To the Graduate Council:

I am submitting herewith a thesis written by Danésha Seth entitled "Effect of inoculum, cultivar, and the biological control fungus *Beauveria bassiana* on damping-off caused by *Rhizoctonia solani* on tomato." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Masters of Science, with a major in Entomology and Plant Pathology.

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Effect of inoculum, cultivar, and the biological control fungus *Beauveria bassiana* on damping-off caused by *Rhizoctonia solani* on tomato

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Danésha Seth
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DEDICATION

This thesis is dedicated my family.
It is especially for my loving parents

Craig and Linda Seth

who have taught me that anything is possible

if you put your heart and mind to it.

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There are many people to whom I am grateful for making my time here in the Department of Entomology and Plant Pathology a rewarding experience. Everyone I had the pleasure of coming into contact with was wonderful, which made it easy to come to here, and a pleasure to remain here.

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ABSTRACT

Rhizoctonia solani Kühn is a common soil inhabiting fungus that causes disease in cultivated plants worldwide. Considering the environmental and economical costs of chemical pesticides, development of additional disease control strategies is necessary. Disease-resistant cultivars and biological control are attractive alternatives.

The main objective of this study was to evaluate 10 tomato (*Lycopersicon esculrntum* Mill.) cultivars for resistance to *R. solani*. The cultivars included Carolina Gold, Celebrity, Hybrid 882, Mountain Belle, Mountain Delight, Mountain Fresh, Mountain Pride, Mountain Spring, Mountain Supreme, and Sunpride. The second objective was to compare two forms of *R. solani* inoculum for ability to cause disease on tomato seedlings. Inoculum of *R. solani* was produced on either rice or cornmeal:sand, and added to planting medium as 0.5 to 1.0-mm sieved rice at 1%, and 4% cornmeal:sand. Infested mixes were placed in 7.6-cm diameter pots and one untreated tomato seed was planted per pot. Stand count data were collected at 7, 14, and 21 days after planting (DAP). At 28 DAP, disease incidence and severity were recorded. There was no difference in percent stand count, or disease incidence for the two inoculum types. Disease severity was significantly higher with the cornmeal:sand inoculum. Pre-emergence damping off was most severe with Celebrity, Mountain Belle, Mountain Spring, and Sunpride. Cultivars that appeared to be less susceptible to damping-off were Hybrid 882, Mountain Fresh, and Mountain Supreme. There were no differences in disease severity of tomato seedlings.

The third objective of this study was to evaluate different forms of *Beauveria bassiana* (Balsamo) Vuillemin, as a biological control agent against *R. solani* on tomato, cultivar Mountain Spring. The six treatments included: 0.02 g conidia per 20 seed; 0.20 g mycelia per 20 seed; 30 g alginate prills per 2 L planting medium; 82 g mycelia per 5 L planting medium; seed treated with conidia + planting medium treated with prills, and seed treated with conidia + planting medium treated with mycelia. The three controls were untreated seed in uninfested soil, and untreated seed and seed treated with methyl cellulose in infested planting medium. Seeds were coated with 0.5% methyl cellulose solution (for adhesion) before addition of conidia or mycelia of *B. bassiana*. All planting mix was infested with 4% w/w cornmeal:sand inoculum of *R. solani*, except the first control group. Treated planting mixes were placed in 7.6-cm diameter pots, and one tomato seed was planted per pot. Stand count data was taken at 7 and 14 DAP. At 31 DAP, disease incidence and severity, and shoot and root weights were determined. Protection against damping-off was greatest with seed treated with mycelia, seed treated with conidia + planting medium treated with mycelia, and seed treated with conidia. However, seed treated with conidia alone delayed germination. Disease incidence was lowest with seed treated with conidia. Seed treated with conidia and medium treated with mycelia had the lowest disease severity. The two treatments with the greatest shoot weight were seed treated with conidia + medium treated with mycelia and medium treated with mycelia. Seed treated with conidia had significantly lower shoot and root weights. Due to delayed germination, plants in this treatment were physiologically younger than

plants from other treatments. The treatment with the highest root weight were the planting medium treated with mycelia, and the seed treated with conidia + planting medium treated with mycelia, although, these were not different from the control groups.

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CAUSAL ORGANISM: *RHIZOCTONIA SOLANI*

Rhizoctonia solani Kühn [teleomorph – *Thanatephorus cucumeris* (Frank) Donk] is a common soil-inhabiting fungus that causes disease in almost every type of cultivated plant worldwide. This destructive pathogen is an opportunist that persists in the soil primarily as a saprophyte on dead plant tissues, actively growing mycelium, or small, loosely formed sclerotia (Agrios, 1997). The pathogen is most virulent when hyphae are very young (Blair, 1943), and if the soil is moist, *R. solani* remains in a state of continuous vegetative growth. Under adverse conditions, the pathogen becomes dormant and thick walled mycelia or sclerotia are formed.

Rhizoctonia solani commonly is divided into many genetically different anastomosis groups (AG), which have been used as a criterion for classification of the species (Parmeter and Whitney, 1970; Sneh et al., 1991). Isolates are similar morphologically; however, each group has different growth requirements, host ranges, and tolerances. Some strains are aggressive pathogens, while others live as saprophytes on plant debris in the soil. In pathogenicity studies with isolates of AG-2-2 and AG-4, sugar beet stand emergence was significantly reduced and AG-2-2 caused severe root rot even in mature plants (Rush et al., 1994). While AG-2-2 and AG-4 cause damping-off in crops such as sunflower, corn, alfalfa, and snap bean (Nelson et al., 1996), they cause only mild disease

in mature plants (Rush et al., 1994). Anastomosis group 5 is commonly associated with severe damping-off of wheat and soybean seedlings, while AG-3 causes only mild lesions on tap roots of adult plants (Sneh et al., 1991). Overall, pathogenicity and virulence of each anastomosis group are variable and depend greatly on the host.

Rhizoctonia solani causes a wide variety of diseases under diverse environmental conditions. These diseases include pre- and post-emergence damping-off of seedlings, wirestem, root and stem rot, stem canker, and fruit rot. Damping-off is the most common disease caused by *R. solani.* Germinating seedlings are most susceptible to damping-off, which is prevalent in both field and greenhouse situations. After emergence, diseased seedlings have water-soaked, soft stems with reddish brown to black lesions at or near the soil line (Agrios, 1997). Lesions eventually coalesce, causing the stem to lose integrity and collapse. The seedlings fall over and die. While damping-off generally occurs in seedlings, wirestem occurs in more mature plants. Wirestem lesions or sunken cankers girdle the stem and have alternating light brown to dark brown bands (Agrios, 1997). The stem becomes wiry and thin at the point of attack, and while these plants are rarely killed, plant growth is stunted, development is significantly delayed, and yield is reduced up to 100% (Baker, 1971; McCarter, 1991; van Bruggen et al., 1986). Root rots occur under adverse soil or environmental conditions. Rhizoctonia root rot is more severe when the roots have been

damaged by nematodes, when there are temperature fluctuations, or when soils are moderately moist and warm (McCarter, 1991).

EFFECTS OF ENVIRONMENT ON DISEASE

Temperature, soil moisture, and plant age are important factors affecting the ability of *R. solani* to cause disease. Disease is usually most severe at lower temperatures, although the fungus can survive under varying soil conditions, which ultimately influence survival and inoculum potential. Doornik (1981) tested several isolates of *R. solani* grown under varied temperatures. Thermophilic isolates (>13°C) and psychrophilic isolates (<13°C) were tested. Thermophilic isolates caused more severe infections at 17°C, and did not infect plants below 13°C. Host symptoms caused by the psychrophilic isolates were significantly more severe at 9°C, although optimal growth was seen at significantly higher temperatures. Leach (1947) grew *R. solani* on solid medium and observed rapid growth at temperatures between 20 and 30°C.

Disease is usually more severe when temperatures are unfavorable for the host. Temperatures between 15 and 18°C (McCarter, 1991) or above 28°C (Brown and McCarter, 1976) may stress plants and favor the pathogen. Cool-weather crops such as spinach and sugar beet are less susceptible to damping-off at lower temperatures (4 to 12°C) (Baker and Martinson, 1970). Leach (1947) found that sugar beet was particularly susceptible to damping-off at temperatures ranging from 16 to 30°C. Benson and Baker (1974) studied pre-emergence

damping-off of radish, which was most severe at 15°C. Warm-weather crops, however, are more affected by damping-off at temperatures that favor the pathogen, such as 20 to 30°C (Baker and Martinson, 1970). In red kidney bean infected by *R. solani*, root-to-shoot ratio is deleteriously affected at temperatures above 18°C, particularly at low soil moisture (van Bruggen et al., 1986).

Mycelial growth increases in soils with low moisture content, while less growth occurs during periods of high moisture (Blair, 1943). Poinsettia stem and root rots were more severe at moisture concentrations below 40%, while at 80% moisture, *R. solani* causes little or no damage (Bateman, 1961). Field tests with two isolates of *R. solani* revealed variation among isolates derived from lettuce and tomato. Disease caused by the lettuce isolate was more severe at significantly higher moisture levels (80 to 90%) than that of the tomato isolate, which caused severe damping-off at moisture concentrations of 25 to 35% (Abdel-Salem, 1933). Disease incidence was less consistent at 100% saturation. Tests on the effect of relative humidity (RH) conducted in growth chambers confirmed that the rate of mycelial growth decreased at or near 96.5% RH (Sherwood, 1970). The relative effects of moisture on disease development depend on both the cultivar of the host and the isolate of the pathogen.

Plant age also may be a significant factor in disease severity. When two AG-2-2 isolates of *R. solani* were tested against two sugar beet cultivars, root disease severity decreased as the age of the plant increased (Englkes and

Windels, 1994). On the other hand, Green et al. (1999) reported that older leaves of tall fescue had increased susceptibility to *R. solani* 30 hours after inoculation.

CONTROL OF *RHIZOCTONIA SOLANI*

This pathogen is difficult to control. Since *R. solani* is widespread and pervasive, control measures often are limited to a very localized area, such as a specific section of a field. Use of a variety of techniques can eradicate the fungus from greenhouse planting media, plant beds, and even field soils on a restricted basis. Traditionally, crop rotation, soil sterilization, and chemical fungicides have provided adequate control.

Crop rotation may be the oldest form of control for *R. solani* as well as other plant pathogens (Leach and Garber, 1970). Since this fungus persists in crop residues, crop rotation to a non-host crop is an effective means of reducing inoculum in field soils (Ruppel, 1985; Rush and Winter, 1990). In fields where root rot incidence is high, crops such as cotton, potato, alfalfa, and corn have been tested as potential non-host crops. In one study (Ruppel, 1985), 3 to 50% more root rot occurred in sugar beet crops when they were followed by another host crop. Rush and Winter (1990) found that sugar beet following alfalfa had a higher percentage of damping-off (47%) than if following wheat (38%). Cotton and sunflower crops had the lowest stand losses with 30% and 19%, respectively.

There has been some disagreement in the scientific community as to which crops are true non-host crops. Rupple (1985) suggested that alfalfa was a non-host crop, and that there was a definite trend towards increased survival of the pathogen in bean and corn crops. Davis and McDole (1979) stated that populations of *R. solani* in the soil were not reduced by crop rotations. With conflicting results in respect to crop rotation, it is important to consider environmental factors and inoculum sources when establishing cropping sequences.

Sterilization of soils with heat is used mainly in greenhouses or nurseries (Boodley, 1998). Most pathogens, *R. solani* included, are killed when subjected to temperatures of 60°C for approximately 30 minutes (Boodley, 1998; Leach and Garber, 1970). Steaming is used primarily to kill disease-causing pathogens and weed seeds; however, there are other advantages, as well. It is a safe and efficient means of controlling pathogens. One drawback of this method is that excess steaming can cause a buildup in the soil of certain minerals such as manganese that can be toxic to plants (Boodley, 1998). In field situations, steamed soil is very susceptible to re-introduction of a pathogen. If the pathogen is introduced into the sterile soil, it multiplies rapidly, as the natural competition has been eliminated.

Chemical fungicides are commonly used to control Rhizoctonia diseases. Effective chemicals include benomyl, captan [N- (trichloromethylthio)-4-cyclohexene-1, 2 dicarboximide], methyl bromide, and terraclor

(pentochloronitrobenzene) (Bateman, 1961; Csinos and Stephenson, 1999; Leach and Garber, 1970). Methyl bromide is a fumigant that has been used to sterilize outdoor seedbeds and field soils under plastic prior to planting high value crops. Due to its effect on the ozone layer, use of methyl bromide has been banned in the U.S. after 2005. Csinos and Stephenson (1999) suggested that carbamate fungicides offer the best control of Rhizoctonia diseases of tobacco seedlings. In the field, flutolanil, iprodine (Burpee, 1998), and tebuconazole were active in reducing Rhizoctonia diseases in tall fescue and tobacco. Davis et al. (1997) reported that cotton seed treated with myclobutanil was effective in reducing Rhizoctonia seed rot, but these seedlings were more susceptible to *Pythium* spp.

