



Biological control of Rhizoctonia root rot of pansy, petunia and basil
by Kathleen Anne Hickey

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

Montana State University

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Abstract:

Rhizoctonia solani Kuhn can cause a serious disease problem in bedding plant production. It frequently kills young plants and can seriously stunt more mature plants. Several commercially available biological control agents are currently labeled for use against R solani, however their widespread popularity and use is limited. A pretransplant plug treatment method was devised to reduce the amount of product required for each application. Several different pre-transplant biological treatments were evaluated and compared to a pre-transplant PCNB (Terraclor™ 75WP) treatment and post-transplant biological and PCNB treatments.

The eight biological control agents evaluated were Deny™ Liquid-Biological Fungicide (Burkholderia cepacia), Deny™ Liquid-Biological Nematicide (B. cepacia), Gliocladium J1446 powder (Gliocladium catenulatum), Kodiak™ (Bacillus subtilis), Mycostop™ (Streptomyces griseoviridis), RootShield™ Drench (Trichoderma harzianum), RootShield™ Granules (T harzianum), and SoilGard™ (G. virens). Interactions and effects associated with two growing media (Sunshine™ Mix #1 and PGC Mix (soil:sand:peat, 1:1:1 v/v), three host plants (pansy, petunia and basil) and four inoculum levels (uninfested, low, medium and high) were also investigated. Plants were assigned a plant marketability score based on plant size, flowering and presence/absence of disease symptoms. Plant dry weights were also measured.

Of the three host plants, petunia was found to be most vigorous in the presence of the pathogen. Sunshine Mix was shown to increase plant health in all tests, and to reduce disease pressure. The biological control agents were shown to be effective in controlling the disease, especially under conditions of low disease pressure. For plants grown in PGC Mix, the pre-transplant chemical treatment and the '2-day' pre-transplant biological treatment were most effective. For plants grown in Sunshine Mix, the post-transplant biological treatment was most effective. Gliocladium J1446 powder, Deny™ Liquid-Biological Nematicide, Kodiak™, Mycostop™ and Deny™ Liquid-Biological Fungicide were most effective. Recommendations based on this research include: prior to using any control for disease, select a good growing medium that maximizes plant health and reduces disease; consider using a pre-transplant biological or chemical treatment in a preventative disease management program, especially if pasteurized growing media are used; and consider using a post-transplant biological treatment, especially if Sunshine Mix is used.

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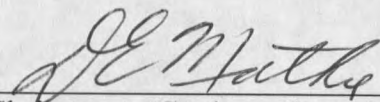
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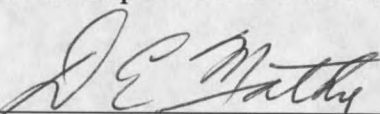
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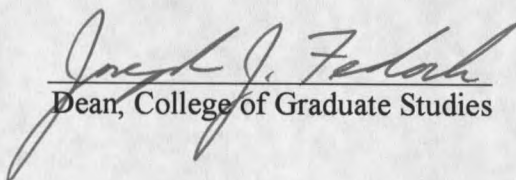
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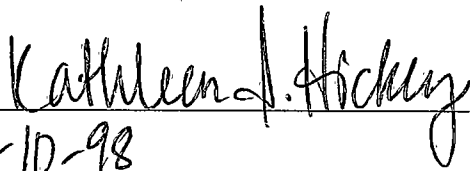
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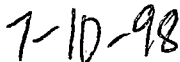
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ABSTRACT

Rhizoctonia solani Kuhn can cause a serious disease problem in bedding plant production. It frequently kills young plants and can seriously stunt more mature plants. Several commercially available biological control agents are currently labeled for use against *R. solani*, however their widespread popularity and use is limited. A pre-transplant plug treatment method was devised to reduce the amount of product required for each application. Several different pre-transplant biological treatments were evaluated and compared to a pre-transplant PCNB (Terraclor™ 75WP) treatment and post-transplant biological and PCNB treatments.

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INTRODUCTION

Bedding plants have been a major component of the U. S. floral and environmental horticulture industries since the late 1940's (20). In 1993, these industries represented 11% of all crop agriculture in the United States. The estimated value of the crops produced by this industry was \$9 billion, which is third in the nation, behind only corn and soybeans (46). Cathey (11) reported in 1994 that gardening is the number one hobby of U. S. citizens. It is a segment of agriculture that can be practiced in all states, by everyone in all communities, with limited resources but with practically guaranteed success. Cathey also reported that the annual growth rate is the fastest in agriculture, at 10% and expected to increase. More than half of this industry is made up of small businesses (9) and has proven to be important to the profitability of the family farm (11).

The U. S. floriculture industry is also important on a world scale. The U. S. ranks within the top three countries in value of production, area in production and size of consumption market (28). In environmental horticulture, "the U. S. is the world's largest producer and market for outdoor landscaping flowers and plants, including trees, shrubs, ground covers, sod or turfgrass, and bedding and garden plants" (18). Grower cash receipts for greenhouse and nursery crops rise steadily each year, a quality which sets it apart from other agricultural crops, in which annual cash receipts vary because of global competition (18).

Bedding plants make up 10.7% of all nursery and floriculture crops, including turfgrass, potted flowering plants, foliage plants and cut flowers and greens (18). In 1992, petunias made up the third highest percentage of flowering plants in the bedding plant crop mix at 9% (53). Impatiens were first with 14% and vegetative geraniums were second with 10%. Pansies were tied for eighth place at 1.6% with nine other bedding plant species. However, pansies are the undisputed most popular annual for winter and spring plantings in the southern U. S. and in other mild climates (31).

Pansies (*Viola x wittrockiana*, family Violaceae) are cool weather plants. Wild violets are shade- and moisture-loving plants. Our common garden variety was developed by crossing a wild European species, *Viola tricolor*, with other closely related Old World species (2). Annual garden pansies are the ideal winter bedding plant. In mild climates, pansies produce a colorful winter display when very few other plants are flowering (4). The ideal temperature for germination is 18 C (65 F) (4,34). Higher temperatures reduce germination and stress mature plants. Production of annual pansy plants from seed requires about 11 weeks. Stress management, sanitation and preventative fungicide use are recommended to produce a well-timed, healthy crop (4). Black root rot, caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris (24), is a problem which has become more serious in recent years (4). Fungicides, applied from transplanting through the production cycle until the crop is finished, are recommended to protect pansies from root and crown diseases.

Petunias (*Petunia x hybrida*, family Solanaceae) were known to be cultivated in America as early as 1880 (10). Petunias are extremely popular plants for summer flower beds, flower boxes and hanging baskets. New varieties are bred for performance in

different climates which has resulted in a multitude of flower shapes, sizes and colors. Of the classes of petunias available, single grandiflora is the most popular because of the size of the flowers, single multiflora is a popular prolific bloomer, and single floribunda have the qualities of a multiflora with added disease and weather tolerance (37). Petunias are native to Brazil, South America (48). The species involved in the formation of the hybrid are disputed, but all were wild species, collected by botanists and spread around the world to nurseries and plant collectors. *P. x hybrida* is a popular research species, primarily used in genetics but more recently included in air pollutant studies, plant biochemistry, protoplast culture and genetic transformation (48). Its relation to tobacco (*Nicotiana*) in the family Solanaceae also makes it valuable for research purposes. In the commercial production of petunias, the ideal germination temperature is 25 C (77 F) (34). Production of petunias from seed requires about 10 weeks. As with pansies, both cultural and chemical means are recommended for control of disease problems.

Sweet basil (*Ocimum basilicum*, family Lamiaceae (44)) is an economically important herb around the world. It is produced in many countries, including the United States. In Italy, where it has been grown year round for over 100 years on small farms, the crop value can reach \$500,000 per ha (\$202,347 per acre) (21). It is valuable fresh, dried, and processed as pesto. In Italy, basil is typically grown in large stands planted directly in the ground, under glass- or plastic-covered greenhouses (21). In the United States, basil is typically produced as a fresh-cut herb or as a potted herb in greenhouses by both large commercial operations and in small family businesses. It is an ideal crop for small businesses due to its high value. Marj Laskey, of Molbak's Greenhouses, Inc., Woodinville, WA (35), reported that sweet basil is their most popular herb to sell and the

least expensive to produce because of its fast growth and quick turnover. Basil is grown, much like bedding plants, from seed with an ideal temperature of 21 C (70 F).

The most important soil-borne diseases of basil are fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *basilici* (Dzidzariya) Armst. & Armst., basal rots, caused most frequently by *Rhizoctonia solani* Kuhn and pythium damping off, caused by *Pythium ultimum* Trow (21). Chemical soil treatments are recommended to avoid disease problems, especially in Mediterranean countries where the crop is intensively cultivated and the same soil is always in continuous production. As with other greenhouse-produced plants, prevention is the key to avoiding disease problems, particularly by the use of certified seed and preventative cultural and chemical means (21). Care has to be taken in the application of any chemical products since basil is a food crop. Residues have to be carefully monitored and few chemicals are actually labeled for use, especially for foliar pests and pathogens. The restrictions associated with applying pesticides to food crops make basil diseases particularly challenging to control successfully.

"Damping-off, crown and root rots are the most common diseases of bedding plants." (43) The three pathogens which are most commonly involved are *Pythium spp.*, *Thielaviopsis basicola* and *Rhizoctonia solani* (29, 43). All of these pathogens can cause serious problems in a greenhouse situation, each with slightly different symptoms and requiring slightly different conditions. The uniform environmental conditions, growing media, crops, and close proximity of plants to each other all lend to a disease easily becoming epidemic. Some production conditions can also exacerbate disease problems. Any type of plant stress can cause latent infections to become rapidly symptomatic and

potentially lethal. The cool, damp conditions of seedling production make it the most common stage plagued by damping off problems. High fertilizer rates have been shown to increase damage due to root rot caused by *P. ultimum* and *P. aphanidermatum* (Edson) Fitzp. in poinsettias (36) and geraniums (22). Gladstone and Moorman (23) attributed the increased losses in seedling geraniums, caused by *P. ultimum*, to nutrient concentration and the level of nutrient salt, regardless of the type of nutrient salt. Conversely, Copes and Hendrix (14) found that a higher $\text{NH}_4\text{:NO}_3$ ratio reduced disease caused by *Thielaviopsis basicola* of pansies grown in sand.

Good sanitation is crucial to disease management, as pathogen survival structures can persist in and on contaminated media, pots, plug flats, tools, benches, floors, and in water supplies. Even as early as 1921, Bewley and Buddin (6) observed that plant pathogens, including *R. solani*, could be isolated from different glasshouse water sources. Sanogo and Moorman (45) showed that *P. aphanidermatum* could be transmitted to healthy cucumber from infested subirrigation nutrient solution. Gutierrez et al. (26) detected viable propagules of *R. solani* in used Styrofoam trays, even after they had been stored for one year and washed with a 1.3% NaOCl solution. Sterilization of the used trays was achieved only with steam or methyl bromide treatments. Other treatments reduced the inoculum density, but not to levels adequate for disease control. In a two-year study of a greenhouse operation in Ohio, Stephens et al. (50) detected both *Pythium* spp. and *R. solani* on used seedling flats, in porous beds and in dust and soil from floors and walkways. In 1994, at the University of Florida, Graham (24) demonstrated that even airborne inoculum of *T. basicola* could infest potting media. Understanding the

pathogens and their ecology is essential to proper disease management, whatever the action taken.

