels of organisms with which they are associated. Many fatty acids and ratios of fatty acids have been identified as particularly useful biomarkers (Cavigelli *et al.*, 1995). The proportion of monounsaturated fatty acids was higher in the barley/ryegrass rotation than the other rotations and the ratios of monounsaturated to saturated fatty acids were higher in both barley rotations than continuous potato. Monounsaturated fatty acids are a biomarker for gram negative bacteria, but some (16:1 $\omega$5c and 18:1 $\omega$9c) are also associated with fungi, and these fatty acids tend to increase in soil with aerobic conditions, organic inputs, and high substrate availability (Larkin, 2003a; Zelles *et al.*,1992, 1995; Bossio and Scow, 1998, Bossio *et al.*, 1998). The proportions of polyunsaturated fatty acids, which are associated with fungi (Larkin, 2003a), were also higher in the barley rotations than the potato rotation in the present study. Hydroxyl group fatty acids are indicators of gram negative bacteria, whereas branched fatty acids are indicators of gram positive bacteria (Larkin, 2003), but

they have also been found in some gram negative bacteria (Cavigelli *et al.*, 1995). Results of this study indicate that the proportion of gram positive bacteria was higher in continuous potato than the barley rotations and the ratio of fungi (polyunsaturated fatty acids) to bacteria (branched and hydroxy fatty acids) was higher in the barley rotations, especially barley/ryegrass, than the continuous potato rotation. These results are similar to those observed between rotated soil and soil from continuous potato treatments in other crop rotation studies in Maine (Larkin, 2003a).

Principal components analysis also revealed differences in soil microbial communities associated with different biocontrol treatments at one of the two locations. However, the biocontrol treatments affected FAME profiles differently within each rotation. Proportions of fatty acid structural classes, class ratios, and biomarkers were also affected by biocontrol treatments, which demonstrated that soil microbial communities were affected by the biocontrol treatments. These differences, however, were not as consistent as those observed among the rotation crops. At one of the locations, fatty acid classes and ratios associated with the biocontrol treatments varied among rotations. Siciliano and Germida (1998) observed that microbial communities in the rhizosphere, measured by FAME analysis, were affected by inoculation with *Pseudomonas* isolates. The biocontrol treatments may have had a greater impact on rhizosphere communities than in bulk soil.

Many management practices for sustainable disease control affect soil microbiology characteristics. Changes in microbial activity or community structure can be mechanisms by which effective rotation crops and biocontrol reduce disease (Larkin, 2004; Huber and Sumner, 1996; Larkin *et al.*, 1998; Cook and Baker, 1983). The

specific effects of cropping systems on soil microbial communities are not well understood (Larkin, 2004), nor is the effect of these microbial communities on soilborne diseases. Larkin (2004) demonstrated that many associations exist between microbial populations, FAME analysis, tuber yield, and Rhizoctonia disease of potato. The incidence and severity of black scurf in potato cropping systems were negatively correlated with FAME principal components and the ratio of monounsaturated to saturated fatty acids. Total and marketable yield were also negatively correlated with scurf incidence and the ratio of monounsaturated to saturated fatty acids and positively correlated with fungal populations. In this study, the barley/ryegrass rotation resulted in a greater proportion of monounsaturated fatty acids, a greater ratio of monounsaturated to saturated fatty acids, and an increase in the ratio of fungi to bacteria over the potato rotation. The barley/ryegrass rotation also increased yield and reduced stem canker, which may be influenced by specific changes in soil microbial communities.

Interactions between crop rotations and biocontrol organisms were detected. Biocontrol treatments did provide some control of black scurf within the barley rotations at one location, although control by specific treatments varied within the different rotations. *L. arvalis* and *B. subtilis* reduced black scurf within the barley/ryegrass rotation, whereas *T. virens* reduced black scurf severity within the barley/clover rotation. *L. arvalis* and *T. virens* treatments increased the percentage of marketable and large size class tubers. Biocontrol by rotation interactions were evident in black scurf results, microbial populations, FAME profiles, and fatty acid structural classes. *L. arvalis* appeared to work better in the barley/ryegrass rotation, whereas *T. virens* was more effective in the barley/clover rotation at location 1.