Considering the environmental and economical costs of using chemicals and the limitations of other control methods due to the ability of *R. solani* to colonize crop residues, development of additional disease control strategies are necessary. In the last 25 years, there has been a marked effort to develop effective alternative methods for controlling Rhizoctonia diseases. Such practices include adding organic amendments to the soil, soil solarization, biological control, and cultivar resistance.

Research has been conducted on biological control using seed and soil drenches to control *R. solani* on tomato and pepper (Mao et al., 1998). Seeds of both plants were treated with the fungus *Gliocladium virens* (Gl-3) and the bacterium *Burkholderia cepacia* (Bc-F) individually and in combination.

Treatments with individual agents reduced damping-off in peppers, but only the combined treatment of GI-3 + BcF resulted in stands that were similar to the uninfested control. After supplementary root drenches, plant fresh weight was significantly greater and disease severity was significantly less than the untreated infested controls. Chickpea seed bacterization with *Pseudomonas fluorescens* NBRI1303 increased germination of seedlings by 25% (Nautiyal, 1997). In addition, chickpea root colonization by *P. fluorescens* decreased root colonization by fungal pathogens. One major concern, however, regarding the use of bacteria as biological control agents is that rhizosphere colonization by bacterial populations has been inconsistent in field trials (Handelsman and Stabb, 1996; Hoitink and Boehm, 1999; Nautiyal, 1997; van Loon et al., 1998).

Biological control is complicated, particularly in the case of root-colonizing microorganisms, due to the complexity of the rhizosphere environment. Concerns have been raised about the proper approaches to sustained use of biological control agents in the soil. When relying on bacteria as a form of biological control, there are many factors that need to be considered. Hoitink and Boehm (1999) suggested that the concentration and availability of nutrients within the rhizosphere play a key role in the ability of a biological control agent to effectively control pathogens.

When available, disease-resistant cultivars are the most promising and cost-effective method of lessening the impact of *R. solani* on a given crop. Assessment of host resistance is based in part on the quantification of

symptoms, growth of the plant, or growth and development of the pathogen (Green et al., 1999). Results of numerous studies have shown that there are host plants that exhibit genetic resistance to a particular pathogen that occurs in nature. There are also a variety of economic crops that are consistently and significantly less susceptible to Rhizoctonia diseases (Keinath and Farnham, 1997; MacNish and Neate, 1996; Wang and Davis, 1997; Yang et al., 1992). In the case of tall fescue cultivars Rhizoctonia blight reduced size and expansion of lesions were two components responsible for the greater level of resistance of the cultivar Kentucky 31, as compared to Mojave (Green et al., 1999). Additionally, Kentucky 31 has a greater leaf width, which resulted in a lower proportion of dead tissue to healthy tissue as compared to the slimmer leaf of the Mojave cultivar.

Keinath and Farnham (1997) assessed resistance in several *Brassica oleracea* cultivars, including cauliflower, broccoli, cabbage, and collard, to wirestem caused by *R. solani*. The authors found that of cauliflower, cabbage, and collard, each group had one cultivar that was more susceptible and one cultivar that was less susceptible to wirestem. However, the two most susceptible cultivars were cauliflower, which had been described previously as being only mildly susceptible to wirestem (Keinath and Farnham, 1997). Of the 12 cultivars screened for wirestem resistance, Arctic cauliflower, Blue Max collard, and Viking broccoli were moderately resistant.

In many of the reports on host resistance, there is significant genetic variation among cultivars in response to susceptibility to Rhizoctonia diseases. Leach and Webb (1993) tested five potato cultivars and 26 clones against black scurf caused by *R. solani*. A moderate degree of resistance was identified in three of the russet lines, which had significantly fewer malformed tubers than other russet types. The round white potato types had the lowest resistance and the greatest disease severity. Four russet types were regarded as significantly more resistant to stem lesions, and two russet types produced tubers virtually sclerotia-free. Overall, Leach and Webb were able to identify one potato clone that was significantly more resistant to black scurf; however, yields were inconsistent, making its marketability low.

Differences in susceptibility to Rhizoctonia fruit rot have been reported among a number of tomato cultivars. Bassi et al. (1979) tested two tomato cultivars for potential resistance to fruit rot caused by *R. solani*. Based on histological studies, Bassi et al. suggested that PI 193407 was resistant. While direct infection of a susceptible cultivar (C-28) occurred with the development of an infection peg, no infection peg was formed with the resistant line. Johnson (1981) also concluded that PI 193407 was partially resistant to fruit rot as measured by a delay in infection and slow lesion development.

ENTOMOPATHOGENS AS CONTROLS FOR PLANT PATHOGENS

Some insect pathogens have been recognized as potential agents for biological control of plant pathogens. The ubiquitous entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has been tested as a biological control agent against a variety of economic insect pests, including Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Jaros-Su et al., 1999), European corn borer (*Ostrinia nubilalis* Hübner) (Bing and Lewis, 1991), Southern corn rootworm (*Diabrotica undecimpunctata howardi* Barber) (Pereira and Roberts, 1990), Japanese beetle (*Popilla japonica* Newman) (Steinhaus, 1975) and whitefly (*Trialeurodes vaporariorum* Westw.) (Poprawski et al., 2000).

There is conclusive evidence that *B. bassiana* can successfully colonize plants and that some protection from insect herbivory is provided. Bing and Lewis (1992) first reported that the fungus persisted on the phylloplane of certain genotypes of corn. Applied to the whorl-stage of corn, *B. bassiana* colonized the plants and moved upward in the pith of the plant where it was most frequently found at the internode below the primary ear, above where the conidial suspension was applied. Although the hyphae were found within the vascular elements of the plant, it apparently caused no adverse effects. In additional field trials on three different growth stages of corn (whorl stage, late-whorl stage and pretassel stages), damage by European corn borer larvae was significantly less on plants treated with *B. bassiana* at the pretassel stage than on plants inoculated at either of the two earlier stages (Bing and Lewis, 1993). Application

of *B. bassiana* significantly reduced larval feeding damage, but protection did not persist throughout the growing season.

Beauveria bassiana also colonizes potato endophytically under field conditions and persists for extended periods of time (Jones, 1994). With the use of light and electron microscopy, Wagner and Lewis (2000) confirmed that *B. bassiana* could live as an endophyte, observing that hyphae of *B. bassiana* occurred between parenchyma cells. *Beauveria bassiana* produces mycotoxins, which have been demonstrated to have general antibiotic and antifungal activity (Copping and Menn, 2000; Genthner et al., 1994; Gupta et al., 1995). Some of the known toxins include beauvericin, oosporein, and cyclosporin. When tested against bacteria, oosporein inhibited growth of *Bacillus subtilis*, *P. aeruginosa*, and *Staphylococcus aureus* (Vining, 1962).

Beauveria bassiana is little studied as a biological control agent of plant pathogens, although it has been shown to inhibit growth of *Fusarium oxysporum* and *Botrytis cinerea in vitro* (Bark et al., 1996). In a study by Bishop (1999), *B. bassiana* had antifungal properties. A methyl cellulose suspension of *B. bassiana* isolate 11-98 was applied to tomato seeds which then were planted in soil infested with *R. solani*. Plants receiving the *B. bassiana* treatment had a significantly greater shoot weight than the infested controls. Pant stands of the tomato cultivar Mountain Spring treated with *B. bassiana* were significantly greater than the infested controls.

Chapter 2

Effect of inoculum rate and form of *Rhizoctonia solani* on disease of tomato seedlings

INTRODUCTION

For studies on disease control or screening cultivars for disease resistance, the optimal level of disease in the untreated control is 50% (Flett and McLaren, 1994). Success in establishing consistent disease incidence and severity with *Rhizoctonia* inoculum has been variable. Differences in mycelial growth rates and sclerotial production among isolates of *R. solani* cause inoculum density and disease-causing potential to be inconsistent (Bishop, 1999; Rollins et al., 1999). A number of different methods of preparing inoculum have been proposed, but few studies have compared forms of inoculum for their ability to cause disease and reported consistent results. For example, McCoy and Kraft (1984) compared techniques and inoculum sources to evaluate pea resistance to stem rot caused by *R. solani.* Although inoculum produced on cornmeal:sand (CM:S) resulted in significant differences between resistant and susceptible pea lines, only sclerotial inoculum could be easily quantified. For this reason, McCoy and Kraft suggested that sclerotial inoculum be used when screening for disease resistance. Keinath (1995) studied the relationship between inoculum density and form on disease incidence in cabbage (*Brassica oleracea* var. *capitata*), using sclerotia and CM:S inoculum. Based on the relationship between wirestem incidence and plant weight to inoculum density, Keinath suggested that 0.5% CM:S or 125 sclerotia/kg of

planting medium was needed to achieve a disease rating of 50% in cabbage. Bishop (1999) compared several forms and rates of *R. solani* inoculum produced on rice and CM:S for effectiveness in causing damping-off and stem cankers in tomato seedlings. Bishop reported significant differences between treatments for both disease incidence and severity. Disease incidence and severity were greatest when the inoculum was added at 2% w/w to the planting medium as 0.5 to 1-mm-size fraction of ground infested rice grains.

The objective of this study was to compare different rates and forms of *R. solani* inoculum produced on rice or CM:S for their effectiveness in causing disease on tomato seedlings.

MATERIALS AND METHODS

Production of *Rhizoctonia* inoculum

Rhizoctonia solani was grown on potato dextrose agar (PDA, 24 g potato dextrose broth and 15 g agar to 1 L deionized water). Plates were incubated for seven days at ambient room temperature (25 to 30°C).

Rice grain inoculum was prepared by the addition of one inoculated Petri dish of PDA to a 500-ml Erlenmeyer flask containing rice grain medium (100 g long grain white rice in 72 ml deionized water; autoclaved on three consecutive days). The fungus was incubated in the rice medium in a growth chamber at 28°C for 12 days. Rice inoculum was air-dried for 48 h under a laminar flow

hood, then the inoculum was ground in a blender and sieved. Whole rice grain inoculum also was produced.

Cornmeal:sand inoculum was prepared with 9 g cornmeal and 300 g clean quartz sand (Bishop, 1999; Keinath, 1997) in 500-ml Erlenmeyer flasks. The flasks containing the CM:S mix were autoclaved for 1 h on two successive days. One-week-old cultures of *R. solani* were flooded with 6 ml of sterile deionized water. The mycelium was scraped loose, and the fungal suspension from one PDA plate was added to a 500-ml Erlenmeyer flask containing CM:S medium. An additional 10 ml of sterile deionized water was added to each flask. The inoculum was incubated in a growth chamber at 28°C for 12 d prior to its addition into planting medium.

Infestation of planting medium

The experiment was designed as a randomized complete block with nine treatments and 10 replicates. The treatments were: 1% 0.5 to 1-mm rice inoculum, 2% 0.5 to 1-mm rice inoculum, 1% 1 to 2-mm rice inoculum, 2% 1 to 2-mm rice inoculum, 4% CM:S inoculum, 8% CM:S inoculum, 1 infested rice grain, 2 infested rice grains, and an uninfested control. With the exception of whole rice grains, inoculum was added to ProMix (Hummert International, Earth City, MO) by weight, mixed thoroughly for 1 min and placed in standard 7.6-cm diameter plastic pots (Hummert International, Earth City, MO). For the whole rice grain inocula, 1 or 2 infested rice grains were added to individual pots of ProMix.

One untreated tomato seed, cv. Better Boy (Novartis Seeds, Downers Grove, IL), was planted per pot. The pots were placed in a growth chamber at 22.5 to 24°C and watered daily. After 2 wk, the plants were removed from the pots and the roots were washed. Disease incidence and severity were recorded. Disease incidence was measured as the percentage of seedlings exhibiting disease symptoms. Disease severity was measured on the following scale: 0 = no symptoms (healthy seedling), 1 = lesion < 2.5 mm, 2 = lesion 2.5 to 5.0 mm, 3 = lesion > 5.0 mm, 4 = lesion girdling the stem, 5 = lesion girdling the stem and constricting the stem (< 75%), 6 = lesion girdling the stem (> 75%), and 7 = dead seedling. Data were analyzed for significance with the mixed procedure of SAS systems software (Version 8.1, SAS Institute, Cary, NC).

RESULTS

In this study, there were significant differences ($P = 0.0001$) between the forms and rates of inoculum for disease incidence and severity (Table 2-1). Disease incidence was greatest with the 1% 0.5 to 1-mm rice inoculum, 1% 1 to 2-mm rice inoculum, and the 4% and 8% CM:S inocula. Disease incidence was significantly lower with the 2% 0.5 to 1-mm rice and 2-rice-grains inocula. Disease severity was greatest with the 1% 1 to 2-mm rice, 4 % CM:S, and the 1-rice grain inocula. Disease severity was lowest with the 2% 0.5 to 1-mm rice and 2% 1 to 2-mm rice inocula. A low level of disease was noted in the uninfested control.