R. solani is the anamorph of the basidiomycete, *Thanatephorus cucumeris*. It is a common inhabitant of soil and can cause disease on a very wide range of hosts (1,12,15). It commonly attacks plant parts in or on the ground. The diseases it causes include damping off, root rot, crown rot, stem rot, stem canker and stem and leaf blights (1,12,15). Young plants are most susceptible to damping off, but mature plants are susceptible to all of the other possible diseases. *R. solani* kills young and mature plants alike. It exists as sterile mycelia which produce resting structures called sclerotia. The fungus persists as mycelia or sclerotia, freely in soil or in infected perennial plant material and residue. In field situations, it may persist indefinitely and prevent the successful growth of susceptible hosts permanently (1). Papavizas et al. (41) showed that *R. solani* is confined to the top 10 cm of field soil, but that it can survive for up to 12 weeks at a temperature of 5 C (41 F) and for up to 40 weeks at a temperature of 10 C (50 F).

Strains of *R. solani* are classified into different anastomosis groups (AG), depending on the ability of the hyphae of different strains to fuse successfully. Most of the strains that cause disease in bedding plants and herbaceous ornamentals are categorized in AG4 and AG1 (15). Some strains exist saprophytically, some parasitically and some as mycorrhizae on various hosts. Perkins and McGee (42) showed that one isolate of *R. solani* (AG6), an orchid symbiont, had a very limited host range and that its distribution was dependent on the distribution of the host. They suggested that they could not detect the fungus away from its host due to an apparent lack of alternate hosts

and probable poor saprophytic survival. Since there are so many different strains of the same species, which vary dramatically in their ecology, the classification scheme for *R. solani* has been investigated by many different scientists in an attempt to determine if further designations are needed. Many of the current approaches used to classify *R. solani* involve molecular techniques (52). These techniques group different isolates definitively, but the new groupings will likely require changes in the current standard classification.

Not all strains of *R. solani* cause disease, but those that do are extremely damaging. Effective control measures have been devised for many plant pathogens, but *R. solani* continues to be difficult to control. Extensive research by scientists at Ohio State University has gone into understanding and developing naturally-suppressive, composted growing media (25, 32, 38, 49). Their research has shown that although several serious pathogens can be sufficiently, if not completely, controlled by using composted bark and peat media, diseases caused by *R. solani* are not controlled consistently enough and that fungicides are still the only reliable control (25). They recommended using fungicides such as thiophanate-methyl (Cleary's™ 3336F), etridiazole-thiophanate-methyl (Banrot™), pentachloronitrobenzene (PCNB) (Terraclor™ 400 and Terraguard™ 50W).

Chemicals have long been known to effectively control diseases caused by *R. solani*. In 1957, Shanks and Link (47) reported that PCNB gave effective control of *R. solani*. Hausbeck and Kusnier (30) reported that damping off in vinca, caused by *R. solani* was prevented by treatments of PCNB (Terraclor™ 400F). In 1993, Becker and Schwinn (3) reported that the status of chemical control of *R. solani* was moderate and

ranked it in moderate need for improvement. Although there are good chemical controls for the pathogen, research into alternate methods of control, including the use of compost-based media and biological control organisms, is providing growers with a broader range of choices for disease management. These alternate methods of control are especially important in the production of food crops such as basil, for reasons stated above.

Pressure by local and national agencies to reduce runoff from greenhouses by reducing chemical use and recycling water (5), and the January 1, 2001 ban imposed on the use of methyl bromide by the U. S. Environmental Protection Agency (51) have initiated an abundance of research on biological control agents. In theory, biological control agents have several advantages over chemical controls (27). They are more disease-specific than chemicals. The use of a disease-specific biological control agent could be more effective than the broad spectrum chemicals commonly used. They may provide long term protection with a single application by growing with the plant and protecting it for life. Their use reduces environmental and water contamination due to chemicals and they present fewer problems associated with worker exposure and re-entry interval requirements.

The biggest challenges associated with the use of biological control agents involve formulation and shelf-life (27). The products are composed of living organisms, which lose their activity when they die. The companies that develop the products have to be just as concerned with keeping the organisms alive as the growers who apply them to their crops. The living organisms, mostly bacteria and fungi, are easily affected by changes in temperature, moisture, the presence of chemical substances, such as

fungicides, herbicides and insecticides, and the presence of competing organisms, such as plant pathogens and other biological control agents. Much success has been achieved by using biological agents for control of soil-borne diseases, since conditions in the rhizosphere are more stable than those around other more exposed plant parts (27). The extensive research in this area has helped make biological control agents more efficient and practical.

The use of biological control agents promises to be most effective in controlling diseases of greenhouse crops. The controlled environment of a greenhouse and the small, uniform types and amounts of growing media provide optimum conditions for the proliferation of living organisms (including plant pathogens, as stated above). As a result, much of the research on biological control agents has originated in the greenhouse and laboratory. Field conditions are much more variable and generally research results are the not same from one test to another. With more and more experience, scientists are continually producing more effective biological control agents. Unfortunately, the production and licensing of these new products is expensive. The main limiting factors in the use of biological control agents are price and efficacy. Efficacy must be as good as the standard chemical control, especially when controlling a pathogen such as *R. solani*, for which there are already sufficiently effective chemical controls.

The purpose of this research was to compare the efficacies of several different biological control agents to a standard chemical control, as applied in the production from seed of two flowering potted plants and a potted herb under normal greenhouse conditions. Different treatment applications, both pre- and post-transplant, were investigated, along with the effects of two different growing media, four inoculum levels

and three host plants. The efficacies of the different treatment applications of each biological control agent and the chemical control are reported. Results of an investigation into measuring the inoculum potential of infested growing media are also reported.

MATERIALS AND METHODS

Biological and Chemical Control Agents

The following biological control agents were evaluated in this research. The rates used are summarized in Table 1. Deny™ Liquid-Biological Fungicide (*Burkholderia cepacia* type Wisconsin) and Deny™ Liquid-Biological Nematicide (*B. cepacia* type Wisconsin) were provided by C. C. T. Corporation, 5115 Avenida Encinas, Suite A, Carlsbad, CA 92008. Both Deny products are liquid formulations of different isolates of the same bacterium in concentrations of at least 2.6×10^8 viable cells/liter (8.8×10^9 viable cells/fl. oz.). They are labeled for use as a seedling transplant drench or in chemigation on vegetables, fruits, nuts, vine crops, herbs, spices, ornamentals, greenhouse crops, turfgrasses, flowers, bulbs, and field crops to control and suppress *Rhizoctonia*, *Fusarium* and *Pythium* (fungicide formulation), and lesion, spiral, lance and sting nematodes (nematicide formulation).

Kodiak™ (*Bacillus subtilis* strain GB03) was provided by Gustafson, Inc., 1400 Preston Rd., Suite 400, Plano, TX 75093. Kodiak is a dry, powder formulation of bacterial spores in a concentration of not less than 5.5×10^{10} viable spores per gram. It is labeled for use as a seed treatment on cotton, seed and pod vegetables, peanuts, soybeans, wheat, barley, corn, and other agricultural seed to suppress *Fusarium*, *Rhizoctonia*, *Alternaria* and *Aspergillus*.

Mycostop™ (*Streptomyces griseoviridis* strain K61) and Gliocladium J1446 powder (*Gliocladium catenulatum* strain J1446) were provided by AgBio Development, Inc., 9915 Raleigh St., Westminster, CO 80030. Mycostop is a dry, powder formulation of fungal spores and mycelia at a concentration of 1×10^8 colony forming units per gram. It is labeled for use as a seed treatment, soil spray, soil drench, transplant dip, cutting dip, and foliage spray on vegetables and floral, foliage and woody ornamentals to control *Fusarium*, *Alternaria* and *Phomopsis*, and to suppress *Botrytis*, *Pythium* and *Phytophthora*.

Gliocladium J1446 powder is an experimental dry, powder formulation of fungal spores and mycelia (concentration not provided). It is recommended for use as a dry or liquid potting mix amendment and soil drench on lettuce, herbs, bedding plants, cucumbers, and tomatoes to control *Rhizoctonia* and *Pythium*.

RootShield™ Granules and RootShield™ Drench (*Trichoderma harzianum* strain T22 (KRL-AG2)) were provided by BioWorks, Inc., 122 N. Genesee St., Geneva, NY 14456. RootShield Granules is a dry, granular formulation of fungal spores and mycelia at a concentration of at least 1×10^7 colony forming units per gram. The granular formulation is labeled for use as a greenhouse or nursery potting mix amendment on all ornamental crops, cabbage, tomato, and cucumber. RootShield Drench is a dry, powder formulation of fungal spores and mycelia also at the concentration of at least 1×10^7 colony forming units per gram. The drench formulation is labeled for use as a soil drench on all ornamental crops, cabbage, tomato, and cucumber. Both formulations protect roots against *Pythium*, *Rhizoctonia* and *Fusarium*.

SoilGard™ (*Gliocladium virens* strain GL-21) was provided by W. R. Grace and Co., 7379 Route 32, Columbia, MD 21044. SoilGard is a granular formulation of fungal spores and mycelia at a concentration of 1×10^6 colony forming units per gram. It is labeled for use as a potting mix amendment on ornamental and food crop plants grown in greenhouses, nurseries, and interiorscapes to control *Pythium* and *Rhizoctonia*.

The chemical fungicide used in this research was Terraclor™ 75WP (Uniroyal Chemical Co., Inc., Middlebury, CT 06749). The active ingredient is pentachloronitrobenzene (PCNB, 75% by weight). The dry, wettable powder is labeled for use as a soil drench and for mechanical incorporation on vegetables, turfgrasses and nursery, greenhouse and field grown ornamentals to control *Rhizoctonia*, *Sclerotium*, *Corticium*, *Plasmodiophora*, *Helminthosporium*, *Sclerotinia*, *Typhula*, *Fusarium*, *Botrytis*, *Stomatina*, *Dothistromia*, *Ovulinia* and *Pellicularia*.

Table 1. Rates of biological and chemical control agents used in the greenhouse efficacy tests.

biological and chemical control agents	rates
Deny™ Liquid - Biological Fungicide	1.25 ml formulation per 1 liter distilled water
Deny™ Liquid - Biological Nematicide	1.25 ml formulation per 1 liter distilled water
Gliocladium J1446 powder	2 g formulation per 1 liter distilled water
Kodiak™	5 g formulation per 1 liter distilled water
Mycostop™	100 mg formulation per 1 liter distilled water
RootShield™ Drench	599 mg formulation per 1 liter distilled water
RootShield™ Granules	low: 350 mg formulation (1/8 tsp) per pot high: 700 mg formulation (1/4 tsp) per pot
SoilGard™	low: 160 mg formulation per pot high: 240 mg formulation per pot
Terraclor™ 75WP (PCNB)	493 mg formulation per 1 liter distilled water

Greenhouse Efficacy Tests

The greenhouse tests were designed to measure the efficacy of the above-mentioned biological control agents under the normal production conditions associated with producing 10 cm (four-inch) potted plants from seed in plug trays. The biological control agents were evaluated on three host plants, in two growing media and at four inoculum levels.

Plants and growing media

The pansy and petunia seeds were provided by Goldsmith Seeds, Inc., P.O. Box 1349, Gilroy, CA 95021. The Genovese basil seeds were purchased from Ornamental Edibles, 3622 Weedin Ct., San Jose, CA 95132. The seeds were sown into #288 square plug trays (TLC Polyform, 13/16" square cells, 1 1/4 " deep) filled with one of the two growing media and kept in an intermittent mist chamber on bottom heat for two weeks. Seedlings were watered by hand with 100 ppm N fertilizer at every irrigation (Peter's Professional General Purpose Fertilizer, 20-20-20) after they were removed from the mist chamber. In order to harden the seedlings, the trays were moved to a plastic-enclosed chamber for the third week, a plastic-enclosed chamber with the top open for the fourth week and then to an open greenhouse bench for the final week. Seedlings were five weeks old when used for experimental purposes. Once transplanted, plants were grown for four weeks or until flowering occurred in both the pansy and petunia.