Both effective crop rotations and the addition of biological antagonists are sustainable disease control alternatives that could reduce losses in tuber yield and quality from Rhizoctonia disease of potato. The use of biocontrol within beneficial rotations could improve efficacy against *R. solani*. This integrated approach demonstrates that microbial antagonists and effective crop rotations can potentially increase yield and suppress Rhizoctonia disease of potato.

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# APPENDICES

## Appendix A

## *RHIZOCTONIA SOLANI* PATHOGENICITY TRIALS

Eight isolates of *R. solani* were screened for virulence on potato shoots by stem canker severity, plant size, and seedpiece black scurf coverage assessments. All isolates came from diseased potato plants in Maine except RSAG3scl24, a member of AG-3 isolated from potato in Alaska. All isolates were characterized by FAME analysis (MIDI-FAME) and confirmed as members of AG-3 except RSN2R95A. The anastomosis group of this isolate is yet to be determined. Levels of inoculum adequate to cause considerable disease were also determined in potting soil (Metro-Mix 200). Isolate RS31B was chosen as the pathogenic isolate for all experiments because it belonged to AG-3 and caused stem canker, stunting, and black scurf. The appropriate concentration of inoculum, which consisted of *R. solani* grown on organic cracked wheat for approximately 7 days, was determined to be 16 g per kg potting mix and 4 g per kg field soil mix (3:1 w/w field soil:sand).

**Table A.1.** Rhizoctonia disease and size ratings of potato plants treated with eight isolates of *R. solani* in trial 1.

| Treatment[a] | Stem canker severity[b] | Relative size | Black scurf[c] (%) |
|---|---|---|---|
| RS31B | 1 | severely stunted | > 5 |
| RSN2R95A | 1 | stunted | 1-5 |
| RSRF4s56 | 1 | stunted | > 5 |
| RSAG3scl24 | 1 | stunted | > 5 |
| RSIAP | 0 | slightly stunted | 1-5 |
| RSIAP-B | 0 | normal | 1-5 |
| RSXVI | 0 | stunted | 0 |
| RSDL | 0 | normal | 1-5 |
| Control | 0 | normal | 0 |
| Wheat control | 0 | normal | 0 |

[a]4 g of organic cracked wheat inoculated with each isolate was added to approximately 100 g of potting soil in a 10 cm pot. The control received no inoculum and the wheat control received 4 g of non-inoculated wheat. All treatments were replicated 3 times and grown in a growth chamber for 3 weeks.
[b]Stem canker severity was determined as follows: 0 = no symptoms; 1 = brown discoloration; 2 = cankers covering <25% of the stem circumference; 3 = 25% to 75% coverage by cankers; 4 = 75% coverage by stem cankers; and 5 = stem completely nipped off or death of the plant.
[c]Black scurf ratings were based on the percent of the seed piece surface covered by sclerotia.

**Table A.2.** Rhizoctonia disease ratings and plant size for potato plants treated with four virulent isolates of *R. solani*.

| Treatment[a] | Stem canker severity[b] | Relative size | Black scurf[c] (%) |
|---|---|---|---|
| RSRF4s56 | 0 | slightly stunted | 1-5 % |
| RSAG3scl24 | 1 | slightly stunted | > 5 % |
| RSN2R95A | - | - | - |
| RS31B | 1 | slightly stunted | > 5 % |
| RS31B (1 g) | 1 | stunted | > 5 % |
| RS31B (2 g) | 1 | stunted | 1-5 % |
| RS31B (8 g) | 1 | stunted | 1-5 % |
| Control | 0 | normal | 0 |
| Wheat control | 0 | normal | 0 |

[a]Treatments were prepared as described above except with five replications of each treatment. RS31B wasalso added at 1, 2, and 8 g of inoculum per sample.
[b]Stem canker severity was determined as follows: 0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25% of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by stem cankers; and 5 = stem completely nipped off or death of the plant. No sprouts emerged from seed pieces treated with RSN2R95A.
[c]Black scurf ratings were based on the percent of the seed piece surface covered by sclerotia.