Table 2-1. Effect of inoculum form and rate on disease incidence and severity of tomato seedlings [a].

	Disease incidence (%)		Disease severity (0-7) [b]	
Inoculum	This study [c]	Bishop (1999)	This study	Bishop (1999)
1% 0.5 to 1 mm	100 a [d]	63 ab	3.3 ab	2.4 b
2% 0.5 to 1 mm	60 c	88 a	1.7 c	3.9 a
1% 1 to 2 mm	100 a	63 ab	3.8 a	2.0 bc
2% 1 to 2 mm	90 ab	19 cd	2.3 bc	0.7 cd
4% cornmeal:sand	100 a	25 cd	3.7 a	0.8 cd
8% cornmeal:sand	100 a	50 bc	3.1 ab	2.3 b
1 rice grain	90 ab	69 ab	3.8 a	2.4 b
2 rice grains	70 bc	69 ab	3.6 ab	2.7 ab
Uninfested control	10 d	13 d	0.1 d	0.3 d

[a] *Rhizoctonia solani* was added before planting.

[b] Disease severity was measured on a scale of 0 to 7 where 0 = no symptoms, 1 = lesion < 2.5 mm, 2 = lesion 2.5 to 5.0 mm, 3 = lesion > 5.0 mm, 4 = lesion girdling the stem, 5 = lesion girdling and constricting the stem (< 75%), 6 = lesion girdling the stem (> 75%), and 7 = dead seedling.

[c] The protocol for both studies was similar; there were 10 replicates of each treatment in this study and 16 in the test conducted by Bishop.

[d] Within each column, values followed by the same letter were not significantly different according to a F-LSD test at $P = 0.05$.

DISCUSSION

Although there are several studies using different types of *R. solani* inoculum, few of these reports have compared the different forms of inoculum for their ability to cause disease. Choosing inocula that are easy to prepare and that yield consistent results is important. Keinath and Farnham (1997) compared the effectiveness of CM:S and sclerotia for screening *B. oleracea* for resistance to Rhizoctonia wirestem. Although they found that both types were equally effective in inciting disease, they chose to introduce the inoculum as sclerotia because they found this method easier. In the present study, rice inoculum and CM:S inoculua were compared for consistency and ease of preparation. The rice inoculum was much more time-consuming because of the preparation required before the inoculum could be used. Also, the rice inoculum requires a 36 to 48-h drying period before it can be blended and sieved, which increases the risk for contamination. The CM:S inoculum can be used directly from the flask in which it is grown and requires no drying or sieving. The CM:S was much less time-consuming to prepare than the rice inoculum, which is consistent with Bishop's report (1999). However, Bishop reported that the use of 4 and 8% CM:S resulted in low levels of disease (25 and 50% disease incidence respectively). In this study, both rates of CM:S resulted in 100% disease incidence. A 60% disease incidence was achieved with the 2% 0.5 to 1-mm rice inoculum. Bishop found that the 2% 0.5 to 1-mm rice inoculum resulted in a higher disease incidence

(88%). Also, the 2% 1 to 2-mm rice led to a 10% disease incidence in Bishop's study and a 90% disease incidence in this study.

A number of factors may have caused these differences in results. For example, Bishop's study was conducted in a greenhouse where constant temperatures were difficult to attain. The present study was conducted in the regulated environment of a growth chamber, where the temperature was kept relatively constant throughout the study. In addition, the discrepancy between disease incidences among the inoculum types in the two studies may be attributed to distribution of the infested particles within the planting medium. In the present study, the infested bags of media were shaken for 1 min prior to planting. A time period for distribution of the inocula into the planting mix was not given in the Bishop study. It is likely that an overall higher disease incidence was achieved in the present study due to a more thorough distribution of infested particles within the planting mix.

Disease severity is a consideration when choosing a form of inoculum, especially when testing host susceptibility. When assessing 20 pea cultivars for potential resistance to Rhizoctonia stem rot, McCroy and Kraft (1984) found that resistance ratings of cultivars differed between plants inoculated with the CM:S and sclerotia inoculum types. In this study, the lower rates of ground and sieved inoculum resulted in higher disease severity than the higher densities. These results do not concur with Bishop's findings, with the exception of a higher disease severity rating with the 1% 1 to 2-mm rice compared with the 2% 1 to 2-

mm rice. It is not known why the lower rates caused a greater disease incidence in the present study. The results of this study are incongruous with what we would expect to find with the different particle sizes. Disease incidence is likely to increase linearly as the particle size of the inoculum increases (Keinath, 1995; van Bruggen and Arenson, 1985). Smaller particles have less infective potential because there is a point where the smaller propagules do not have enough energy to be an actively infective unit.

There are a number of factors that can affect the inoculum potential of *Rhizoctonia*. Environment may affect how well the fungus colonizes the medium, which in turn contributes to differences in inoculum density. During disease development, uncontrolled differences in temperature and moisture will result in differences in disease incidence and severity. Although the number of rates of CM:S was limited in this study, due to the ease of CM:S inoculum preparation, the disease-causing potential of lower rates of CM:S inoculum should be explored further in order to achieve a 50% disease incidence.

Chapter 3

Effect of inoculum and cultivar on damping-off of tomato seedlings caused by *Rhizoctonia solani*

INTRODUCTION

Diseases caused by *R. solani* are many and diverse. They include pre- and post-emergence damping-off of seedlings, wirestem, root and stem rot, and stem canker. Damping-off is the most common disease caused by *R. solani* and germinating seedlings are most susceptible to damping-off. In field studies, *R. solani* also significantly delayed seedling emergence and development (van Bruggen et al., 1986). In cases where fungicides are not available or are impractical as a means of controlling disease, disease-resistant cultivars are the most promising and cost-effective method of lessening the impact of *R. solani* on a given crop. Assessment of host resistance is based in part on the quantification of symptoms, growth of the plant, or growth and development of the pathogen (Green et al., 1999). Results of numerous studies have shown that there are naturally occurring host plants that exhibit genetic resistance to a particular pathogen. Specifically, a variety of economic cultivars are consistently less susceptible to Rhizoctonia diseases (Green et al., 1999; Keinath and Farnham, 1997; MacNish and Neate, 1996; Yang et al., 1992).

Keinath and Farnham (1997) assessed resistance in several *Brassica oleracea* cultivars, including cauliflower, broccoli, cabbage, and collard, to wirestem caused by *R. solani*. They found that of cauliflower, cabbage, and

collard, each group had one cultivar that was more susceptible and one cultivar that was less susceptible to Rhizoctonia diseases. Leach and Webb (1993) tested five potato cultivars and 26 clones against *R. solani* black scurf. The white potato types had the lowest resistance, and the greatest disease severity. Four russet types were regarded as significantly more resistant to stem lesions, and two russet types produced tubers virtually sclerotia-free. Overall, Leach and Webb were able to identify one clone that was significantly more resistant to black scurf, however, yields were inconsistent making its marketability low.

Rhizoctonia solani is the primary cause of fruit rots in tomato in the southern United States and screening of tomato lines for resistance has been done. Wagner et al. (1979) studied the host-pathogen interaction of seven different tomato lines in screening for potential resistance to Rhizoctonia fruit rot. Seven fruits from each line were placed in infested soil and kept in dark chambers at 29ºC and 95% RH. After nine days, the fruits were assessed for lesion development. The percentage of infected fruit ranged from 14.3 (Yellow Doyle) to 54.3 (PI 193407) although there was no significant cultivar-pathogen interaction, only varying degrees of aggressiveness on the part of the pathogen. Based on histological studies, Bassi et al. (1979) suggested that PI 193407 was resistant. While direct infection of a susceptible cultivar occurred with the development of an infection peg, no infection peg was formed with the resistant line. Johnson (1981) also concluded that PI 193407 was partially resistant to fruit rot as measured by a delay in infection and slow lesion development.

Bishop (1999) reported significant differences in damping-off of the tomato cultivar Mountain Pride when compared to cultivars Celebrity and Mountain Spring. When challenge-inoculated with *R. solani*, seedling survival rates of Mountain Pride were 27 and 73% greater than Celebrity, and 35 and 75% greater than Mountain Spring, in Trials 1 and 2, respectively.

The objectives of this study were (1) to evaluate two forms of *R. solani* inoculum for ability to cause disease in tomato seedlings and (2) to evaluate 10 commercial tomato cultivars for susceptibility to damping-off caused by *R. solani*.

MATERIALS AND METHODS

Production of *R. solani* inoculum and infestation of planting mix

Rhizoctonia solani was grown on potato dextrose agar (PDA; 24 g potato dextrose broth and 15 g agar to 1 L deionized water). Plates were incubated for seven days at ambient room temperature (25 to 30°C).

Rice grain inoculum was prepared by the addition of one culture of *R. solani* on PDA to a 500-ml Erlenmeyer flask containing rice grain medium (100 g long grain white rice in 72 ml deionized water; autoclaved on three consecutive days). The fungus was incubated in the rice medium in a growth chamber at 28°C for 12 days. Rice inoculum was air-dried for 48 h under a laminar flow hood, then the inoculum was ground in a blender and sieved. Particles from the 0.5 to 1-mm fraction were used to infest the planting mix.

Erlenmeyer flasks (500-ml) containing 3% cornmeal:sand (9 g cornmeal and 300 g quartz sand) (CM:S) substrate were autoclaved for 1 h on two successive days. One-week-old cultures of *R. solani* were flooded with 6 ml of sterile deionized water. A sterile loop was used to scrape the mycelium loose, and the fungal suspension was extracted with a 25-ml pipette. The suspension was then added to a single 500-ml Erlenmeyer flask containing the CM:S medium. An additional 10 ml of sterile deionized water was added to each flask. The inoculum was incubated in a growth chamber at 28°C for 12 d prior to its addition into planting medium.

Approximately 60 L of ProMix-BH (Premier Horticulture, Red Hill, PA) was added to a large plastic bucket and mixed with 12 L of deionized water until a uniform texture was achieved. The moistened ProMix-BH was divided into thirds and placed into separate plastic bags. Infested rice inoculum was added at 1% w/w to one bag, infested CM:S was added to the second bag at 4% w/w, and the ProMix-BH in the third bag was not infested. Each bag was mixed thoroughly by shaking for 3 min to assure even distribution of inoculum.

Experimental design and treatments

Trial 1 was conducted in two parts. The first part was designed as a 3×5 factorial in a randomized complete block with 50 replicates, three soil treatments, and five tomato cultivars. The cultivars were Celebrity, Mountain Delight, Mountain Spring, Mountain Supreme, and Sunpride (Table 3-1). The soil

Table 3-1. Source of untreated tomato seeds.

Cultivar	Source
Carolina Gold	Seeds for the South, Graniteville, SC
Celebrity	Novartis Seeds [a], Downers Grove, IL
Hybrid 882	Seedway, Hall, NY
Mountain Belle	Seeds for the South, Graniteville, SC
Mountain Delight	Seeds for the South, Graniteville, SC
Mountain Fresh	Seeds for the South, Graniteville, SC
Mountain Pride	Novartis Seeds, Downers Grove, IL
Mountain Spring	Novartis Seeds, Downers Grove, IL
Mountain Supreme	Seeds for the South, Graniteville, SC
Sunpride	Seeds for the South, Graniteville, SC

[a] Novartis Seeds is now Syngenta.

treatments were rice and CM:S inoculum of *R. solani* and an uninfested control. The second part was set up identically to the first except that there were 15 replicates and six cultivars tested. The cultivars were Carolina Gold, Hybrid 882, Mountain Belle, Mountain Fresh, Mountain Pride, and Mountain Spring (Table 3-1).

For the second and third trials, the experiment was designed as a 3×10 factorial in a randomized complete block with three soil treatments, 10 tomato cultivars, and 10 replicates. The cultivars tested were Carolina Gold, Celebrity, Hybrid 882, Mountain Belle, Mountain Delight, Mountain Fresh, Mountain Pride, Mountain Spring, Mountain Supreme, and Sunpride. For all trials, uninfested potting mix or mix infested with rice and CM:S inoculum of *R. solani* was placed in standard 7.6-cm diameter plastic pots (Hummert International, Earth City, MO) and one untreated seed was planted per pot. For all trials, the experiment was conducted in a greenhouse and watered once per day.

Data collection and analysis

Emergence data were recorded every seven days after planting (DAP). At 28 DAP, the plants were removed from pots and the roots were washed free of soil. Disease incidence was determined as the percentage of seedlings exhibiting disease symptoms. Disease severity (0 to 7) was measured on the following scale: 0 = no symptoms (healthy seedling), 1 = lesion < 2.5 mm, 2 = lesion 2.5 to 5.0 mm, 3 = lesion > 5.0 mm, 4 = lesion girdling the stem, 5 = lesion girdling the

stem and constricting the stem (< 75%), 6 = lesion girdling the stem (> 75%), and 7 = dead seedling. Data for the two parts of Trial 1 were combined for analysis. Data were analyzed for significance with the GLM or mixed procedures of SAS systems software (Version 8.1, SAS Institute, Cary, NC). Significant effects were further analyzed with Fisher's-protected least significant difference (F-LSD) test at $P = 0.05$.