The plants were grown in one of three different greenhouses, under standardized conditions. Daylength conditions of 14 hour days (lights on 6am-10am and 4pm-8pm) were maintained with metal halide 1000W lamps. The temperature was maintained at 22

C (72 F) day and 18 C (65 F) night. Plants were fertilized at every irrigation with 100 ppm N, Peter's Professional General Purpose Fertilizer (20-20-20 or 20-10-20).

The two growing media used were Sunshine™ Mix #1 (Canadian sphagnum peat moss, perlite, dolomitic limestone, and wetting agent; Fison's Horticulture, Inc., Bellevue, WA 98004) and a soil-based medium (Bozeman silt loam: washed concrete sand: Canadian sphagnum peat, 1:1:1 v/v) prepared by the Plant Growth Center, Montana State University, Bozeman. The soil-based medium (PGC Mix) was pasteurized (Lindig aerated steam system, 90 C (194 F) for 45 min, 22 C (72 F) for 45 min) and passed through a #6 (3.35 mm) sieve prior to use. Sunshine Mix was used unaltered.

Pathogen and inoculum levels

The pathogen, *Rhizoctonia solani* (AG4), is registered with the American Type Culture Collection (ATCC 28268). Inoculum was prepared by adding several large plugs of the fungus grown for several days on PDA (potato dextrose agar, Difco Laboratories, Detroit, MI 48232-7058), to 1 liter canning jars filled with sterilized, moist canola seeds and incubating at room temperature for two weeks. The infested canola seeds were air-dried for several days and stored in a paper bag at room temperature until used. All of the prepared inoculum was used within six months and tested for viability prior to use by placing several infested seeds on PDA and observing the rate of growth.

The growing media were infested to achieve four inoculum levels. Each level was established by counting individual seeds of infested canola (inoculum units) into 10 cm (four-inch) square pots (Press-Fill, 4" black, T. O. Plastics, Inc., 2901 East 78th St., Minneapolis, MN 55425) pre-filled with growing medium and mixing thoroughly. The

four inoculum levels were: high = 25 inoculum units; medium = 15 units; low = 5 units; and an uninfested check = 0 units. Media were infested approximately 24 hours prior to transplant for the tests in which the biological control agents were applied as drenches. Media were infested approximately 48 hours prior to transplant for the tests in which the biological control agents were applied as dry soil amendments.

Experimental design

Two experiments were conducted, each within a different three month period in 1997 (Table 2). Within experiment one there were five tests (tests 1-5) and within experiment two there were eight tests (tests 6-13). One biological control agent was used to make the biological treatments in each test; the test and corresponding biological control agent are listed in Table 2. The same chemical control agent was used to make the chemical treatment(s) in each test. Each test included three replications of each growing medium-host plant-inoculum level-treatment combination. Plants were arranged in three completely randomized blocks with one replication per block.

Table 2. Dates of the experiments and tests included in the greenhouse efficacy tests.

experiment	test	biological control agent	test date
1	1	Deny™ Liquid - Biological Fungicide	4/17/97-7/3/97
1	2	Deny™ Liquid - Biological Nematicide	4/26/97-7/11/97
1	3	Gliocladium J1446 powder	5/26/97-8/5/97
1	4	Kodiak™	5/12/97-8/7/97
1	5	Mycostop™	5/1/97-7/21/97
2	6	Deny™ Liquid - Biological Fungicide	9/19/97-12/2/97
2	7	Deny™ Liquid - Biological Nematicide	9/11/97-11/25/97
2	8	Gliocladium J1446 powder	9/5/97-11/19/97
2	9	Kodiak™	8/21/97-11/4/97
2	10	Mycostop™	8/29/97-11/12/97
2	11	RootShield™ Drench	9/26/97-12/10/97
2	12	RootShield™ Granules	10/3/97-12/17/97
2	13	SoilGard™	10/10/97-12/24/97

Treatment application

The treatments were designed to determine the maximum efficacy of the control agents by adjusting the amount of product applied and the time of application in relation to the time of transplant. The pre-transplant treatments were devised to determine if smaller amounts of product, applied early in production at a time that lends well to mass plant treatment, were equally effective as the standard post-transplant treatments. The pre-transplant treatments were applied to plants while still in plug trays. The post-transplant treatments were made to plants in 10 cm square pots.

Table 3 summarizes the biological and chemical treatments used in each test. For the pre-transplant drench treatments ('7-day', '2-day', '1-day', '0-day', 'chem'), two hundred ml of each formulation were prepared using non-sterile distilled water. Two ml were then applied to each seedling plug with an automatic pipetman. For post-transplant drench treatments ('post', 'pchem'), eight l of each formulation were prepared using non-sterile distilled water, and one hundred ml were then applied to each 10 cm pot. The dry soil amendments ('low', 'high') were added to the growing medium in each 10 cm pot.

Every test included biological, chemical and untreated check treatments. For the test and the corresponding biological control agent used to make the biological treatments, refer to Table 2. In experiment one, tests one through five were conducted (Table 3). The five treatments in each test were (i) a '2-day' pre-transplant biological treatment applied at 48 and 24 hours prior to transplant, so that a total of 4 ml was applied over two days; (ii) a '1-day' pre-transplant biological treatment applied once 24 hours prior to transplant; (iii) a '0-day' pre-transplant biological treatment applied just

prior to transplant; (iv) a chemical pre-transplant treatment ('chem') also applied just prior to transplant; and (v) an untreated check ('none').

Table 3. Biological and chemical treatments applied in each greenhouse efficacy test.

exp	test ¹	biological treatment	chemical treatment	untreated check
1	1	2-day, 1-day, 0-day	chem	none
1	2	2-day, 1-day, 0-day	chem	none
1	3	2-day, 1-day, 0-day	chem	none
1	4	2-day, 1-day, 0-day	chem	none
1	5	2-day, 1-day, 0-day	chem	none
2	6	7-day, 2-day, 0-day, post	chem	none
2	7	7-day, 2-day, 0-day, post	chem	none
2	8	7-day, 2-day, 0-day, post	chem	none
2	9	7-day, 2-day, 0-day, post	chem	none
2	10	7-day, 2-day, 0-day, post	chem	none
2	11	7-day, 2-day, 0-day, post	chem	none
2	12	low, high	chem, pchem	none
2	13	low, high	chem, pchem	none

¹ For definitions of tests, refer to Table 2.

In experiment two, tests six through thirteen were conducted. The six treatments in tests six through eleven were (i) a '7-day' pre-transplant biological treatment applied once 7 days prior to transplant; (ii) a '2-day' pre-transplant biological treatment; (iii) a '0-day' pre-transplant biological treatment; (iv) a chemical pre-transplant treatment ('chem'); (v) a biological post-transplant treatment applied just after transplant to the growing medium in the 10 cm pots ('post'); and (vi) an untreated check ('none').

Tests twelve and thirteen were also included in experiment two, but the biological treatments were applied as dry soil amendments. For two of the five treatments in these tests, the (i) low and (ii) high recommended rates of each biological control product (refer to Table 1) were added to the 10 cm square pots containing the infested growing media 24 hours after the pots were infested and 24 hours prior to seedling transplant. The other

three treatments were (iii) a chemical pre-transplant treatment ('chem'); (iv) a chemical post-transplant treatment ('pchem'); and (v) an untreated check ('none').

Data collection and analysis

At the end of the four-week growing period, plants were assigned a visually-based marketability score. The basil was scored according to height, based on the height of the tallest basil plant in the three blocks of the experiment. The greatest height was divided into three approximately equal units, equivalent to the short, medium and tall heights. The basil plant vigor scores ranged from one to five: 1 = dead plant; 2 = barely alive; 3 = short height; 4 = medium height; and 5 = tall height.

The pansy and petunia plants were scored according to size, the presence and/or absence of disease symptoms, and flower status. Plants were grouped into three size categories, based on the number of leaves and the size of the canopy area. The size categories correspond to the base plant vigor scores, ranging from one to four: 1 = dead plant; 2 = small size; 3 = medium size; and 4 = large size. Disease presence was determined by examining the lowest leaves of each plant. Plants with circular leaf spots or leaf lesions characteristic of *Rhizoctonia* leaf spot/blight (12,15) were considered diseased, those without were considered non-diseased. To incorporate disease presence/absence into the plant vigor score, the scores for the non-diseased plants were increased by one point while the scores for the diseased plants remained unchanged. Plant flower status was rated according to the most advanced stage of flowering. The three assigned stages of flowering were flower buds, closed flowers and open flowers. To incorporate flower status into the plant vigor score, plants with buds received one

additional point, plants with closed flowers received two additional points, and plants with open flowers received three additional points. According to this scoring method, the lowest possible score for pansy and petunia was 1 (dead) and the highest was 8 (large plant = 4, +1 = no disease, and +3 = open flower).

After scoring, all above-ground plant parts were removed and placed into a drying oven (45-50 C; 113-122 F) in individual paper bags. Soil and roots were discarded. After plant tissue was dry (one week in the oven), plant dry weights were measured and recorded. The data were analyzed using SASTM (The Statistical Analysis SystemTM for WindowsTM, release 6.12, copyright 1989-1996, Cary, NC 27513) proc GLM, and means were separated using the Student-Newman-Keul's multiple comparison test.

Each greenhouse efficacy test produced one large set of data with several factors: two growing media, three host plants, four inoculum levels, five to six treatments and three replications. Each large data set (test) contained only one biological control agent, and was analyzed individually to investigate the statistical significance of the different factors. None of the test data sets was statistically analyzed in combination with any other. In order to investigate individual factors within a test data set, it was necessary to either average values over the factors not in question or to exclude them temporarily. In some cases, it made more sense to exclude data rather than to average over it. For example, in order to investigate the effects of the treatments in Sunshine Mix for a particular test, all of the data from plants grown in PGC Mix were temporarily excluded rather than averaged into the remaining data. Once the Sunshine Mix data were obtained, the same methods were used to obtain the data for PGC Mix alone and only then were the two compared (not statistically).

This same approach was applied to all of the different factors in each test data set. The statistical data obtained for each factor were compared within each test, never between tests. However, by excluding data in order to narrow down test data sets to investigate individual factors, the number of observations were decreased, which decreased the statistical power to detect significant differences. Since there were thirteen very large test data sets and it was necessary to narrow them down as described above, thus eliminating most statistically significant differences, the interpretation of the statistical analyses was approached in a step-by-step manner.

The data were first analyzed for each general factor, i.e. the growing media, the host plants, and the inoculum levels. Each test was statistically analyzed individually and no data were excluded. Next, data representing disease pressure were analyzed in order to determine if each test had sufficient disease pressure to be included in data interpretation. Once disease pressure was proven, the data for inoculum level from tests with proven disease pressure were analyzed, separated by host plant and growing medium, in order to determine if the disease had been controlled in the test. Lastly, the disease control treatment data from each test in which there was disease control were analyzed, separated by host plant, growing medium, and inoculum level, in order to determine which treatment was responsible for the observed disease control. Based on the results from these analyses and interpretations, conclusions and recommendations for use were made.

Measurement of Inoculum Potential

The inoculum potential of each inoculum level was measured in order to estimate the change in potential over the four week growing period and to compare the potential of growing medium without plants to the potential of growing medium with plants. To measure the inoculum potential of each inoculum level, a modified version of the beet seed assay, as described by Stephens et al. (50) and Ko and Hora (33) was used. Ten cm square pots of PGC Mix were infested to four inoculum levels, as described above. This assay was not done with Sunshine Mix due to the large number of fast-growing contaminant fungi present. Pots were kept on a greenhouse bench and watered lightly, once a day until they were assayed. After 24 hours incubation, three pots of each level were emptied into individual small plastic bags, each containing 100 autoclaved sugar beet seeds. The bags were kept at room temperature for 48 hours. At least 60 beet seeds were recovered from each bag by sieving the mixture with a #7 (2.80 mm) sieve. The seeds were placed into petri plates containing acidified PDA (pH 4.0, adjusted with lactic acid). Twenty seeds were placed on each plate.