**Table A.3.** Rhizoctonia disease ratings and size of potato plants treated with the two most virulent isolates, RS31B and RSN2R95A at three rates.

| Treatment[a] | Stem canker | Size | Black scurf |
|---|---|---|---|
| RS31B (1 g) | 1 | stunted | > 5 |
| RS31B (2 g) | 1 | very stunted | > 5 |
| RS31B (4 g) | 1 | stunted | > 5 |
| RSN2R95A (1 g) | 1 | stunted | 0 |
| RSN2R95A (2 g) | 2 | stunted | 0 |
| RSN2R95A (4 g) | 1 | stunted | 0 |
| Control | 0 | normal | 0 |
| Wheat control | 0 | normal | 0 |

[a]Treatments were prepared as described above with five replications of 1, 2, or 4 g of inoculum per pot for both isolates.
[b]Stem canker severity was determined as follows: 0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25 % of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by stem cankers; and 5 = stem completely nipped off or death of the plant.
[c]Black scurf ratings were based on the percent of the seed piece surface covered by sclerotia.

## Appendix B

## PATHOGENICITY OF *RHIZOCTONIA SOLANI* AG-3 ON RED CLOVER

Potato plants preceded by red clover had stem cankers and inoculum levels as severe as treatments preceded by potato. The possibility that clover contributes to increased populations of *R. solani* and high levels of Rhizoctonia disease of potato because it is also a host of *R. solani* AG-3 was investigated. Clover seed inoculated with *Rhizobium* inoculant was added to potting soil (Metro-Mix 200) infested with *R. solani* AG-3 (isolate RS31B) and to non-infested potting soil. In the first trial, clover plants grown in the infested soil were much smaller and had less developed root systems than plants grown in the non-infested soil. These plants also had stem discoloration and lesions that yielded *R. solani* upon surface sterilization and placement on Flower's media and PDA. No lesions were found on clover plants in the non-inoculated treatments. In a second trial conducted in the greenhouse, neither clover plants nor potato plants had any symptoms of Rhizoctonia disease. An antagonist, *Trichoderma*, was found to have colonized the potting soil used in this trial and prevented disease. In a third trial, potato plants had severe canker, but there was no difference between inoculated and non-inoculated clover plants.

*R. solani* AG-3 is able to cause stem lesions on clover, but perhaps only under specific environmental conditions. The first trial was conducted in a growth chamber under high relative humidity and cooler temperatures that favor infection by *R. solani.* The other trials were conducted in the greenhouse where it is warmer and less humid. It is likely that *R. solani* was still able to colonize clover roots in the third trial, but unable

to cause lesions. This could explain why clover plants in rotation treatments did not appear symptomatic, even though they contributed to increases in *R. solani* populations and Rhizoctonia disease of potatoes.

## Appendix C

## *RHIZOCTONIA ZEAE* PATHOGENICITY TRIALS

*R. zeae* has been shown to cause disease of turfgrasses (Burpee and Martin, 1992; Martin and Lucas, 1983) and minor disease symptoms of potato (Carling and Leiner, 1990). The pathogenicity of several field isolates of *R. zeae* collected from plots where potatoes and grasses had grown in Newport, ME, and a known isolate RZ504 (obtained from Roseann Leiner, University of Alaska, Fairbanks) were investigated for pathogenicity on barley, ryegrass, and potato.

Three field isolates, RZRF27I, RZLRNE17D, and RZLRNE17E, were tested for pathogenicity on barley and ryegrass. Plants were assessed for plant stand, plant size, and root, stem, or leaf necrosis or lesions. However, there were no disease symptoms present on any plants or in any of the treatments. *R. zeae* isolates were also tested on potato and there were indications that they could cause disease (Table C.1 and Table C.2). However, isolation from diseased plants indicated that only two out of twelve plants were actually infected by *R. zeae*. These were plants inoculated with RZRF7I and RZ504. All other cultures grown from cankers were *R. solani*. Stems must have become infected by tuberborne inoculum of *R. solani*.

Since tuberborne inoculum apparently caused most of the cankers in the first trial, this experiment was repeated and all seed pieces were surface sterilized in 2 % formaldehyde in water for 2 min. In the second trial, *R. zeae* was able to cause some stem canker on potato plants (Table C.2), although symptoms and incidence were generally less severe than *R. solani* AG-3. The symptoms seen in the control treatments must be the result of soil contamination or the surface sterilization procedure for the seed

pieces was not completely effective. Perhaps, sclerotia or mycelia deep within an eye were not reached by the sterilization solution.