RESULTS

Effect of inoculum

At 7, 14, and 21 DAP, the main effect of inoculum on percent survival of tomato seedlings was significant for Trial 1 ($P = 0.0364$; $P = 0.0364$; $P = 0.0045$), Trial 2 ($P < 0.0001$), and Trial 3 ($P < 0.0001$) (Table 3-2). For all sampling times, the uninfested control had significantly higher survival than the plants exposed to the CM:S or the rice inoculum. There was no significant difference in the percent survival of tomato seedlings planted with CM:S and rice inoculum.

At 28 DAP, the main effect of inoculum on disease incidence and severity of surviving tomato seedlings was significant for all trials ($P < 0.0001$) (Table 3-3). Both infested treatments resulted in significantly greater disease incidence and severity than the uninfested control. A 99 to 100% disease incidence was recorded with both types of inoculum in all trials. A low incidence of disease (1.3

Table 3-2. Effect of inoculum on percent survival of tomato at 7, 14, and 21 days after planting (DAP)[a].

Inoculum	7 DAP			14 DAP			21 DAP		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Uninfested	82 a[b]	80 a	85 a	82 a	80 a	85 a	82 a	92 a	87 a
Cornmeal:sand	63 b	35 b	52 b	63 b	35 b	52 b	56 b	53 b	55 b
Rice	60 b	32 b	56 b	61 b	32 b	56 b	52 b	43 b	63 b

[a] *Rhizoctonia solani* was added before planting.
[b] Within each column, values followed by the same letter are not significantly different according to a F-LSD at $P = 0.05$.

Table 3-3. Effect of inoculum on disease incidence and severity of tomato seedlings at 28 days after planting (DAP)[a].

Inoculum	Disease incidence (%)			Disease severity (0-7)[b]		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Uninfested	2 b[c]	1.3 b	2.5 b	0.02 c	0.0 c	0.1 c
Cornmeal:sand	100 a	100 a	100 a	4.9 a	4.7 a	3.4 a
Rice	99 a	100 a	99 a	4.0 b	3.6 b	2.6 b

[a] *Rhizoctonia solani* was added before planting.
[b] Disease rating was measured on a scale of 0 to 7 with 0 = no symptoms, 1 = lesion < 2.5 mm, 2 = lesion 2.5 to 5.0 mm, 3 = lesion > 5.0 mm, 4 = lesion girdling the stem, 5 = lesion girdling the stem and constricting the stem (< 75%), 6 = lesion girdling the stem (> 75%), and 7 = dead seedling.
[c] Within each column, values followed by the same letter are not significantly different according to a F-LSD at $P = 0.05$.

to 2.5%) was noted for the uninfested control in all three trials. Disease severity of seedlings was greater for CM:S than the rice inoculum in all trials.

Effect of tomato cultivar

The main effect of cultivar on percent survival of germinated tomato seedlings at 21 DAP was significant for Trial 1 ($P < 0.0001$) and Trial 2 ($P = 0.0007$), but not for Trial 3 (Table 3-4). Hybrid 882, Mountain Delight, Mountain Fresh, and Mountain Supreme performed equally well in Trial 1 with seedling survival ranging from 75% for Hybrid 882 to 62% for Mountain Fresh. Mountain Spring and Celebrity had significantly less seedling survival with 44 and 56%, respectively. Cultivars Carolina Gold, Mountain Belle, Mountain Pride, and Sunpride were intermediate in survival. The cultivars that achieved the highest survival rates in Trial 2 included Hybrid 882 and Mountain Supreme. Again, Hybrid 882 had the highest survival rate (87%) of all the tested cultivars. Celebrity, Mountain Belle, Mountain Spring, and Sunpride had the lowest seedling survival ranging form 43 to 50%. Carolina Gold, Mountain Delight, Mountain Fresh, and Mountain Pride were intermediate in seedling survival with 63 to 70%.

The effect of cultivar on disease incidence of surviving seedlings at 28 DAP was not significant in Trials 1, 2, and 3 (data not shown). Disease severity was significant only for Trial 2 ($P < 0.0001$) (Table 3-5). In the surviving seedlings, disease was most severe on Celebrity, Mountain Pride, and Sunpride.

Table 3-4. Effect of cultivar on percent survival of tomato seedlings at 21 days after planting (DAP) [a].

Cultivar	Seedling survival (%)		
	Trial 1	Trial 2	Trial 3
Carolina Gold	60 bc [b]	67 abc	67
Celebrity	56 cd	43 d	53
Hybrid 882	75 a	87 a	80
Mountain Belle	59 bc	50 cd	73
Mountain Delight	71 ab	63 bcd	63
Mountain Fresh	62 abc	70 abc	77
Mountain Pride	59 bc	67 abc	77
Mountain Spring	44 d	50 cd	57
Mountain Supreme	67 abc	80 ab	73
Sunpride	58 bc	50 cd	63

[a] *Rhizoctonia solani* was added before planting.

[b] Within each column, values followed by the same letter or no letter are not significantly different according to a F-LSD at $P = 0.05$.

Table 3-5. Effect of cultivar on disease severity on tomato seedlings at 28 days after planting (DAP) [a].

	Disease Severity (0-7) [b]		
Cultivar	Trial 1	Trial 2	Trial 3
Carolina Gold	3.8	2.5 cde [c]	2.1
Celebrity	2.4	3.6 ab	2.3
Hybrid 882	3.9	2.4 de	2.3
Mountain Belle	3.5	1.6 e	1.4
Mountain Delight	2.3	2.9 bcd	2.3
Mountain Fresh	3.8	2.4 de	1.6
Mountain Pride	3.9	3.9 a	2.0
Mountain Spring	3.3	2.7 bcd	2.0
Mountain Supreme	3.0	2.4 de	2.2
Sunpride	1.8	3.2 abc	2.2

[a] *Rhizoctonia solani* was added before planting.

[b] Disease rating was measured on a scale of 0 to 7 where 0 = no symptoms, 1 = lesion < 2.5 mm, 2 = lesion 2.5 to 5.0 mm, 3 = lesion > 5.0 mm, 4 = lesion girdling the stem, 5 = lesion girdling the stem and constricting the stem (< 75%), 6 = lesion girdling the stem (> 75%), and 7 = dead seedling.

[c] Within each column, values followed by the same letter or no letter are not significantly different according to a F-LSD at $P = 0.05$.

Mountain Delight and Mountain Spring were intermediate and Carolina Gold, Hybrid 882, Mountain Belle, Mountain Fresh, and Mountain Supreme had the lowest disease severity ratings.

Effect of the interaction of inoculum and tomato cultivar

At 21 DAP, the effect of the interaction of inoculum and cultivar on percent survival of tomato seedlings was significant in Trials 1 ($P < 0.0001$) and 2 ($P = 0.0304$). For both trials, there was no difference in seedling survival among the cultivars planted in uninfested soil (Tables 3-6 and 3-7). In Trial 1, cultivars with the highest survival rates with rice inoculum were Hybrid 882 and Sunpride. Cultivars in the intermediate range included Carolina Gold, Mountain Belle, Mountain Delight, and Mountain Fresh. Cultivars with the lowest survival were Celebrity, Mountain Pride, Mountain Spring, and Mountain Supreme. With the CM:S inoculum, cultivars with the greatest survival rate were Hybrid 882 and Mountain Supreme. Cultivars in the intermediate range included Carolina Gold, Mountain Belle, Mountain Delight, and Mountain Pride. Four cultivars had the poorest survival: Celebrity, Mountain Fresh, Mountain Spring, and Sunpride.

For Trial 2 (Table 3-7) with the rice inoculum, Hybrid 882 and Mountain Supreme had the greatest survival rates (90%). Carolina Gold, Mountain Delight, Mountain Fresh, Mountain Pride, and Sunpride were intermediate and Celebrity, Mountain Belle, and Mountain Spring had significantly lower seedling survival rates. Greatest survival with the CM:S inoculum was achieved with Carolina

Table 3-6. Effect of inoculum and cultivar on percent survival of tomato seedlings at 21 days after planting (DAP) [a]. Trial 1.

Cultivar	Inoculum		
	Rice	Cornmeal:sand	Uninfested
Carolina Gold	58 abc [b]	54 abc	87
Celebrity	40 cd	47 bc	80
Hybrid 882	78 a	72 a	80
Mountain Belle	54 abc	62 abc	67
Mountain Delight	53 abc	60 abc	100
Mountain Fresh	68 ab	46 c	93
Mountain Pride	44 bcd	68 ab	80
Mountain Spring	26 d	45 c	80
Mountain Supreme	47 bcd	73 a	80
Sunpride	73 a	20 d	80

[a] *Rhizoctonia solani* was added before planting.

[b] Within each column, values followed by the same letter or no letter are not significantly different according to a F-LSD at $P = 0.05$.

Table 3-7. Effect of inoculum and cultivar on percent survival of tomato seedlings at 21 days after planting (DAP) [a]. Trial 2.

Cultivar	Inoculum		
	Rice	Cornmeal:sand	Uninfested
Carolina Gold	40 bc [b]	80 a	80
Celebrity	20 c	30 b	80
Hybrid 882	90 a	70 ab	100
Mountain Belle	10 c	50 ab	90
Mountain Delight	40 bc	50 ab	100
Mountain Fresh	60 ab	60 ab	90
Mountain Pride	30 bc	70 ab	100
Mountain Spring	20 c	30 b	100
Mountain Supreme	90 a	60 ab	90
Sunpride	30 bc	30 b	90

[a] *Rhizoctonia solani* was added before planting.
[b] Within each column, values followed by the same letter are not significantly different according to a F-LSD at $P = 0.05$.

Gold, Hybrid 882, and Mountain Pride (70 to 80%). Four cultivars had lower but not significantly different survival rates. These were Mountain Belle and Mountain Delight with 50%, and Mountain Fresh and Mountain Supreme with 60% survival. Celebrity, Mountain Spring, and Sunpride had the lowest survival, ranging from 10 to 30%.

Seedling survival for each cultivar varied between inoculum types. In Trial 1 (Figure 3-1), cultivars Celebrity, Mountain Delight, Mountain Fresh, and Mountain Spring had seedling survival rates that were significantly reduced in the mix infested with both types of *R. solani* inoculum. Two cultivars Mountain Pride, and Sunpride, had lower survival rates with one inoculum type, but not the other. The seedling survival rate of four cultivars in mix infested with *R. solani* was not significantly different than seedlings in uninfested mix. These were Carolina Gold, Hybrid 882, Mountain Belle, and Mountain Supreme.

In Trial 2 (Figure 3-2), seedling survival of infested treatments was not different than the uninfested control for Hybrid 882, Mountain Fresh, and Mountain Supreme. Survival rates were significantly lower in both types of infested mix than the uninfested control with Celebrity, Mountain Belle, Mountain Delight, Mountain Spring, and Sunpride. Survival rates in the uninfested control and the CM:S treatment were not significantly different, but were significantly greater than the rice treatment with Carolina Gold.