Plates were examined visually and microscopically within 24 hours incubation at room temperature. The visual identifying characteristics used included the appearance of small, compact colonies with highly branched, white mycelia. The microscopic identifying characteristics used included the occurrence of crosswalls and right-angled branching within the mycelia. Colonies that had the identifying characteristics were counted positive. Percent colonization was calculated. This was repeated at two weeks and four weeks after infestation.

In order to determine if there was a difference in inoculum potential in PGC Mix which had contained plants, this test was repeated once using the PGC Mix from test twelve (the RootShield Granules test) after plants had been scored and removed for drying. PGC Mix from pots that had contained an untreated check plant at each inoculum level was tested. The root systems of removed plants were shaken free so that only growing medium was added to the plastic bags containing sterile beet seeds. Percent colonization was determined as described above.

RESULTS

Greenhouse Efficacy Tests

The results are presented first by focusing on the effects of the general factors of the experiment, i.e. the growing media, inoculum levels and host plants, and then narrowing to the main interest of the research, the disease control treatments. The data from each test were statistically analyzed individually. The test means were combined into experiment one, experiment two, and a combined total of both experiments in Tables 4, 5 and 6. The means for each general factor illustrate the effects of each on plant growth in the presence of the pathogen.

Plants grown in Sunshine Mix were healthier and had both higher marketability scores and dry weights than plants grown in PGC Mix (Table 4). This was true in both of the experiments as well as in the combined total. In each of the individual statistical analyses of the data (each test), plants grown in Sunshine Mix had statistically higher marketability scores and dry weights than plants grown in PGC Mix (data not shown). In Table 4, the data for inoculum level, host plant, and disease control treatment were averaged into the mean values rather than excluded.

Table 4. Effect of growing medium on disease caused by *R. solani*.

experiment		growing medium ¹	
		Sunshine Mix #1	PGC
1	score ²	4.11	3.10
	plwt ²	0.95	0.54
2	score	5.14	2.82
	plwt	0.60	0.15
1 & 2	score	4.75	2.93
	plwt	0.73	0.30

¹Growing medium, mean plant vigor score and plant dry weight data were averaged over host plant, inoculum level and treatment. ² score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g).

Petunia had higher marketability scores and dry weights than either pansy or basil (Table 5). This observation suggested that petunia was the healthiest host plant, and that the differences were due to either a reduced susceptibility of petunia to the pathogen or the inherently different sizes and growth habits of the different plants. The same observation was statistically supported in each individual analysis (data not shown). Based on the marked differences between the growing media and the host plants, the tests were separated into different data sets based on the six growing medium-host plant combinations for further data analysis and interpretation. As in Table 4, the data in Table 5 for inoculum level, growing medium, and disease control treatment were averaged into the mean values rather than excluded.

Table 5. Effect of disease caused by *R. solani* on host plant.

experiment		host plant ¹		
		pansy	petunia	basil
1	score ²	2.98	4.68	3.17
	plwt ²	0.34	1.12	0.78
2	score	4.01	4.44	3.51
	plwt	0.30	0.52	0.30
1 & 2	score	3.62	4.53	3.38
	plwt	0.32	0.75	0.49

¹Host plant, mean plant vigor score and plant dry weight data were averaged over growing medium, inoculum level and treatment. ² score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g).

Plant growth at the various inoculum levels was as expected, best in the uninfested level and worst at the high level (Table 6). The same decreasing gradient was present in both experiments and the combined total, although the range of marketability score and dry weight values was larger and the differences between the uninfested level and the infested levels more pronounced in experiment one than in experiment two. The differences in the data suggested a difference in disease pressure between the two experiments. As in Tables 4 and 5, the data in Table 6 for host plant, growing medium and disease control treatment were averaged into the mean values rather than excluded.

Table 6. Effect of inoculum level on disease caused by *R. solani*.

experiment		inoculum level ¹			
		uninfested	low	medium	high
1	score ²	6.27	3.65	2.92	2.59
	plwt ²	1.16	0.79	0.56	0.46
2	score	4.39	4.06	3.85	3.64
	plwt	0.41	0.39	0.36	0.34
1 & 2	score	4.73	3.90	3.50	3.24
	plwt	0.70	0.54	0.44	0.39

¹Inoculum level, mean plant vigor score and plant dry weight data were averaged over growing medium, host plant and treatment. ² score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g).

The plant dry weights for all factors were reduced between experiments one and two, most likely due to a seasonal decrease in natural day length. The plant marketability score values did not reflect the same decrease due to the human error associated with detecting small differences in plant growth and assigning consistent vigor scores between tests performed as much as four months apart.

Prior to any further data interpretation, the suspected change in disease pressure was investigated by calculating difference values for each of the tests in both experiments. The 'clean' data means are made up of three observations, each from an uninfested growing medium and receiving no treatment (the untreated check). The 'high' data means are also made up of three observations, each from a growing medium infested to the high inoculum level and receiving no treatment (the untreated check). The difference values, which represent disease pressure, are presented for the host plants in both Sunshine Mix (Table 7) and PGC Mix (Table 8).

The difference values in experiment one (tests 1-5) were larger than those in experiment two, confirming that the disease pressure did change and that the change was a reduction in disease pressure between experiment one and two. In calculating difference values for each test, it was discovered that some tests had a zero or negative disease pressure. In order to avoid false interpretations of the results, the test data in which there was no disease pressure, based on either plant dry weight or plant marketability score, were eliminated from further results.

In Sunshine Mix (Table 7), four data sets were eliminated for pansy, seven for petunia and four for basil. Fourteen of the fifteen data sets eliminated were from experiment two, another piece of evidence that confirmed the reduced disease pressure

between the two experiments. More data sets were eliminated for petunia than for pansy or basil, suggesting that the differences observed in plant growth presented in Table 5 were not due to inherent plant growth differences, but to petunia's lower susceptibility to the pathogen.

In PGC Mix (Table 8), one data set was eliminated for pansy, three for petunia and two for basil. Again, the same trends suggesting reduced disease pressure in experiment two and reduced susceptibility in petunia were observed. A total of fifteen data sets were eliminated from the host plant-Sunshine Mix combinations (Table 7), while only six were eliminated from PGC Mix (Table 8). This suggested that there was reduced disease pressure in Sunshine Mix and that a difference in disease pressure existed between experiments. The highest disease pressure occurred in PGC Mix within experiment one, while the lowest occurred in Sunshine Mix within experiment two. The disease pressures in Sunshine Mix within experiment one and in PGC Mix within experiment two were intermediate.

Table 7. Disease pressure of tests one through thirteen for Sunshine Mix.

tests ¹		pansy			petunia			basil		
		clean ⁴	high ⁵	differ- ence	clean	high	differ- ence	clean	high	differ- ence
1	score ²	5.00	1.33	3.67	6.00	3.67	2.33	4.67	2.33	2.34
	plwt	0.80	0.15	0.65	1.43	0.89	0.54	1.21	0.44	0.77
2	score	5.33	1.33	4.00	5.33	3.33	2.00	4.33	2.00	2.33
	plwt	0.60	0.70	0.10	0.76	0.61	0.15	0.82	0.24	0.58
3	score	6.33	1.00	5.33	5.67	6.33	-0.66 ✕	4.67	2.00	2.67
	plwt	0.82	0.00	0.82	1.07	1.25	-0.18 ✕	1.48	0.35	1.13
4	score	6.67	1.00	5.67	7.67	6.00	1.67	4.67	2.00	2.67
	plwt	1.27	0.00	1.27	2.16	1.92	0.24	1.94	0.52	1.42
5	score	6.00	1.00	5.00	7.00	4.00	3.00	5.00	2.33	2.67
	plwt	1.31	0.00	1.31	2.02	1.03	0.99	2.10	0.73	1.37
6	score	6.33	5.33	1.00 – ³	6.00	5.33	0.67 –	4.67	4.00	0.67
	plwt	0.45	0.38	0.07 –	0.58	0.56	0.02 –	0.55	0.34	0.21
7	score	6.00	5.67	0.33 ✕	5.00	5.33	0.33 ✕	4.00	4.33	-0.33 ✕
	plwt	0.44	0.55	-0.11 ✕	0.91	1.03	-0.12 ✕	0.54	0.56	-0.02 ✕
8	score	5.67	5.00	0.67	8.00	7.00	1.00 ✕	4.00	4.33	-0.33 ✕
	plwt	0.63	0.49	0.14	1.00	1.29	-0.29 ✕	0.63	0.71	-0.08 ✕
9	score	6.00	2.33	3.67	7.33	5.33	2.00 –	4.33	4.33	0.00 ✕
	plwt	0.93	0.23	0.70	1.05	1.04	0.01 –	1.03	0.88	0.15 ✕
10	score	3.33	1.67	1.66 –	6.67	6.00	0.67 ✕	5.00	4.33	0.67
	plwt	0.14	0.10	0.04 –	0.99	1.08	-0.09 ✕	1.03	0.79	0.24
11	score	2.33	2.00	0.33 ✕	4.33	5.00	-0.67 ✕	4.67	3.67	1.00
	plwt	0.04	0.04	0.00 ✕	0.55	0.56	-0.01 ✕	0.40	0.26	0.14
12	score	5.67	6.00	-0.33 ✕	5.67	5.67	0.00 ✕	4.67	4.33	0.34
	plwt	0.46	0.42	0.04 ✕	0.66	0.52	0.14 ✕	0.45	0.23	0.22
13	score	5.33	5.67	-0.34 ✕	5.67	5.67	0.00 ✕	4.67	4.33	0.34 ✕
	plwt	0.41	0.43	-0.02 ✕	0.75	0.55	0.20 ✕	0.46	0.46	0.00 ✕

¹ Refer to Table 2 for definitions of tests. ² score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g). ³ – indicates low disease pressure; ✕ indicates no disease pressure. ⁴ clean = untreated (check) at unfested inoculum level. ⁵ high = untreated (check) at high inoculum level.