**Table C.1.** Stem canker severity and incidence from trial 1 of potato plants treated with isolates of *R. zeae*.

| Treatment[a] | Stem canker severity | Stem canker incidence (%) |
|---|---|---|
| RZ504 | 1.5 | 75 |
| RZRF7I | 1 | 75 |
| RZLRNE17E | 2 | 100 |
| RS31B (*R. solani* AG-3) | 5 | 100 |
| control | 0.25 | 25 |
| wheat control | 1 | 50 |

[a]Stem canker severity was determined as follows: 0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25% of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by stem cankers; and 5 = stem completely nipped off or death of the plant.

**Table C.2.** Incidence and severity of stem canker of potato plants treated with isolates of *R. zeae* in trial 2.

| Treatment | Stem canker severity[a] | Stem canker incidence (%) |
|---|---|---|
| RZRF7I | 1.20 | 90 |
| RZLRNE17E | 0.67 | 67 |
| RS31B (*R. solani* AG-3) | 2.12 | 100 |
| control | 0.41 | 30 |
| wheat control | 0 | 0 |

[a]Stem canker severity was determined as follows: 0 = no symptoms; 1 = brown discoloration; 2 = cankers covering < 25% of the stem circumference; 3 = 25 % to 75 % coverage by cankers; 4 = 75 % coverage by stem cankers; and 5 = stem completely nipped off or death of the plant.

## Appendix D

## *PENICILLIUM* SP. IN VITRO INHIBITION TRIALS

These trials were conducted to determine if potential biocontrol isolates of a *Penicillium* sp. associated with potato roots were able to inhibit *R. solani* in vitro. Agar disks, 6 mm in diameter, of *Trichoderma virens* GL-21 and five isolates of *Penicillium* sp. associated with potato roots in Maine (Larkin, 2003a) were placed on PDA 5 cm from agar disks of isolates of *R. solani* RS31B. Each isolate was replicated 3 times. After 3 days of growth at room temperature, the radius of each *R. solani* colony was measured in the direction towards the potential antagonist (inhibition zone) and in the direction perpendicular to the antagonist. The level of inhibition by all Penicillium isolates was similar, but marginal with growth of *R. solani* about 85 % of that of the control (Table D.1). The inhibition caused by *R. solani* on itself was even greater. Only *T. virens* showed a substantial inhibition zone.

*T. virens* and *R. solani* inhibited *R. solani* more effectively than *Penicillium* sp. After four days the *R. solani* plugs had covered the majority of the plate and seemed to inhibit the YG isolates. In these trials, *Penicillium* sp. did not appear to have antibiotic activity towards *R. solani*. However, the cultures of *R. solani* and *Penicillium* sp. did not converge, so either or both fungi must have produced some inhibitory substance.

**Table D.1.** Levels of in vitro inhibition of *R. solani* by *Penicillium* sp. isolates.

| Biocontrol isolate | Inhibition zone radius[a] / Perpendicular radius[b] | Percent Inhibition (%) |
|---|---|---|
| *Penicillium* sp. YG1 | 0.84 | 15.7 |
| *Penicillium* sp. YG2 | 0.84 | 15.8 |
| *Penicillium* sp. YG3 | 0.84 | 15.7 |
| *Penicillium* sp. YG4 | 0.84 | 16.3 |
| *Penicillium* sp. YG5 | 0.86 | 13.9 |
| *Trichoderma virens* | 0.61 | 38.6 |
| *Rhizoctonia solani* | 0.73 | 26.6 |

[a]The radius of the *R. solani* colony in the direction of the potential biocontrol organism.
[b]The radius of *R. solani* in the direction perpendicular to the potential biocontrol organism.

## BIOGRAPHY OF THE AUTHOR

Marin Talbot Brewer was born in Mentor, Ohio on April 7, 1976. She graduated from Mentor High School in 1994. Then she attended the University of Cincinnati and graduated in 1998 with a Bachelor of Science in Biology. She moved to Maine in 1999 to work for the USDA, ARS, New England Plant, Soil, and Water Laboratory as a Biological Science Technician and entered the Plant, Soil, and Environmental Sciences graduate program at The University of Maine in the fall of 2001.

Marin has accepted a position as a Research Associate in the Crop and Horticulture Science Department at The Ohio State University, which will begin in December of 2003. Marin is a candidate for the Master of Science degree in Plant, Soil, and Environmental Sciences at The University of Maine in December, 2003.