For the interaction between inoculum type and cultivar, disease incidence was not significant for any of the three trials (data not shown). For disease

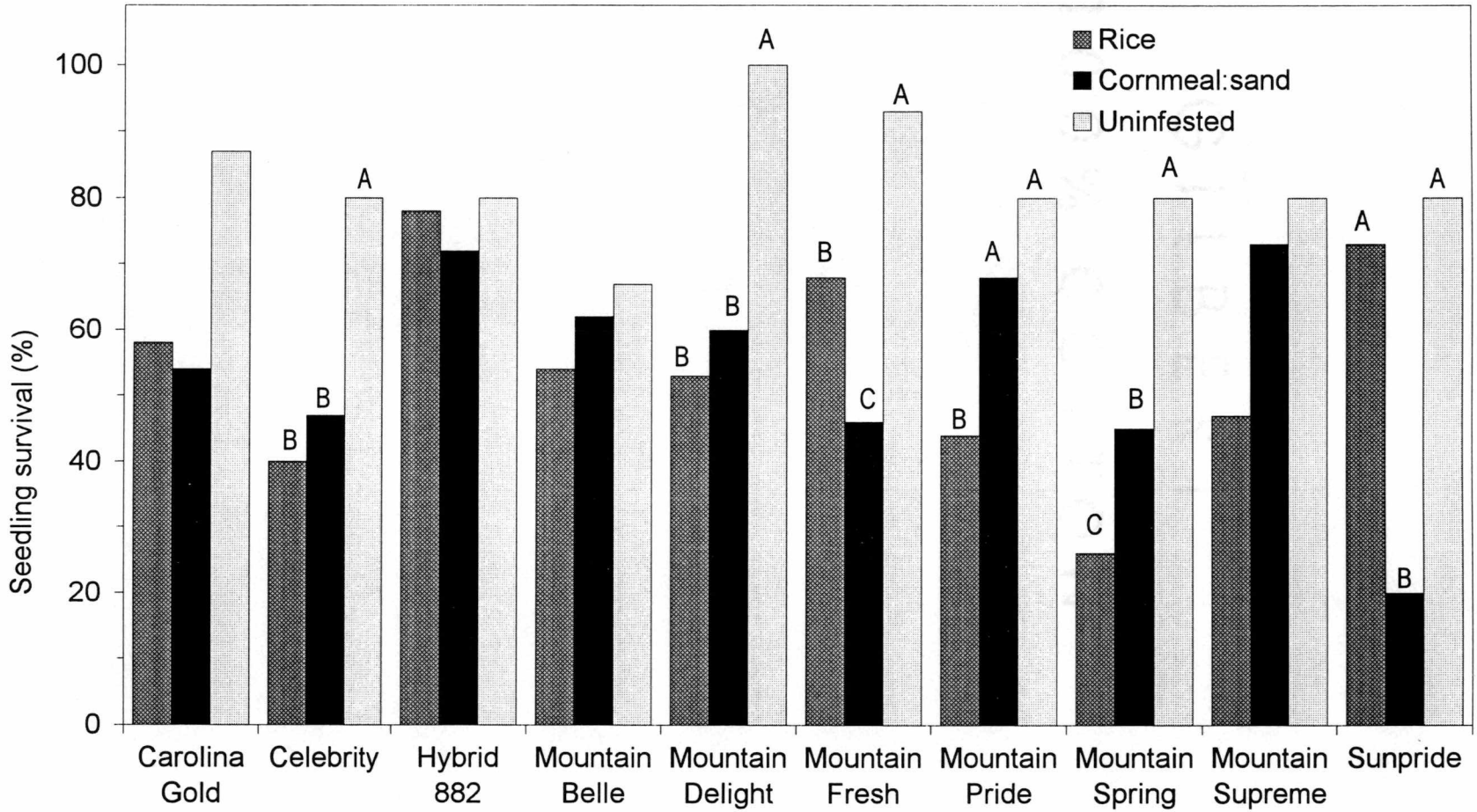

Figure 3-1. Effect of inoculum and tomato cultivar on seedling survival at 21 days after planting (DAP), Trial 1. Within each cultivar, bars with the same letter or no letter are not significantly different according to a F-LSD at $P = 0.05$.

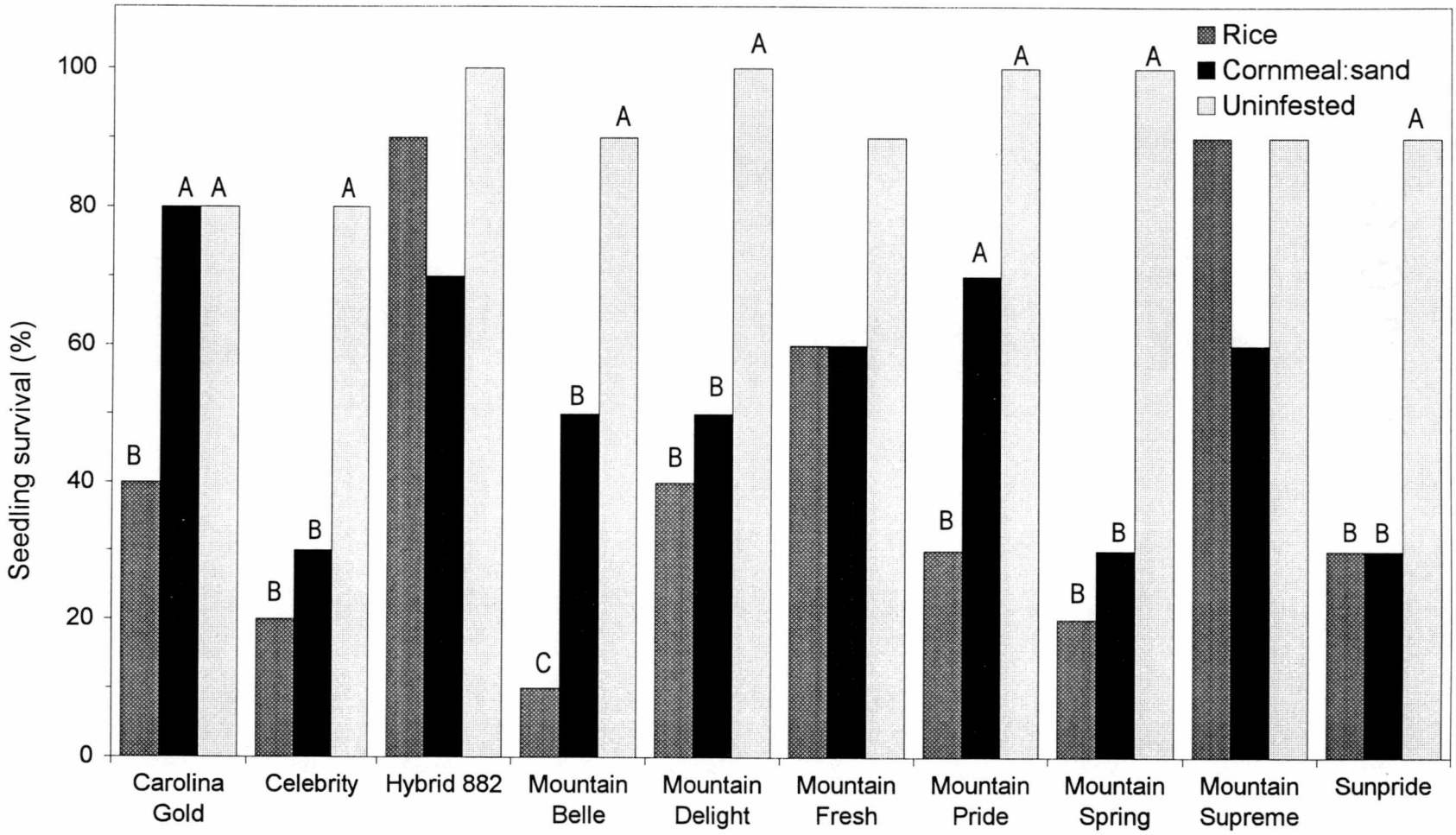

Figure 3-2. Effect of inoculum and tomato cultivar on seedling survival at 21 days after planting (DAP), Trial 2. Within each cultivar, bars with the same or no letter are not significantly different according to a F-LSD at $P = 0.05$.

severity, there were no significant differences between surviving plants in Trial 3. The interaction was significant for disease severity in Trial 1 ($P = 0.0173$) and Trial 2 ($P < 0.0001$) (Figures 3-3 and 3-4). With all cultivars in both trials the disease rating of one or both inoculated treatments was significantly greater than the uninfested control. In Trial 1 (Figure 3-3), there was a difference between rice and CM:S inoculum for five cultivars. For Celebrity, disease severity was greater with rice inoculum, while it was greatest with CM:S inoculum for Carolina Gold, Mountain Belle Mountain Fresh, and Mountain Spring. For Trial 2 (Figure 3-4) the CM:S inoculum resulted in a significantly greater disease rating in Hybrid 882, Mountain Belle, Mountain Delight, Mountain Supreme, and Sunpride. Disease severity was greater in Mountain Pride with rice inoculum. Mountain Belle was the only cultivar where the rice treatment and the uninfested control were not significantly different from one another.

DISCUSSION

The first objective of this study was to establish which form of *R. solani* inoculum, rice or cornmeal:sand, was more reproducible and would yield consistent results. Different forms of inocula have been described as more or less effective in inciting disease, or easier and faster to prepare. In 1999, Bishop reported that CM:S was less time-consuming to prepare than rice inoculum. He also reported that although both forms of inocula were equally effective in inciting

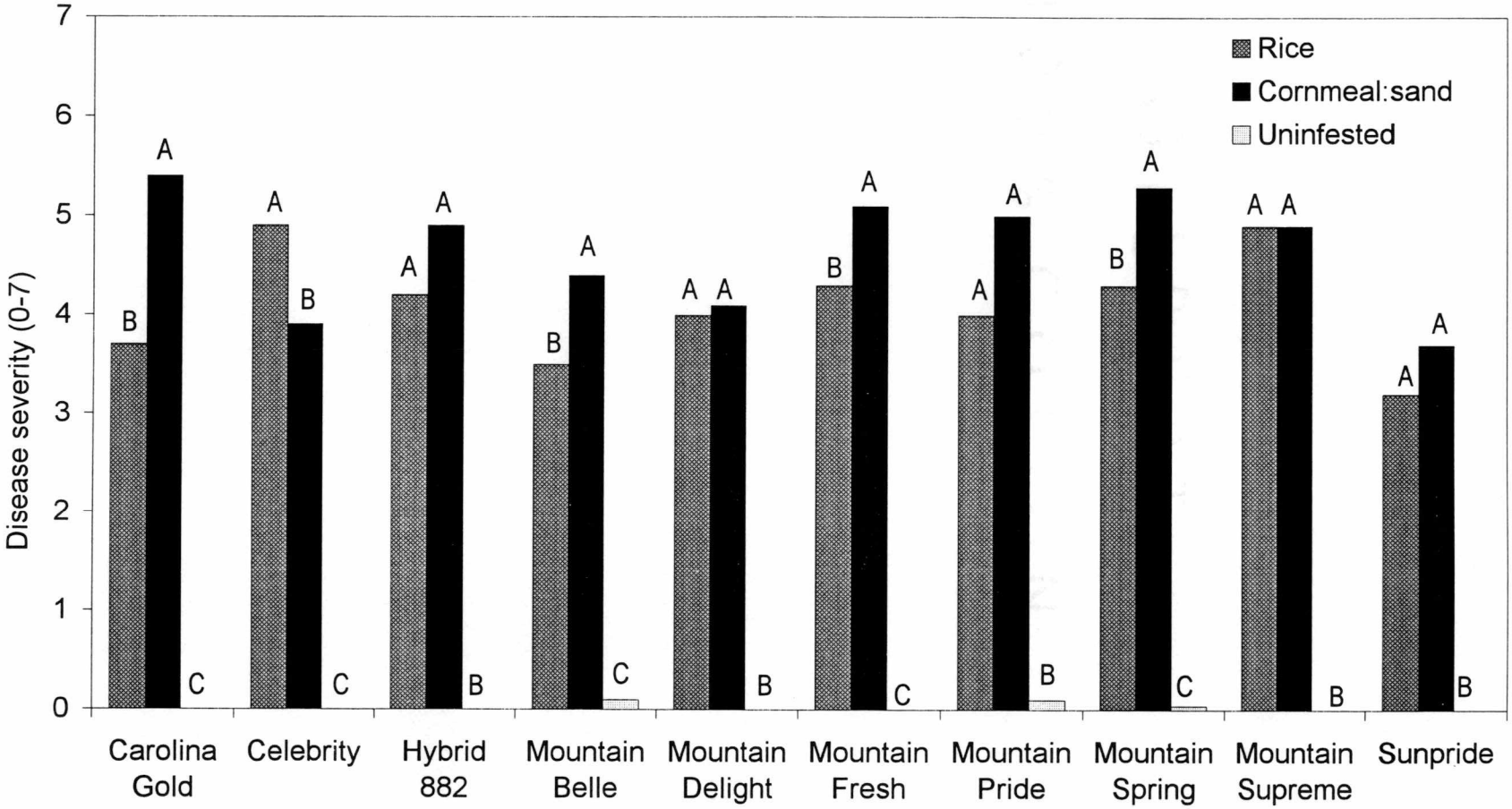

Figure 3-3. Effect of inoculum and tomato cultivar on disease severity of seedlings at 28 days after planting (DAP), Trial 1. Within each cultivar, bars with the same letter or no letters are not significantly different according to a F-LSD at $P = 0.05$.