Table 8. Disease pressure of tests one through thirteen for PGC Mix.

tests ¹		pansy			petunia			basil		
		clean ⁴	high ⁵	differ- ence	clean	high	differ- ence	clean	high	differ- ence
1	score ²	3.67	1.67	2.00	6.00	1.00	5.00	4.67	1.33	3.34
	plwt	0.41	0.08	0.33	1.62	0.00	1.62	1.03	0.03	1.00
2	score	5.67	2.00	3.67	4.00	1.00	3.00	5.00	1.00	4.00
	plwt	0.75	0.12	0.63	0.43	0.00	0.43	0.30	0.00	0.30
3	score	4.67	1.00	3.67	6.67	7.00	-0.33 ✕	4.33	1.00	3.33
	plwt	0.32	0.00	0.32	1.87	1.39	0.48 ✕	1.03	0.00	1.03
4	score	3.67	1.00	2.67	5.33	1.00	4.33	4.67	1.00	3.67
	plwt	0.29	0.00	0.29	1.11	0.00	1.11	1.53	0.00	1.53
5	score	5.67	1.00	4.67	6.67	2.33	4.34	4.67	2.00	2.67
	plwt	0.66	0.00	0.66	1.48	0.50	0.98	1.34	0.32	1.02
6	score	4.67	2.00	2.67	5.00	3.33	1.67	3.67	2.33	1.34 -
	plwt	0.20	0.09	0.11	0.35	0.25	0.10	0.15	0.07	0.08 -
7	score	3.33	1.67	1.66 - ³	3.00	1.67	1.33 -	3.00	1.67	1.33
	plwt	0.07	0.02	0.05 -	0.05	0.01	0.04 -	0.18	0.03	0.15
8	score	2.67	1.67	1.00 -	4.00	3.33	0.67 -	3.33	2.33	1.00 -
	plwt	0.15	0.08	0.07 -	0.49	0.40	0.09 -	0.18	0.13	0.05 -
9	score	3.33	1.67	1.66 -	4.67	4.67	0.00 ✕	3.67	2.67	1.00
	plwt	0.14	0.08	0.06 -	0.50	0.62	-0.12 ✕	0.27	0.16	0.11
10	score	3.33	1.67	1.66 -	4.00	2.67	1.33	3.00	3.00	0.00 ✕
	plwt	0.14	0.10	0.04 -	0.49	0.21	0.28	0.12	0.10	0.02 ✕
11	score	2.33	2.00	0.33 ✕	3.33	2.67	0.66 -	3.00	1.67	1.33 -
	plwt	0.04	0.04	0.00 ✕	0.15	0.13	0.02 -	0.02	0.00	0.02 -
12	score	3.00	1.33	1.67	3.00	3.67	-0.67 ✕	3.00	2.33	0.67 -
	plwt	0.12	0.02	0.10	0.13	0.16	-0.03 ✕	0.03	0.02	0.01 -
13	score	3.00	2.33	0.67 -	4.33	2.67	1.66	3.00	2.00	1.00 ✕
	plwt	0.09	0.08	0.01 -	0.24	0.14	0.10	0.06	0.06	0.00 ✕

¹ Refer to Table 2 for definitions of tests. ² score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight. ³ - indicates low disease pressure; ✕ indicates no disease pressure. ⁴ clean = untreated (check) at uninfested inoculum level. ⁵ high = untreated (check) at high inoculum level.

To determine which of the data sets with proven disease pressure had effective disease control, the data for the inoculum levels are presented in Tables 9-14, along with the statistical significance values assigned by the Student-Newman-Keul's multiple comparison test. The data are separated by host plant and growing medium and include the disease control treatment data in the mean values. It was assumed that the disease

was controlled in those tests in which the plant dry weight data for the uninfested inoculum level were not statistically different from the data for the high inoculum level. Due to the higher disease pressure in experiment one, data sets from tests in experiment one in which the data for the uninfested inoculum level were not statistically different from the data for the medium inoculum level, were considered to have effective disease control.

Based on the lack of statistical significance between the plant dry weight in the uninfested inoculum level and the plant dry weight in the high inoculum level, tests 6, 8 and 10 contained effective disease control for the pansy-Sunshine Mix data sets (Table 9). For the pansy-PGC Mix data sets, disease was controlled in tests 7, 8, 12 and 13 (Table 10). For the petunia-Sunshine Mix data sets, disease was controlled in tests 1, 4, 5, 6 and 9 (Table 11). For the petunia-PGC Mix data sets, disease was controlled in tests 4, 6, 7, 8, 10 and 11 (Table 12). For the basil-Sunshine Mix data sets, disease was controlled in tests 3, 6, 10, 11 and 12 (Table 13). Lastly, for the basil-PGC Mix data sets, disease was controlled in tests 8 and 12 (Table 14). More of the tests with effective disease control came from experiment two, suggesting that control was more effective in a lower disease pressure environment. A summary of the tests with effective disease control is presented in Figure 1.

Table 9. Disease control in data sets with proven disease pressure for pansy in Sunshine Mix.
 inoculum levels ⁴

exp	tests ¹		inoculum levels			
			uninfested	low	medium	high
1	1	score ³	5.20 a ²	4.80 ab	3.60 bc	2.93 c
		pltwt	0.86 a	0.68 ab	0.52 b	0.39 b
	2	score	5.67 a	4.73 a	2.87 b	2.40 b
		pltwt	0.63 a	0.57 a	0.29 b	0.14 c
	3	score	5.73 a	3.33 b	2.40 b	2.07 b
		pltwt	0.67 a	0.39 b	0.20 c	0.13 c
	4	score	6.07 a	3.13 b	2.33 bc	1.87 c
		pltwt	1.10 a	0.56 b	0.29 b	0.23 b
	5	score	6.27 a	4.07 b	2.13 c	2.67 c
		pltwt	1.31 a	0.77 b	0.26 c	0.41 c
2	6 * ⁵	score	5.89 a	5.56 ab	5.50 ab	5.11 b
		pltwt	0.45 a	0.44 a	0.42 a	0.36 a
	8	score	5.83 a	5.78 a	5.94 a	5.61 a
		pltwt	0.64 a	0.64 a	0.65 a	0.61 a
	9	score	6.17 a	5.89 a	5.33 a	4.50 b
		pltwt	0.79 a	0.75 a	0.69 ab	0.57 b
	10 *	score	5.78 a	6.00 a	5.56 a	5.06 a
		pltwt	0.70 a	0.71 a	0.59 a	0.61 a

¹ For definitions of tests, refer to Table 2. ² Row means followed by the same letter are not significantly different ($P=0.05$) according to the Student-Newman-Keul's multiple comparison test. ³ score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); pltwt = plant dry weight (g). ⁴ Inoculum level data were averaged over disease control treatment. ⁵ * indicates low disease pressure.

Table 10. Disease control in data sets with proven disease pressure for pansy in PGC Mix.

exp	tests ¹		inoculum levels ⁴			
			uninfested	low	medium	high
1	1	score ³	3.53 a ²	2.60 b	1.33 c	1.40 c
		pltwt	0.41 a	0.25 b	0.06 c	0.05 c
	2	score	4.20 a	1.60 b	1.47 b	1.40 b
		pltwt	0.29 a	0.06 b	0.04 b	0.03 b
	3	score	4.20 a	1.33 b	1.40 b	1.67 b
		pltwt	0.23 a	0.05 b	0.03 b	0.06 b
	4	score	4.40 a	1.47 b	1.27 b	1.13 b
		pltwt	0.46 a	0.08 b	0.05 b	0.01 b
	5	score	5.27 a	2.07 b	1.47 b	1.67 b
		pltwt	0.55 a	0.14 b	0.06 b	0.09 b
2	6	score	3.94 a	4.22 a	2.67 b	2.44 b
		pltwt	0.19 ab	0.24 a	0.14 bc	0.12 c
	7 * ⁵	score	3.06 a	1.94 b	1.72 b	1.72 b
		pltwt	0.05 a	0.03 a	0.04 a	0.03 a
	8 *	score	3.28 a	2.89 a	2.17 b	1.94 b
		pltwt	0.18 a	0.18 a	0.12 a	0.12 a
	9 *	score	3.44 a	3.06 ab	2.50 bc	1.94 c
		pltwt	0.22 a	0.26 a	0.20 a	0.10 b
	10 *	score	3.39 a	2.67 b	2.11 c	1.72 c
		pltwt	0.15 a	0.13 ab	0.10 ab	0.08 b
	12	score	2.93 a	2.33 a	2.67 a	2.20 a
		pltwt	0.10 a	0.07 a	0.09 a	0.06 a
	13 *	score	3.13 a	2.87 a	2.73 a	2.27 b
		pltwt	0.08 a	0.07 a	0.07 a	0.06 a

¹ For definitions of tests, refer to Table 2. ² Row means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³ score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g). ⁴ Inoculum level data were averaged over disease control treatment. ⁵ * indicates low disease pressure.

Table 11. Disease control in data sets with proven disease pressure for petunia in Sunshine Mix.

exp	tests ¹		inoculum levels ⁴			
			uninfested	low	medium	high
1	1	score ³	5.87 a ²	5.07 ab	4.40 b	3.73 b
		pltwt	1.49 a	1.30 ab	1.09 ab	0.90 b
	2	score	5.87 a	4.87 ab	3.87 b	3.33 b
		pltwt	1.02 a	0.91 a	0.59 b	0.45 b
	4	score	7.73 a	6.33 b	6.00 b	6.13 b
		pltwt	2.48 a	2.03 a	1.93 a	1.98 a
	5	score	7.07 a	5.07 b	4.87 b	4.60 b
		pltwt	2.16 a	1.50 a	1.45 a	1.61 a
2	6 * ⁵	score	5.83 a	5.83 a	5.28 a	5.50 a
		pltwt	0.58 a	0.61 a	0.58 a	0.52 a
	9 *	score	7.17 a	6.11 b	5.44 b	5.56 b
		pltwt	0.97 a	1.01 a	0.91 a	0.92 a

¹ For definitions of tests, refer to Table 2. ² Row means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³ score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); pltwt = plant dry weight (g). ⁴ Inoculum level data were averaged over disease control treatment. ⁵ * indicates low disease pressure.

Table 12. Disease control in data sets with proven disease pressure for petunia in PGC Mix.

exp	tests ¹		inoculum levels ⁴			
			uninfested	low	medium	high
1	1	score ³	6.00 a ²	4.33 b	3.07 c	2.67 c
		pltwt	1.63 a	1.30 a	0.66 b	0.54 b
	2	score	5.13 a	3.40 b	2.60 b	2.33 b
		pltwt	0.69 a	0.43 ab	0.32 b	0.18 b
	4	score	6.00 a	4.73 a	4.80 a	2.93 b
		pltwt	1.80 a	1.42 a	1.35 a	0.73 b
	5	score	5.87 a	4.47 b	3.20 c	3.00 c
		pltwt	1.18 a	1.10 a	0.64 b	0.57 b
2	6	score	4.44 a	3.94 ab	3.94 ab	3.56 b
		pltwt	0.34 a	0.32 a	0.36 a	0.27 a
	7 * ⁵	score	2.50 a	2.00 ab	1.72 b	1.50 b
		pltwt	0.07 a	0.05 a	0.05 a	0.02 a
	8 *	score	3.89 a	3.44 a	2.78 b	2.67 b
		pltwt	0.38 a	0.45 a	0.35 a	0.31 a
	10	score	3.78 a	2.89 b	2.94 b	2.72 b
		pltwt	0.37 a	0.29 a	0.33 a	0.30 a
	11 *	score	5.50 a	5.17 a	5.28 a	5.11 a
		pltwt	0.40 a	0.37 ab	0.38 ab	0.33 b
	13	score	3.60 a	2.80 b	3.00 b	2.53 b
		pltwt	0.16 ab	0.11 b	0.18 a	0.11 b

¹ For definitions of tests, refer to Table 2. ² Row means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³ score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); pltwt = plant dry weight (g). ⁴ Inoculum level data were averaged over disease control treatment. ⁵ * indicates low disease pressure;

Table 13. Disease control in data sets with proven disease pressure for basil in Sunshine Mix.

exp	tests ¹		inoculum levels ⁴			
			uninfested	low	medium	high
1	1	score ³	4.80 a ²	4.33 ab	3.47 b	2.33 c
		pltwt	1.47 a	1.25 ab	0.84 b	0.32 c
	2	score	4.53 a	3.40 b	3.00 b	2.80 b
		pltwt	0.85 a	0.60 ab	0.46 b	0.41 b
	3	score	4.33 a	3.00 bc	4.00 ab	2.20 c
		pltwt	1.38 a	0.78 bc	1.21 ab	0.43 c
	4	score	4.60 a	2.87 b	2.87 b	2.40 b
		pltwt	2.15 a	1.03 b	0.99 b	0.70 b
	5	score	4.93 a	4.60 a	2.93 b	3.20 b
		pltwt	2.09 a	1.77 a	0.86 b	1.10 b
2	6	score	4.56 a	3.78 b	4.22 ab	4.17 ab
		pltwt	0.41 a	0.26 b	0.35 ab	0.33 ab
	10	score	4.56 a	4.39 a	4.39 a	3.78 b
		pltwt	0.82 a	0.78 a	0.70 a	0.59 a
	11	score	4.39 a	4.22 a	4.00 a	3.67 a
		pltwt	0.36 a	0.35 a	0.28 a	0.29 a
	12	score	4.53 a	4.40 a	4.33 a	4.07 a
		pltwt	0.39 a	0.33 ab	0.30 ab	0.24 b

¹ For definitions of tests, refer to Table 2. ² Row means followed by the same letter are not significantly different ($P=0.05$) according to the Student-Newman-Keul's multiple comparison test. ³ score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g). ⁴ Inoculum level data were averaged over disease control treatment.