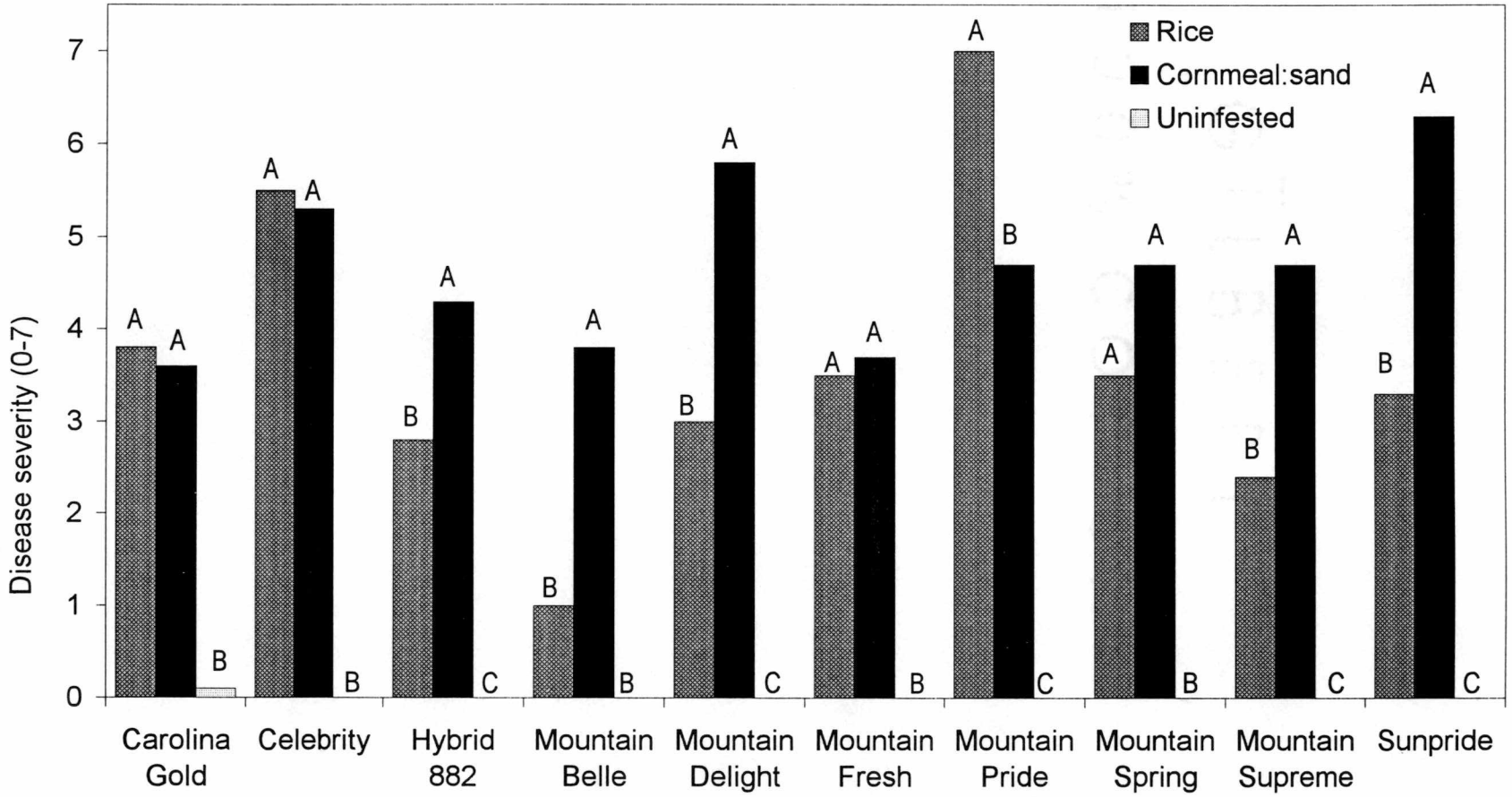

Figure 3-4. Effect of inoculum and tomato cultivar on disease severity of seedlings at 28 days after planting (DAP), Trial 2. Within each cultivar, bars with the same letter or no letter are not significantly different according to a F-LSD at $P = 0.05$.

disease. In the present study there was no difference in disease incidence for rice and CM:S inoculum. Keinath (1995) tested the effectiveness of CM:S and sclerotia produced on autoclaved green beans for screening direct-seeded crucifer crops for tolerance to Rhizoctonia pre- and post-emergence damping-off. With both the CM:S and sclerotia inocula, pre-emergence damping-off of cabbage seedlings was more severe at the higher inoculum densities. Keinath found that results were more reproducible with the sclerotial inoculum, and he suggested that sclerotia be used in order to achieve a consistent 50% disease incidence.

In the present study, disease severity ratings were generally higher with CM:S inoculum. This is consistent with results reported in Chapter 2 for CM:S at 4% and rice inoculum at 1%. The CM:S inoculum resulted in a disease severity rating of 3.7 while rice at 1% produced a rating of 3.3. In contrast, Bishop (1999) found that rice at 1% produced a much higher disease severity rating (2.4) than did the 4% CM:S (0.8). There are a number of reasons that the disease incidence and severity in this study may differ from other studies. Environment may affect how well a fungus is able to colonize a substrate and thus contribute to differences in inoculum density, disease incidence, and severity. The number of rates of CM:S were limited in both the present study and in the study in Chapter 2. In the future, lower rates of inoculum should be evaluated in order to achieve a 50% disease incidence and more consistent disease severity ratings.

The second objective of this study was to evaluate 10 commercial tomato cultivars for susceptibility to *R. solani*. For the most part, cultivars tested in this study varied in response to disease incited by this pathogen but none were substantially resistant. However, significant differences were observed between cultivars throughout all three trials. The cultivar that performed best in the infested treatments was Hybrid 882. In each trial, overall seedling survival for Hybrid 882 was greater than any other cultivar. In addition, Hybrid 882 was the only cultivar that consistently grew out of severe stem lesions in the first few weeks after planting. In some instances, the stem was 100% girdled, but new roots emerged above the diseased tissue and the seedling survived. Similar symptoms would most likely cause other cultivars to die. Besidess Hybrid 882, four cultivars had survival rates above 40% with both inocula in two trials: Carolina Gold, Mountain Delight, Mountain Fresh, and Mountain Supreme. Cultivars that appeared highly susceptible to pre-emergence damping-off were Celebrity, Mountain Spring, and Sunpride. Two cultivars, Mountain Belle and Mountain Pride, gave inconsistent results. Disease incidence was not different in any of the trials, and was fairly uniform throughout the 10 cultivars. Disease severity was also fairly uniform; however, Mountain Belle had a low disease severity rating in all three trials.

While some of the variation between cultivars may be due to different rates of germination and development, seedling survival rate is still an important factor when considering potential resistance in field crops. As most of the

significant differences between cultivars are established in the first 7 to 14 DAP with pre-emergence damping-off, it is important to note which cultivars have higher survival rates and which perform poorly under disease pressure.

While none of the cultivars tested in the present study had been previously evaluated for resistance to *R. solani*, some of the cultivars have been tested for resistance to other soilborne pathogens. Carolina Gold, Mountain Belle, Mountain Fresh, Mountain Pride, and Mountain Spring have resistance to race 1 of *Verticillium dahliae* and races 1 and 2 of *F. oxysporum* f. sp. *lycopersici* (Gardner, 1982; Gardner, 1992; Gardner, 1993; Gardner, 1999; Gardner, 2000). Mountain Supreme has some resistance to *Alternaria solani* (Gardner and Shoemaker, 1999). A few tomato cultivars have been tested for fruit rot resistance. However, when assessing potential fruit rot resistance, Bassi et al. (1979) and Johnson (1981) used different criteria to assess resistance. The present study and previous studies on *Brassica* sp. by Keinath (1995) and Keinath and Farnham (1997) used seedling pre- and post-emergence seedling survival as a measure for potential resistance in tomato, while Bassi et al. (1979) and Johnson (1981) measured resistance as a delay in infection and lesion development.

The cultivars evaluated in this study should be retested with lower rates of *R. solani* inoculum. Rhizoctonia diseases are largely controlled by fungicides at a detriment to the health of the soil and the environment (Castillo et al., 2000; De Jong, 1998; Sittig, 1985; Sultan et al., 2001). When available, disease-resistant

cultivars are the most promising and cost effective method of lessening the impact of *R. solani* on a given crop.

Chapter 4

Effect of *Beauveria bassiana* on control of pre- and post-emergence damping-off caused by *Rhizoctonia solani* on tomato

INTRODUCTION

As chemical pesticides become increasingly less economical and environmentally unsound, interest in alternative pest control methods has been growing. The ubiquitous entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has been tested as an effective biological control agent against a variety of insect pests including Colorado potato beetle (*Leptinotarsa decemlineata*) (Jaros-Su et al., 1999), European corn borer (*Ostrinia nubilalis*) (Bing and Lewis, 1991), and Japanese beetle (*Popilla japonica*) (Steinhaus, 1975).

Beauveria bassiana is known to endophytically colonize a variety of field crops (Jones, 1994; Bing and Lewis, 1991; Bing and Lewis, 1992; Wagner and Lewis, 2000). For example, *B. bassiana* has been reported to colonize potato under field conditions and persist for extended periods of time (Jones, 1994). Field trials conducted by Bing and Lewis (1992) demonstrated that *B. bassiana* was able to endophytically colonize corn on three different growth stages; whorl stage, late-whorl stage, and pretassel stage. Damage by European corn borer larvae was significantly less on plants treated with *B. bassiana* at the pretassel stage than either of the other two stages. With the use of light and electron microscopy, Wagner and Lewis (2000) confirmed that *B. bassiana* could live as

an endophyte in corn. Internal examination of the leaf showed that hyphae of *B. bassiana* are able to grow between parenchyma cells.

Beauveria bassiana produces mycotoxins, which have general antibiotic and antifungal activity (Copping and Menn, 2000; Genthner et al., 1994; Gupta et al., 1995). Some of the known toxins include beauvericin, oosporein, and cyclosporin. When tested against bacteria, oosporein inhibited growth of *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Vining, 1962).

Beauveria bassiana is little studied as a biological control agent of plant pathogens, although it has been shown to inhibit growth of *Fusarium oxysporum* Schlecht.: Fr. and *Botrytis cinerea* Pres.: Fr. *in vitro* (Bark et al., 1996; Langbauer et al., 1996). Reisenzein and Tiefenbrunner (1997) tested five isolates of *B. bassiana* against three plant pathogens, *F. oxysporum*, *Armillaria mellea* (Vahl.: Fr.) Kumm., and *Rosellinia necatrix* Prill. Mycelial growth of each of the three pathogens was significantly reduced. In 1999, Bishop found *B. bassiana* had biological control activity against *R. solani*. *Beauveria bassiana* isolate 11-98 was applied to tomato seed, which were planted in soil infested with *R. solani*. Plants receiving the *B. bassiana* treatment had greater shoot weight than the infested control. Plant stands from cultivars Mountain Pride and Mountain Spring that were treated with *B. bassiana* were significantly greater than their respective infested controls.

The objective of this study was to test the effectiveness of *B. bassiana* as a seed treatment, soil treatment, and a combination seed and soil treatment with

conidia and mycelia for protection of tomato seedlings against damping-off caused by *R. solani*.

MATERIALS AND METHODS

Production of *B. bassiana* mycelia

Yeast extract and dextrose were added at 1% (2.5 g) to four 500-ml Erlenmeyer flasks with 250 ml deionized water. The flasks were autoclaved once for 35 min. The broth was inoculated with spores of *B. bassiana* isolate 11-98. Flasks were placed on a shaker for 3 days at ambient room temperature.

Yeast extract (80 g) and dextrose (80 g) were added to 8 L of deionized water in a Bioflo 2000 Modular fermentor vessel (New Brunswick Scientific, Edison, NJ). The fermentor vessel was autoclaved for 90 min, then was inoculated with one shaker culture of *B. bassiana*. The inoculum was incubated for 4 to 5 days at 24°C with agitation (200) and aeration (3 L/min). The culture was harvested on a 1000-µm sieve (Baxter, McGaw, IL) covered with Whatman #4 filter paper. The filter paper was dampened with deionized water, and then 1 L of the *B. bassiana* inoculum was poured over the filter paper until most of the moisture was removed using vacuum suction. The filter paper with the mycelia was placed on aluminum foil and allowed to partially dry under a laminar flow hood for 10 min.

Preparation of *B. bassiana* alginate prills

A sodium alginate solution (5 g sodium alginate, 10 ml ethanol, and 500 ml deionized water) was prepared as described by MacKenzie et al. (2000). Wheat bran (50 g) was added to 250 ml deionized water. Both the sodium alginate and the wheat bran suspensions were autoclaved for 30 min. Eighty-two g of the fungal mycelium were mixed with the sodium alginate and wheat bran in a high-speed blender for 45 s. The mixture was then added drop-wise to 1 L of gellant solution (0.25 M $CaCl_2$, pH 5.4), which was stirred constantly. Droplets formed small uniform beads upon entering the gellant solution. The beads were separated from the solution with sieving and air-dried for 24 h. This yielded approximately 65 g of dried prills.

Incorporation of *B. bassiana* into planting medium

Twenty liters of dry ProMix (Hummert International, Earth City, MO) and 2.5 L deionized water were mixed. For treatments with alginate prills of *B. bassiana*, 30 g of prills were added per 2 L of moistened planting medium and shaken vigorously for 1 min in a large plastic bag. Bags were placed in an incubator for 2 days at 24 to 27°C.

For treatments with mycelia of *B. bassiana*, 82 g *B. bassiana* mycelia were added to 1.25 L deionized water. The mixture was stirred for 1 min to achieve a uniform suspension and then added to 5 L dry planting medium in a large plastic bag. The bag was shaken for 2.5 min. The inoculated planting medium was then

divided into two equal parts and placed in large plastic bags. The bags were placed in the incubator for 2 days at 24 to 27°C.

Preparation of *B. bassiana* seed treatments

Beauveria bassiana was grown on Sabouraud dextrose agar – yeast (SDAY, 1 L deionized water, 65 g SDA powder, 5 g yeast extract). Plates were incubated at ambient room temperature for 7 days (25 to 30°C). Conidia were harvested aseptically and placed in an airtight container, which was stored at 10°C.

A methyl cellulose solution was prepared by autoclaving 1 L deionized water for 30 min. The hot water was placed on a stir plate with heat and stirring. Twenty g methyl cellulose was added. After a uniform suspension was formed, the mixture was placed in an ice bath until the solution was no longer cloudy, approximately 40 min. Tomato seeds, cultivar Mountain Spring, received methyl cellulose only (0.12 ml methyl cellulose per 20 seeds), conidia and methyl cellulose (0.02 g conidia + 0.12 ml methyl cellulose per 20 seeds), or mycelia and methyl cellulose (0.20 g mycelia + 0.12 ml methyl cellulose per 20 seeds). The seeds were mixed to ensure an even distribution of methyl cellulose and conidia or mycelia. The treated seeds were air-dried under a laminar flow hood for 2-3 h.

Production of *R. solani* inoculum

Rhizoctonia solani was grown on potato dextrose agar (PDA, 24 g potato dextrose broth and 15 g agar to 1 L deionized water). Plates were incubated for 7 days at ambient room temperature (25 to 30°C).

Cornmeal:sand inoculum was prepared with 9 g cornmeal and 300 g clean quartz sand (Bishop, 1999; Keinath, 1997) in 500-ml Erlenmeyer flasks. The flasks containing the CM:S medium were autoclaved for 1 h on two successive days. One-week-old cultures of *R. solani* were flooded with 6 ml of sterile deionized water. The mycelia was scraped loose, and the fungal suspension from one PDA plate was added to a 500-ml Erlenmeyer flask containing CM:S medium. An additional 10 ml of sterile deionized water was added to each flask. The inoculum was incubated in a growth chamber at 28°C for 12 d prior to its addition into planting medium.

Experimental design and treatments

The test was designed as a randomized complete block with 10 replicates and nine treatments. The treatments included: seed treated with conidia, seed treated with mycelia, planting medium treated with alginate prills, planting medium treated with mycelia, seed treated with conidia + planting medium treated with prills, and seed treated with conidia + planting medium treated with mycelia. The three controls were untreated seed in uninfested medium, untreated

seed in infested medium, and seed treated with methyl cellulose in infested medium.

Potting mix treated with *Rhizoctonia* and *B. bassiana,* and controls were placed in 7.6-cm pots and one tomato seed was planted per pot. With the exception of the uninfested control, all planting mix was infested with CM:S inoculum of *R. solani* (4% w/w).

Data collection and analysis

Emergence data were recorded 7, 14, and 21 days after planting (DAP). After 31 days, plants were removed from pots and roots were washed free of planting medium. Fresh shoot and root weights, and disease incidence and severity were recorded. Disease incidence was measured as the percentage of seedlings exhibiting disease symptoms. Disease severity (0 to 7) was measured on the following scale: 0 = no symptoms/healthy seedling, 1 = lesion < 2.5 mm, 2 = lesion 2.5 to 5.0 mm, 3 = lesion > 5.0 mm, 4 = lesion girdling the stem, 5 = lesion girdling the stem and constricting the stem (< 75%), 6 = lesion girdling the stem (> 75%), and 7 = dead seedling. Data were analyzed with SAS systems software (Version 8.1, SAS Institute, Cary, NC). Significant effects were further analyzed with Fisher's-protected least difference test ($P = 0.05$).

RESULTS

Effect of treatment on percent survival of tomato seedlings

The effect of treatment was significant for seedling survival at 7 (P = 0.0064) and 14 (P = 0.0045) DAP, but not for 21 DAP. At 7 DAP, the uninfested control and seed treated with *B. bassiana* conidia + planting medium treated with mycelia had the highest seedling survival rates with plant stands of 80% (Table 4-1). Although not significantly different from either the uninfested or the infested control, seed treated with mycelia, planting medium treated with mycelia, and seed treated with conidia + planting medium treated with prills resulted in plant stands of 40 to 60% at 7 DAP. The three treatments with the *B. bassiana* mycelia produced plant stands that were relatively high (60 to 80%), but only significantly greater than seed treated with *B. bassiana* conidia. The germination of seed treated with *B. bassiana* conidia was delayed by at least 2 wks. At 21 DAP, germination in this treatment increased to 70%.

Effect of treatment on disease incidence and severity of tomato seedlings

The effect of treatment was significant for disease incidence ($P = 0.0022$) and severity ($P = 0.0191$). The uninfested control (1%) was significantly different in disease incidence from all other treatments except seed treated with *B. bassiana* conidia (Figure 4-1). The treatments with the greatest disease incidence were the infested control and the seed treated with *B. bassiana* conidia

Table 4-1. Effect of treatment on percent survival of tomato seedlings at 7, 14, and 21 days after planting (DAP) [a].

Treatment	7 DAP	14 DAP	21 DAP
Uninfested Control	80 a [b]	80 a	80
Infested Control	50 ab	40 abc	40
Methyl cellulose Control	40 abc	50 ab	50
Seed with Conidia	0 c	0 c	70
Seed with Mycelia	60 ab	80 a	80
Medium with Prills	30 bc	50 ab	40
Medium with Mycelia	60 ab	60 ab	60
Seed with Conidia + Medium with Prills	40 abc	30 bc	40
Seed with Conidia + Medium with Mycelia	80 a	70 ab	60

[a] *Rhizoctonia solani* was added before planting.

[b] Within each column, values followed by the same letter or no letter are not significantly different according to a F-LSD at $P = 0.05$.

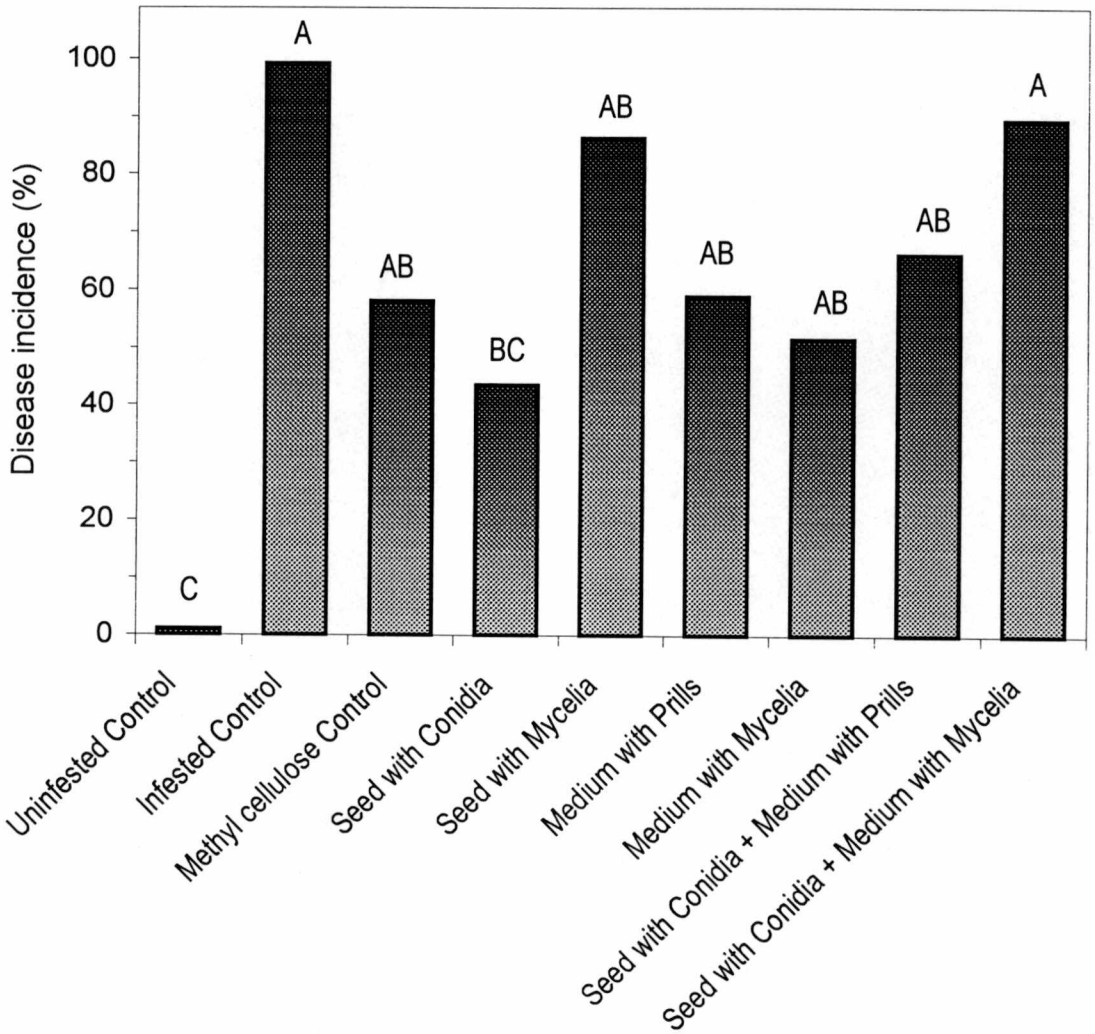

Figure 4-1. Effect of treatment on disease incidence of surviving seedlings at 31 days after planting. *Rhizoctonia solani* was added before planting. Bars with the same letter are not significantly different according to a F-LSD at $P = 0.05$.

+ planting medium treated with mycelia. Disease incidence was lower but not significantly different for these treatments: seed treated with methyl cellulose, seed treated with mycelia, planting medium treated with prills, planting medium treated with mycelia, and seed treated with conidia + planting medium treated with prills.

Disease severity was 0 for the uninfested control (Figure 4-2). Disease severity of the surviving seedlings was greatest for seed treated with mycelia (3.9), but was not significantly different from the two infested controls, or the planting medium treated with prills, seed treated with conidia + planting medium treated with prills, and seed treated with conidia + planting medium treated with mycelia treatments, which ranged from 3.6 to 1.8. The two infested treatments with the lowest disease severity ratings were the seed treated with *B. bassiana* conidia (1.4) and the planting medium treated with mycelia (1.3). Both of these treatments were not significantly different than either the infested or uninfested controls.

Effect of treatment on fresh shoot and root weight

The effect of treatment on fresh shoot weight was significant ($P < 0.0001$). The treatment with the greatest shoot weight was the seed treated with *B. bassiana* conidia + planting medium treated with mycelia. However, it was not significantly different from either the uninfested or infested controls, and the two media treatments (planting medium treated with mycelia and planting medium