Table 14. Disease control in data sets with proven disease pressure for basil in PGC Mix.

exp	tests ¹		inoculum levels ⁴			
			uninfested	low	medium	high
1	1	score ³	4.73 a ²	3.40 b	2.20 c	1.93 c
		pltw	1.15 a	0.63 b	0.26 c	0.23 c
	2	score	4.27 a	2.60 b	1.67 c	1.40 c
		pltw	0.58 a	0.32 b	0.10 c	0.07 c
	3	score	4.40 a	3.07 b	1.87 c	2.13 c
		pltw	1.07 a	0.67 b	0.25 b	0.35 b
	4	score	4.33 a	2.93 b	1.67 c	1.53 c
		pltw	1.44 a	0.76 b	0.17 c	0.18 c
	5	score	4.67 a	2.60 b	2.67 b	2.07 b
		pltw	1.16 a	0.43 b	0.45 b	0.26 b
2	6 * ⁵	score	3.56 a	3.11 a	2.56 b	2.11 b
		pltw	0.15 a	0.12 ab	0.09 bc	0.05 c
	7	score	3.28 a	2.61 b	2.22 b	2.11 b
		pltw	0.17 a	0.10 b	0.06 b	0.05 b
	8 *	score	3.22 a	3.00 a	3.22 a	2.44 b
		pltw	0.18 a	0.17 a	0.17 a	0.10 a
	9	score	3.39 a	3.00 ab	2.72 ab	2.28 b
		pltw	0.23 ab	0.26 a	0.16 ab	0.13 b
	11 *	score	3.00 a	2.83 a	1.94 b	1.94 b
		pltw	0.04 a	0.03 ab	0.02 b	0.01 b
	12 *	score	3.07 a	3.07 a	2.60 a	2.47 a
		pltw	0.06 a	0.07 a	0.04 a	0.04 a

¹ For definitions of tests, refer to Table 2. ² Row means followed by the same letter are not significantly different ($P=0.05$) according to the Student-Newman-Keul's multiple comparison test. ³ score = plant marketability score (1 is dead, 5 is maximum for basil, 8 is maximum for pansy and petunia); plwt = plant dry weight (g). ⁴ Inoculum level data were averaged over disease control treatment. ⁵ * indicates low disease pressure.

For each of the data sets with proven disease pressure and effective disease control, plant dry weight data for each of the treatments in each of the inoculum levels are presented, along with the statistical values assigned by the Student-Newman-Keul's multiple comparison test, in Tables 15-20. The treatment data were the mean of only three observations. Because of the low number of observations in the statistical analysis, the statistical power to detect differences between the data was very low and statistical differences rare. The differences between the plant dry weight data were more precise

than the differences between the plant marketability score data due to the whole number nature of assigned score values compared to plant dry weights measured to the hundredth of a gram. For that reason, only plant dry weight data are analyzed in the treatment means and due to the lack of statistical significance, the rank of the data is considered in lieu of statistical significance.

For the pansy-Sunshine Mix data sets (Table 15), the post-transplant biological treatment (test 6 = Deny-Fungicide, 8 = Gliocladium J1446 and 10 = Mycostop) was most effective, along with the '0-day' pre-transplant biological treatment (10 = Mycostop). For the pansy-PGC Mix data sets (Table 16), the '7-day' pre-transplant biological treatment (test 7 = Deny-Nematicide) and the pre-transplant chemical treatment (tests 8, 12 and 13) were most effective.

For the petunia-Sunshine Mix data sets (Table 17), the '1-day' pre-transplant biological treatment (test 1 = Deny-Fungicide), the pre-transplant chemical treatment (tests 4 and 5), the '2-day' pre-transplant biological treatment (test 5 = Mycostop), and the post-transplant biological treatment (test 6 = Deny-Fungicide and 8 = Kodiak) were most effective. For the petunia-PGC Mix data sets (Table 18), the '1-day' pre-transplant biological treatment (test 4 = Kodiak), the '0-day' pre-transplant biological treatment (test 6 = Deny-Fungicide), and the '2-day' pre-transplant biological treatment (test 7 = Deny-Nematicide, 8 = Gliocladium J1446, 10 = Mycostop and 11 = RootShield Drench) were most effective.

For the basil-Sunshine Mix data sets (Table 19), the pre-transplant chemical treatment (tests 3 and 12), and the post-transplant biological treatment (test 6 = Deny-Fungicide, 10 = Mycostop and 11 = RootShield Drench) were most effective. Lastly, for

the basil-PGC Mix data sets (Table 20), the post-transplant biological treatment (test 8 = Gliocladium J1446) and the high rate biological treatment (test 12 = RootShield

Granules) were most effective. These results are summarized in Figures 2, 3 and 4.

Table 15. Treatment plant dry weight (g) means separated by inoculum level for pansy in Sunshine Mix.

exp	test ¹	treatment	inoculum level			
			uninfested	low	medium	high
2	6 * ³	7-day	0.443 a ²	0.377 a	0.317 b	0.270 a
		2-day	0.467 a	0.560 a	0.293 b	0.370 a
		0-day	0.410 a	0.363 a	0.383 b	0.293 a
		post	0.487 a	0.520 a	0.663 a	0.513 a
		chem	0.423 a	0.370 a	0.447 b	0.357 a
		none	0.447 a	0.423 a	0.427 b	0.380 a
	8	7-day	0.627 b	0.640 a	0.660 a	0.750 a
		2-day	0.547 b	0.547 a	0.690 a	0.520 a
		0-day	0.540 b	0.620 a	0.653 a	0.597 a
		post	0.923 a	0.827 a	0.587 a	0.820 a
		chem	0.567 b	0.523 a	0.617 a	0.467 a
		none	0.627 b	0.687 a	0.717 a	0.490 a
	10 *	7-day	0.560 a	0.713 a	0.583 a	0.390 ab
		2-day	0.617 a	0.560 a	0.553 a	0.143 b
		0-day	0.807 a	0.930 a	0.637 a	0.810 ab
		post	0.730 a	0.790 a	0.617 a	0.883 a
		chem	0.727 a	0.527 a	0.630 a	0.743 ab
		none	0.750 a	0.763 a	0.513 a	0.700 ab

¹The biological control agent in test six is Deny Liquid-Biological Fungicide; in test eight - Gliocladium J1446 powder; in test ten - Mycostop. ²Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³* indicates low disease pressure.

Table 16. Treatment plant dry weight (g) means separated by inoculum level for pansy in PGC Mix.

exp	test ¹	treatment	inoculum level			
			uninfested	low	medium	high
2	7 * ³	7-day	0.147 a ²	0.137 a	0.147 a	0.090 a
		2-day	0.010 a	0.007 b	0.000 b	0.000 a
		0-day	0.030 a	0.003 b	0.003 b	0.003 a
		post	0.010 a	0.023 b	0.043 b	0.023 a
		chem	0.020 a	0.007 b	0.007 b	0.010 a
		none	0.073 a	0.000 b	0.047 b	0.017 a
	8 *	7-day	0.287 a	0.203 a	0.023 a	0.050 a
		2-day	0.220 a	0.227 a	0.140 a	0.103 a
		0-day	0.160 a	0.160 a	0.153 a	0.197 a
		post	0.090 a	0.167 a	0.043 a	0.137 a
		chem	0.157 a	0.203 a	0.170 a	0.147 a
		none	0.150 a	0.143 a	0.163 a	0.083 a
	12	high	0.073 a	0.047 a	0.057 a	0.080 ab
		low	0.087 a	0.073 a	0.050 a	0.040 ab
		chem	0.117 a	0.103 a	0.177 a	0.113 a
		pchem	0.093 a	0.040 a	0.093 a	0.053 ab
		none	0.120 a	0.100 a	0.070 a	0.017 b
	13 *	high	0.113 a	0.070 a	0.073 a	0.050 a
		low	0.080 a	0.073 a	0.063 a	0.080 a
		chem	0.077 a	0.077 a	0.090 a	0.050 a
		pchem	0.053 a	0.040 a	0.047 a	0.043 a
		none	0.093 a	0.087 a	0.087 a	0.080 a

¹The biological control agent in test seven is Deny Liquid-Biological Nematicide; in test eight - Gliocladium J1446 powder; in test twelve - RootShield Granules; in test thirteen - SoilGard. ² Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³* indicates low disease pressure.

Table 17. Treatment plant dry weight (g) means separated by inoculum level for petunia in Sunshine Mix.

exp	test ¹	treatment	inoculum level			
			uninfested	low	medium	high
1	1	2-day	1.627 a ²	1.123 a	1.490 a	0.837 a
		1-day	1.650 a	1.653 a	0.707 a	0.450 a
		0-day	1.283 a	1.237 a	1.090 a	1.050 a
		chem	1.483 a	1.473 a	1.067 a	1.290 a
		none	1.430 a	1.020 a	1.120 a	0.893 a
	4	2-day	2.410 a	2.303 a	1.513 a	1.313 a
		1-day	2.513 a	2.087 a	2.063 a	1.827 a
		0-day	2.640 a	2.220 a	2.137 a	1.990 a
		chem	2.663 a	2.167 a	2.357 a	2.420 a
		none	2.160 a	1.397 a	1.560 a	1.920 a
	5	2-day	2.550 a	1.620 a	1.163 a	2.170 a
		1-day	1.997 a	0.640 a	1.637 a	1.380 a
		0-day	2.323 a	1.74 a	0.753 a	1.363 a
		chem	1.923 a	1.960 a	1.977 a	2.117 a
		none	2.023 a	1.547 a	1.693 a	1.027 a
2	6 * ³	7-day	0.550 a	0.623 a	0.647 a	0.460 a
		2-day	0.490 a	0.683 a	0.570 a	0.503 a
		0-day	0.543 a	0.603 a	0.423 a	0.510 a
		post	0.780 a	0.680 a	0.793 a	0.630 a
		chem	0.507 a	0.473 a	0.443 a	0.433 a
		none	0.583 a	0.607 a	0.593 a	0.557 a
	9 *	7-day	0.993 a	1.077 a	0.620 a	0.673 a
		2-day	1.097 a	1.053 a	1.037 a	0.850 a
		0-day	1.020 a	0.913 a	0.747 a	1.060 a
		post	0.900 a	1.207 a	1.063 a	1.157 a
		chem	0.770 a	0.970 a	0.893 a	0.720 a
		none	1.053 a	0.853 a	1.083 a	1.043 a

¹ The biological control agent in test one is Deny Liquid-Biological Fungicide; in test four - Kodiak; in test five - Mycostop; in test six - Deny Liquid-Biological Fungicide; in test nine - Kodiak. ² Column means followed by the same letter are not significantly different ($P=0.05$) according to the Student-Newman-Keul's multiple comparison test. ³ * indicates low disease pressure.

Table 18. Treatment plant dry weight (g) means separated by inoculum level for petunia in PGC Mix.

exp	test ¹	treatment	inoculum level			
			uninfested	low	medium	high
1	4	2-day	2.193 a ²	0.903 a	1.257 a	0.827 a
		1-day	2.047 a	2.160 a	1.620 a	1.417 a
		0-day	1.847 a	1.157 a	0.863 a	0.630 a
		chem	1.807 a	1.640 a	1.373 a	0.783 a
		none	1.110 a	1.233 a	1.627 a	0.000 a
2	6	7-day	0.250 a	0.323 a	0.290 a	0.253 a
		2-day	0.383 a	0.247 a	0.343 a	0.237 a
		0-day	0.480 a	0.553 a	0.487 a	0.370 a
		post	0.263 a	0.267 a	0.430 a	0.273 a
		chem	0.313 a	0.247 a	0.420 a	0.233 a
		none	0.347 a	0.257 a	0.203 a	0.253 a
	7 * ³	7-day	0.270 a	0.047 a	0.070 a	0.003 b
		2-day	n/a	0.123 a	0.083 a	0.003 b
		0-day	0.050 a	0.007 a	0.000 a	0.023 b
		post	0.035 a	0.043 a	0.033 a	0.000 b
		chem	0.010 a	0.027 a	0.033 a	0.077 a
		none	0.045 a	0.047 a	0.077 a	0.013 b
	8 *	7-day	0.490 a	0.480 a	0.213 a	0.077 a
		2-day	0.557 a	0.550 a	0.650 a	0.480 a
		0-day	0.260 a	0.487 a	0.207 a	0.280 a
		post	0.347 a	0.212 a	0.330 a	0.207 a
		chem	0.157 a	0.410 a	0.443 a	0.390 a
		none	0.490 a	0.543 a	0.237 a	0.400 a
	10	7-day	0.293 a	0.463 a	0.280 a	0.467 a
		2-day	0.510 a	0.177 a	0.517 a	0.217 a
		0-day	0.227 a	0.243 a	0.267 a	0.537 a
		post	0.257 a	0.113 a	0.283 a	0.170 a
		chem	0.450 a	0.463 a	0.400 a	0.180 a
		none	0.487 a	0.283 a	0.230 a	0.210 a
	11 *	7-day	0.173 a	0.213 a	0.107 ab	0.173 a
		2-day	0.190 a	0.217 a	0.067 b	0.067 a
		0-day	0.163 a	0.210 a	0.280 a	0.060 a
		post	0.127 a	0.147 a	0.130 ab	0.177 a
		chem	0.080 a	0.173 a	0.147 ab	0.097 a
		none	0.150 a	0.060 a	0.043 b	0.133 a

¹ The biological control agent in test four is Kodiak; in test six - Deny Liquid-Biological Fungicide; in test seven - Deny Liquid-Biological Nematicide; in test eight - Gliocladium J1446 powder; in test ten - Mycostop; in test eleven - RootShield Drench. ² Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³ * indicates low disease pressure.

Table 19. Treatment plant dry weight (g) means separated by inoculum level for basil in Sunshine Mix.

exp	test ¹	treatment	inoculum level			
			uninfested	low	medium	high
1	3	2-day	1.467 a ²	0.847 a	1.680 a	0.000 b
		1-day	1.427 a	0.463 a	0.793 a	0.043 b
		0-day	1.313 a	0.897 a	0.893 a	0.370 ab
		chem	1.237 a	0.827 a	1.540 a	1.363 a
		none	1.480 a	0.873 a	1.133 a	0.350 ab
2	6	7-day	0.260 a	0.053 b	0.320 a	0.323 a
		2-day	0.407 a	0.290 ab	0.407 a	0.230 a
		0-day	0.437 a	0.290 ab	0.393 a	0.280 a
		post	0.503 a	0.517 a	0.320 a	0.440 a
		chem	0.313 a	0.227 b	0.380 a	0.380 a
		none	0.547 a	0.197 b	0.270 a	0.340 a
	10	7-day	0.787 ab	0.480 a	0.463 a	0.620 a
		2-day	0.507 b	0.447 a	0.510 a	0.533 a
		0-day	0.780 ab	1.040 a	0.833 a	0.587 a
		post	0.940 ab	1.073 a	0.877 a	0.437 a
		chem	0.870 ab	0.780 a	0.680 a	0.563 a
		none	1.030 a	0.880 a	0.837 a	0.790 a
	11	7-day	0.313 a	0.190 a	0.233 a	0.280 a
		2-day	0.303 a	0.463 a	0.213 a	0.367 a
		0-day	0.343 a	0.300 a	0.293 a	0.230 a
		post	0.487 a	0.533 a	0.387 a	0.320 a
		chem	0.323 a	0.300 a	0.223 a	0.260 a
		none	0.400 a	0.327 a	0.317 a	0.260 a
	12	high	0.417 a	0.363 a	0.300 b	0.247 a
		low	0.347 a	0.253 a	0.433 a	0.230 a
		chem	0.357 a	0.373 a	0.320 b	0.293 a
		pchem	0.357 a	0.370 a	0.267 b	0.210 a
		none	0.453 a	0.277 a	0.187 b	0.230 a

¹ The biological control agent in test three is Gliocladium J1446 powder; in test six - Deny Liquid-Biological Fungicide; in test ten - Mycostop; in test eleven - RootShield Drench; in test twelve - RootShield Granules. ² Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test.

Table 20. Treatment plant dry weight (g) means separated by inoculum level for basil in PGC Mix.

exp	test ¹	treatment	inoculum level			
			uninfested	low	medium	high
2	8 * ³	7-day	0.100 a ²	0.153 a	0.153 a	0.043 ab
		2-day	0.203 a	0.180 a	0.203 a	0.000 b
		0-day	0.223 a	0.143 a	0.187 a	0.117 ab
		post	0.213 a	0.210 a	0.213 a	0.217 a
		chem	0.147 a	0.117 a	0.137 a	0.120 ab
		none	0.177 a	0.210 a	0.140 a	0.127 ab
	12 *	high	0.063 a	0.103 a	0.090 a	0.013 a
		low	0.070 a	0.043 a	0.013 a	0.093 a
		chem	0.087 a	0.053 a	0.047 a	0.033 a
		pchem	0.040 a	0.067 a	0.027 a	0.047 a
		none	0.030 a	0.107 a	0.043 a	0.020 a

¹ The biological control agent in test eight is Gliocladium J1446 powder; in test twelve - RootShield™ Granules. ² Column means followed by the same letter are not significantly different (P=0.05) according to the Student-Newman-Keul's multiple comparison test. ³ * indicates low disease pressure.

Figure 1 summarizes the proven disease pressure and effective disease control in each data set for the tests in experiments one and two. The higher disease pressure in PGC Mix and the lower disease pressure on petunia were again evident. The previously stated difference in disease pressure between PGC Mix experiment one (highest) and Sunshine Mix experiment two (lowest), with Sunshine Mix experiment one and PGC Mix experiment two intermediate, was also evident by taking into account the incidence of both disease pressure and effective disease control. Based on this difference of disease pressure, the effective control treatments were summarized in Figures 2-4, based on the high, moderate and low disease pressures as stated above.

Figure 1. Tests with effective disease control treatments for host plant – growing medium combinations.

		host plant – growing medium combination					
exp	test ¹	Sunshine Mix #1			PGC Mix		
		pansy	petunia	basil	pansy	petunia	basil
1	1	dp ²	dp – C	dp	dp	dp	dp
	2	dp	dp	dp	dp	dp	dp
	3	dp		dp – C	dp		dp
	4	dp	dp – C	dp	dp	dp – C	dp
	5	dp	dp – C	dp	dp	dp	dp
2	6	dp – C	dp – C	dp – C	dp	dp – C	dp
	7				dp – C	dp – C	dp
	8	dp – C			dp – C	dp – C	dp – C
	9	dp	dp – C		dp		dp
	10	dp – C		dp – C	dp	dp – C	
	11			dp – C		dp – C	dp
	12			dp – C	dp – C		dp – C
	13				dp – C	dp	

¹ For definitions of tests, refer to Table 2. ² dp = proven disease pressure, C = effective disease control.

When tested under high disease pressure (Figure 2), only a biological control agent gave effective disease control. Kodiak (test 4) was the only example of a biological control agent specific to one host plant. It was the only agent effective under high disease pressure and was most effective on petunia under low disease pressure (Figure 4). Under high disease pressure it was most effective when applied as a pre-transplant drench and under low disease pressure as a post-transplant drench, taking into account that the post-transplant biological treatment was not included in the tests performed under high disease pressure (experiment one).

Figure 2. Most effective disease control treatments for tests under high disease pressure associated with PGC Mix – experiment one.

exp	test	biological control agent	disease control treatment				bca total
			biological			chemical	
			2-day	1-day	0-day	chem	
1	1	Deny™ Liquid – Biological Fungicide					
	2	Deny™ Liquid – Biological Nematicide					
	3	Gliocladium J1446 powder					
	4	Kodiak™		x			1
	5	Mycostop™					
treatment total				1			

Under conditions of moderate disease pressure (Figure 3), both biological and chemical control agents were among the most effective treatments. The most effective chemical treatment was the pre-transplant drench, taking into account that the post-transplant chemical treatment was only included in experiment two. The post-transplant chemical treatment was not among the most effective treatments in any of the tests under any disease pressure category. The most effective of the biological treatments was the '2-day' pre-transplant drench. There were five instances in which it was the most effective treatment in a data set, which makes it easily comparable to the six instances of the pre-transplant chemical treatment. The most frequently effective biological control agents included Gliocladium J1446 powder, Deny Liquid-Biological Nematicide, Mycostop and Deny Liquid-Biological Fungicide. Of the sixteen cases in which most effective treatments were listed, five were in Sunshine Mix, eleven in PGC Mix, nine for petunia, four for pansy and three for basil, suggesting better control in PGC Mix and for petunia. Eight of the eleven effective cases in PGC Mix were biological treatments,

while three of the five for Sunshine Mix were chemical, suggesting better biological control in PGC Mix. Seven of the nine effective control treatments for petunia were pre-transplant biological treatments, along with only one of four for pansy and two of three for basil. Under moderate disease pressure, the best biological control was obtained in PGC Mix. Of the three host plants, the most effective biological control was on petunia, applied as a '2-day' pre-transplant drench.

Figure 3. Most effective disease control treatments for tests under moderate disease pressure associated with PGC Mix – experiment two and Sunshine Mix #1 – experiment one.

exp	test	biological control agent	disease control treatment								bca total	
			biological					chemical				
			7-day	2-day	1-day	0-day	post	high	low	chem		pchem
1	1	Deny™ Liquid – Biological Fungicide	na ¹		x		na	na	na		na	1
	2	Deny™ Liquid – Biological Nematicide	na				na	na	na		na	0
	3	Gliocladium J1446 powder	na				na	na	na	x	na	0
	4	Kodiak™	na				na	na	na	x	na	0
	5	Mycostop™	na	x			na	na	na	x	na	1
2	6	Deny™ Liquid – Biological Fungicide			na	x		na	na		na	1
	7	Deny™ Liquid – Biological Nematicide	x	x	na			na	na		na	2
	8	Gliocladium J1446 powder		x	na		x	na	na	x	na	2
	9	Kodiak™			na			na	na		na	0
	10	Mycostop™		x	na			na	na		na	1
	11	RootShield™ Drench		x	na			na	na		na	1
	12	RootShield™ Granules	na	na	na	na	na	x		x		1
	13	SoilGard™	na	na	na	na	na			x		0
treatment total			1	5	1	1	1	1	0	6	0	

¹ 'na' indicates that treatment was not included in test.