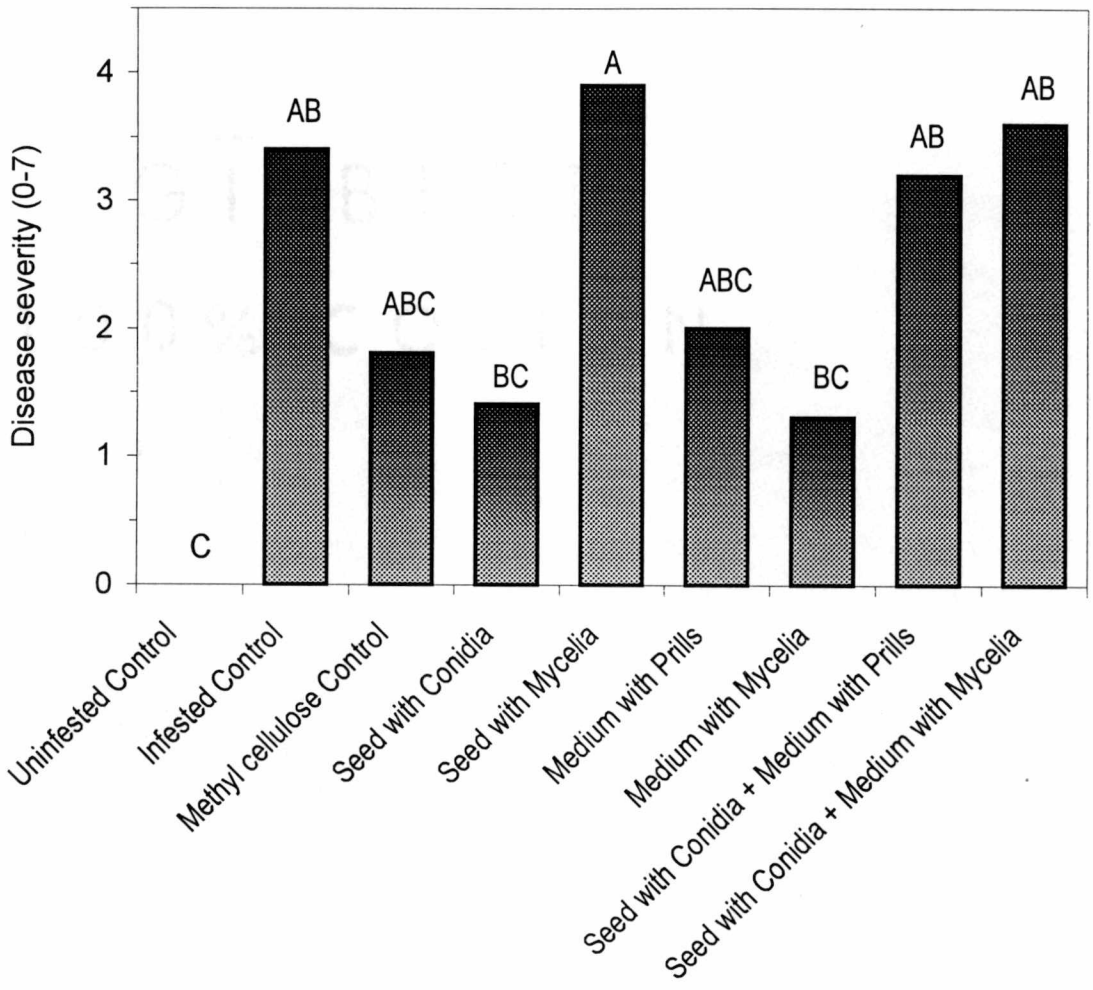

Figure 4-2. Effect of treatment on disease severity of tomato seedlings at 31 days after planting. *Rhizoctonia solani* was added before planting. Bars with the same letter are not significantly different according to a F-LSD at $P = 0.05$.

treated with prills) (Figure 4-3). The methyl cellulose control, seed treated with *B. bassiana* conidia, seed treated with mycelia, and seed treated with mycelia + planting medium treated with prills treatments had lower shoot weights. However, only the seed treated with *B. bassiana* conidia was significantly lower than the controls.

The treatment effect was significant for root weight at $P = 0.0002$. The treatments with the greatest root weight were the planting medium treated with mycelia (1.4 g) and the seed treated with conidia + planting medium treated with mycelia (1.3 g) (Figure 4-4). These were not significantly different from the three control groups. With the exception of the methyl cellulose treatment, these two treatments were significantly greater than the other four treatments. The seed treated with *B. bassiana* conidia and the seed treated with *B. bassiana* conidia + planting medium treated with prills had the smallest root weights and were significantly lower than the infested and uninfested controls.

DISCUSSION

In this study, there was variation in the effectiveness of different forms of *B. bassiana* in reducing pre-emergence damping-off of tomato seedlings grown in medium infested with *R. solani*. Survival of tomato seedlings in infested medium was greatest in the combination treatment of seed treated with *B. bassiana* conidia + planting medium treated with mycelia, and seed treated with mycelia which had the highest survival. The seed mycelial treatment increased

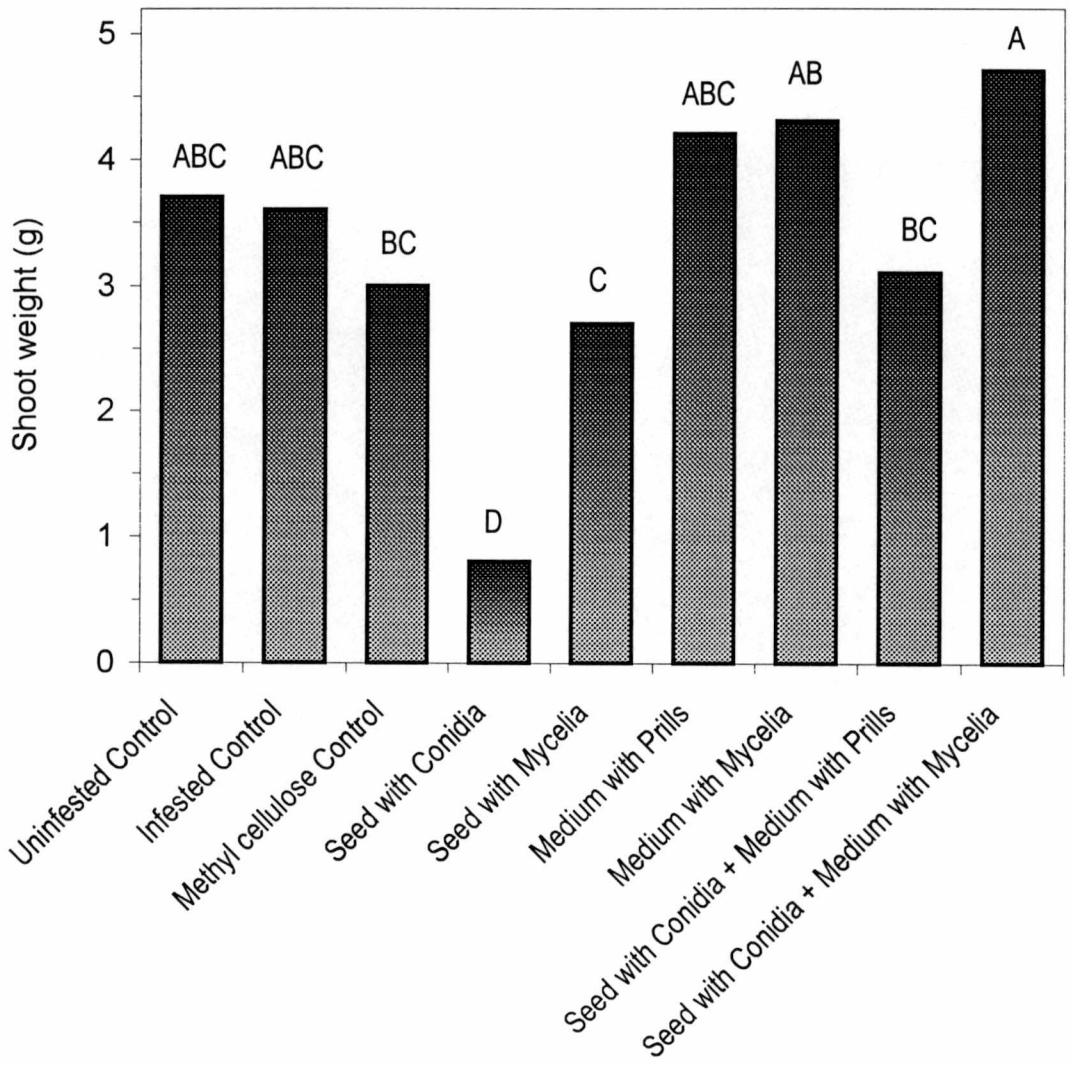

Figure 4-3. Effect of treatment on fresh shoot weight of surviving tomato seedlings at 31 days after planting. *Rhizoctonia solani* was added before planting. Bars with the same letter are not significantly different according to an F-LSD at $P = 0.05$.

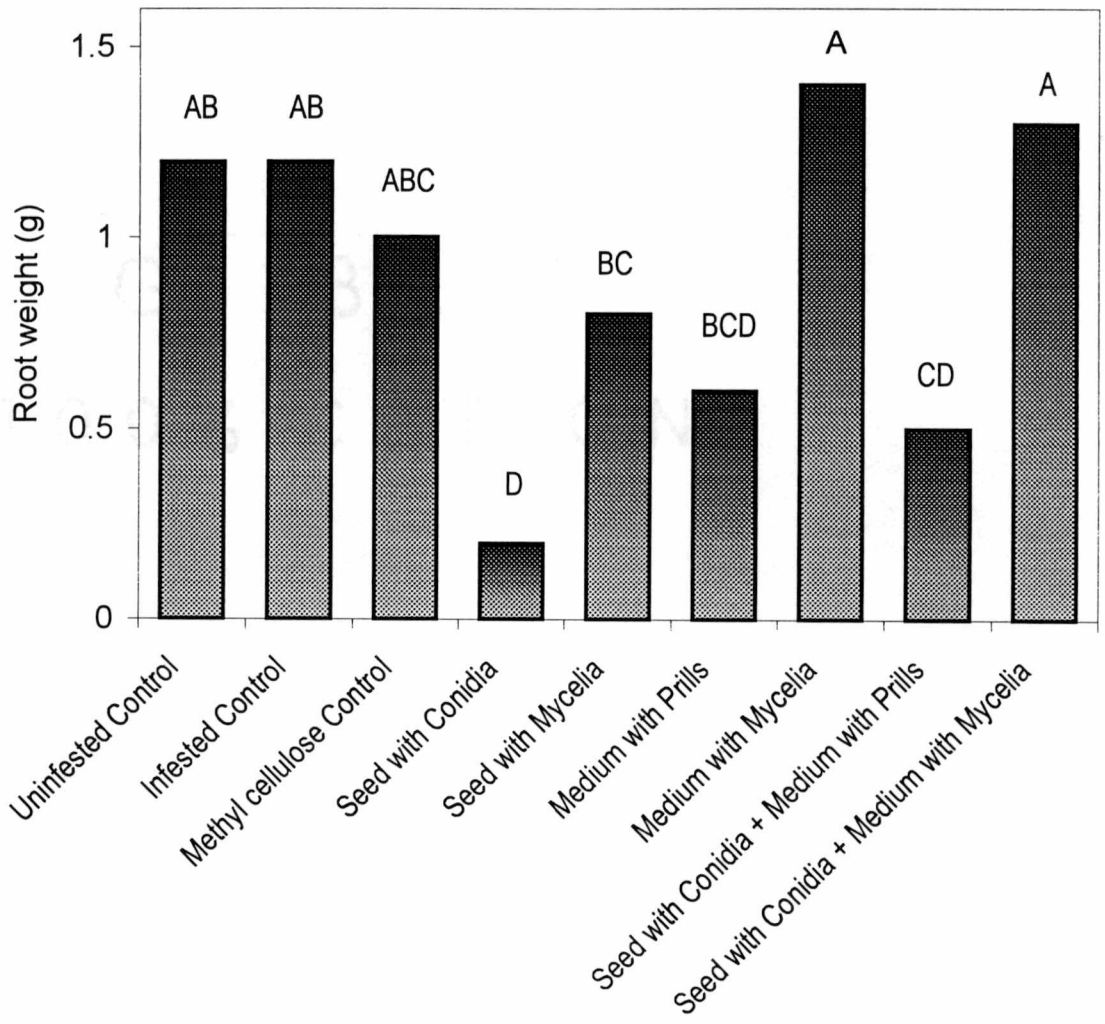

Figure 4-4. Effect of treatment on fresh root weight of surviving tomato seedlings at 31 days after planting. *Rhizoctonia solani* was added before planting. Bars with the same letter are not significantly different according to an F-LSD at $P = 0.05$.

seedling survival from 60 to 80% from 7 to 14 DAP. It is possible that the mycelial seed treatment protected the seeds from both pre- and post-emergence damping-off as the *B. bassiana* became more established in the planting medium. Lewis and Papavizas (1985) tested mycelial and conidial preparations of various species of *Trichoderma* as biological control agents against Rhizoctonia damping-off. Plant stands of cotton and sugar beet were significantly increased when soil infested with *R. solani* was treated with mycelial preparations of *Trichoderma* isolates, but not with the conidial treatments. In addition, Lewis and Papavizas found that mycelial preparations reduced survival of *R. solani* in the soil al least 50%.

Although the conidial seed treatment led to 70% seedling survival at 21 DAP, germination was significantly delayed. It is not known why germination was delayed in these seeds. Compounds produced by *B. bassiana* conidia may have had phytotoxic effects on seedlings. Studies have shown that other biological control fungi also have this effect on plants at high inoculation concentrations. *Trichoderma virens* Pers.: Fr. produces the compounds viridin and gliotoxin, which inhibit germination and root growth of mustard seed at 1 ppm (Bailey and Lumsden, 1998; Lumsden et al., 1992). Phytotoxicity and delayed germination have been reported also in cotton seed treated with high concentrations of *T. virens* (Howell and Stipanovic, 1994). *Beauveria bassiana* has not been reported to be toxic to host plants (Edgington et al, 2000; Roberti et al, 1993; Wagner and Lewis, 2000). However, these studies focused on aerial treatments and not seed

or soil treatments.

In this study, *B. bassiana* was evaluated for disease control under very high disease pressure; therefore, treatments should be re-evaluated with a lower rate of *R. solani* inoculum. In addition, even though the seed conidia treatment resulted in delayed germination of seedlings, this treatment had low disease incidence and severity, and high seedling survival. Further studies should include lower rates of conidia on seed.

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