Under conditions of low disease pressure (Figure 4), again both biological and chemical control agents were effective. The most effective chemical treatment was the

pre-transplant drench. The most effective biological treatment was the post-transplant drench. The most effective biological control agents included Deny Liquid-Biological Fungicide and Mycostop. Of the ten cases in which most effective treatments were listed, two were for petunia, four for pansy and four for basil. Contrary to the results from moderate disease pressure, under conditions of low disease pressure, pansy and basil appeared to be more amenable to disease control than petunia. Eight of the ten effective treatments were post-transplant biological treatments, suggesting that under low disease pressure the post-transplant biological treatment was most effective, even more so than the most effective pre-transplant chemical treatment.

Figure 4. Most effective disease control treatments for tests under low disease pressure associated with Sunshine Mix #1 – experiment two.

exp	test	biological control agent	disease control treatment								bca total
			biological						chemical		
			7-day	2-day	0-day	post	high	low	chem	pchem	
2	6	Deny™ Liquid – Biological Fungicide				xxx ¹	na ²	na		na	3
	7	Deny™ Liquid – Biological Nematicide					na	na		na	0
	8	Gliocladium J1446 powder				x	na	na		na	1
	9	Kodiak™				x	na	na		na	1
	10	Mycostop™			x	xx	na	na		na	3
	11	RootShield™ Drench				x	na	na		na	1
	12	RootShield™ Granules	na	na	na	na			x		0
	13	SoilGard™	na	na	na	na					0
treatment total			0	0	1	8	0	0	1	0	

¹ The number of marks for each treatment reflects the number of times they were the most effective treatments. In this case, the post-transplant treatment was most effective for test 6 for all three host plants. ² 'na' indicates treatment not included in test.

Measurement of Inoculum Potential

The results from the beet seed inoculum potential assay are summarized in Table 21. None of the beet seeds was infected with *R. solani* 24 hours after infestation. The uninfested growing media continued to be *R. solani*-free for the span of the four week experiment, including the growing media from the RootShield Granules test. By two weeks after infestation, the growing media with low, medium and high inoculum levels were all infested. The inoculum potential continued to increase over the next two weeks, as reflected by the increased percent of beet seed infection measured at four weeks after infestation. In the two weeks between the second week after infestation and the fourth week after infestation, the inoculum potential of the low and medium inoculum levels doubled. Both the high and medium inoculum levels reached maximum percent infection by the fourth week after infestation (98 % infection). The results from the growing media from the RootShield Granules test were the same as the results from the fourth week after *in vitro* infestation of growing media without plants, suggesting that the presence of plants did not affect the change in inoculum potential. Both the medium and high inoculum levels had reached maximum percent infection by the end of the test (96-98 % infection).

Table 21. Percent beet seed infection at the four inoculum levels by *R. solani* after 0, 2 and 4 weeks incubation and at the end of the RootShield Granules test.

inoculum level	0 weeks	2 weeks	4 weeks	4 weeks-RSg ¹
uninfested	0 %	0 %	0 %	0 %
low	0 %	9.3 %	41 %	15 %
medium	0 %	57.3 %	98.7 %	96 %
high	0 %	86.7 %	98 %	98 %

¹ RSg = RootShield Granules test.

DISCUSSION

Data interpretation was approached in the manner presented in order to take into account first the 'big picture' effects of the general factors, i.e. host plants, growing media, and inoculum levels. It was then narrowed down to the 'small' effects of the different disease control treatments, while weeding out unimportant or false results in the process. In this manner, a large quantity of data was analyzed and interpreted, and the important results selected for discussion. The gradient in disease pressure served as an interesting means of segregating the effective control treatments for presentation and discussion.

The change in disease pressure between experiments one and two added an unexpected, but not detrimental, factor to the results of the research. It was used to solidify the data interpretation, by eliminating data sets without disease pressure to avoid false positive interpretations, and as a basis for the presentation of the treatment results. The fact that there was a decreasing trend in plant dry weight and plant vigor score from the uninfested inoculum level to the high inoculum level in both experiments (Table 6), confirmed that the experimental design was sound enough to proceed with the analysis.

The decrease in disease pressure between the two experiments was found to be directly related to the length of time that had passed since the canola-based inoculum was first prepared. Although the inoculum was tested *in vitro* prior to use in the greenhouse

tests and appeared to be potent, it lost effectiveness over time and by the end of experiment two, was significantly less potent than it had been in experiment one.

The seasonal effect noted in Tables 4-6 did not affect the results since it was consistent in each test and the tests were each analyzed individually. It was however, one of the reasons that tests with the same biological control organisms could not be compared statistically. The other reason was that the treatments were different between tests in experiment one and experiment two (refer to Table 3), regardless of the biological control agent tested.

One of the most interesting results of the research was that Sunshine Mix #1 produced plants that were statistically healthier than did PGC Mix in all of the tests. In a test conducted at Ohio State University which included soil, composted bark and a variety of peat-based growing mixes, Sunshine Mix #4 and a similar peat-based mix, Fafard 3-B, were shown to be the best growing mixes based on flowering and rooting criteria of *Hippeastrum* (17).

Sunshine Mix and PGC Mix are two very different growing media, based on all of the properties of a growing medium, as presented by Fonteno in Bedding Plants IV (19). The porosity, density, moisture content, soluble salts and cation exchange capacity differ for each. Although both soil-based and soilless growing media can produce acceptable plants, they require different irrigation and fertility regimes. Most growers use peat-based mixes especially for annuals and bedding plants. Peat-based media have been shown to perform better than soil-based media because of uniformity, lighter weight, quicker drainage, better aeration, and better water and nutrient holding capacities (19).

Peat-based media have also been shown to be suppressive to disease, not only due to their good, plant stress-reducing qualities, but also due to their capacity to support a wide range of naturally antagonistic microorganisms (7, 8). Much research has gone into determining the cause of the natural suppressiveness of peat-based mixes. Boehm et al. (8) determined that peat in different stages of composting supports different antagonistic microflora and that the appropriate antagonists can greatly reduce disease incidence, especially disease caused by *Pythium* sp. to short lived crops such as bedding plants. Further research into suppressive mixes led to the discovery that composted hardwood bark is also a good option and was even shown to be more suppressive in combination with some peat than peat-based mixes alone (32).

Research has since led to investigations that involve sterilizing these mixes and reintroducing beneficial microbes and biological control agents. Many biological control agents are organisms that were originally isolated from suppressive growing media and seemingly would be expected to recolonize sterile mixes quickly and efficiently. Nelson and Hoitink (38) found that sterile composted hardwood bark recolonized with *Trichoderma harzianum* was suppressive of *R. solani* to levels equal to or greater than unsterile composted hardwood bark. This suggests that if the medium has the ability to support microorganisms, then a biological control agent introduced into that sterile medium without competitors may be very successful in colonization and preventing pathogens from causing disease.

The cause of the greater effectiveness of Sunshine Mix in this research was not determined. Speculation that it is due to the antagonistic action of suppressive microorganisms is supported by two observations. The fact that the Sunshine Mix data

sets involved lower disease pressure than the PGC Mix data sets and that the observed difference in disease pressure (Tables 7 and 8) was present, suggests that some quality of the Sunshine Mix either directly reduced the disease pressure on the host plants by affecting the pathogen, or directly affected the host plant by stimulating defense responses or promoting growth.

Microorganisms have been shown to both compete with pathogens and increase plant health directly (16). Also, the fact that the inoculum potential test could not be completed with Sunshine Mix due to the large number of fast-growing contaminant microorganisms compared to PGC Mix suggests microorganism competition as the means of disease pressure reduction. Therefore, plants grown in Sunshine Mix were expected to be healthier than those in PGC Mix regardless of the disease control treatment. The results presented in Table 4 support the theory. Plants grown in Sunshine Mix were healthier than those in PGC Mix, regardless of the plant, growing medium, inoculum level or disease control treatment. However, the results presented in Figure 3 showed that the healthiest plants, resulting from the most effective disease control treatments, were grown in PGC Mix.

Under high disease pressure (Figure 2), only *B. subtilis* (Kodiak) was effective. The disease pressure in PGC Mix within experiment one was so high that none of the treatments was effective. Kodiak appeared to have worked, but the results regarding its true efficacy were unclear. If the apparent efficacy under high disease pressure were real, then it should have easily outperformed the other biological control agents at the lower disease pressures, but this was not observed. Although the results at high disease pressure were interesting, they were unsubstantiated by the results from the other levels

of disease pressure. Perhaps Kodiak increased the vigor of petunia consistently at all three disease pressure levels, but was out-performed by the other biological control agents at the lower levels. These results were from a data set in PGC Mix, which suggests that Kodiak may have been more effective when introduced into a pasteurized environment. A study by Olsen and Baker (40) showed that *B. subtilis* was an effective antagonist against *R. solani*, especially in heat-treated soils, but that its specificity to particular soils and particular isolates of *R. solani* made it unpredictable.

Under moderate disease pressure (Figure 3), results from both growing media were included, the only case in which effective treatments could be compared along with their associated growing media. In support of the observation under high disease pressure that biological control agents may be more effective when introduced into pasteurized environments, eleven of the sixteen effective treatments listed were from PGC Mix data sets. Also, the majority of the effective treatments listed (nine of sixteen) involved petunia, and five of those were from PGC Mix. The most effective biological control agents were *Gliocladium catenulatum* (Gliocladium J1446 powder), *Burkholderia cepacia* (Deny Liquid-Biological Nematicide), *Streptomyces griseoviridis* (Mycostop), and *B. cepacia* (Deny Liquid-Biological Fungicide).

From these results, it appears that PGC Mix is much better for supporting biological control agents, but more than one third of the listed effective treatments (six of sixteen) were the pre-transplant chemical treatment. Under moderate disease pressure, the pre-transplant biological treatments outperformed all others, especially the post-transplant biological treatment, which was listed only once as a most effective treatment. Particularly effective was the '2-day' pre-transplant biological treatment. It was the only

pre-transplant treatment in which plants received twice as much biological control agent, suggesting that in this case, more might be better. Six of eight most effective pre-transplant biological treatments were in PGC Mix, suggesting that not only does PGC Mix support biological control agents, but it is particularly supportive of pre-transplant biological treatments. Petunia seemed to be particularly amenable to protection by biological control agents with seven of the nine most effective treatments listed involving biological treatments. However, within the scope of this research, the reasons for petunia's better performance were not determined. The increased health may have been due to the physical characteristics of the roots which made it more habitable to microorganisms, the root exudates which may have stimulated microorganism activity, or simply that petunia was less susceptible to the pathogen and thus under lower disease pressure than the other two host plants.

Interestingly, under low disease pressure (Figure 4), only one of the ten most effective control treatments listed was the chemical treatment. Unfortunately, based on the way the data were summarized, only Sunshine Mix was included in the low disease pressure category so no further investigation into effectiveness related to growing medium was possible. The most effective biological control agents under low disease pressure were *B. cepacia* (Deny Liquid-Biological Fungicide) and *S. griseovirides* (Mycostop). The overwhelmingly most effective treatment under these conditions was the post-transplant biological treatment. It was the treatment listed eight times of the ten total. It appears that under low disease pressure, when using a peat-based growing medium, specifically Sunshine Mix #1, that the post-transplant treatment greatly outperformed both the pre-transplant biological and chemical treatments that were so

successful under moderate disease pressure. Perhaps at low disease pressure, the natural suppressiveness of the Sunshine Mix was no longer stifled by high disease pressure and along with the biological control agents, plant health was vastly increased.

A study done by Nemec et al. (39) tested the efficacies of several biological control agents in Fafard Superfine (peat-based) mix to control root diseases of vegetables and citrus. They found that *B. subtilis* (Kodiak) and *T. harzianum* (RootShield) were most efficient at colonizing plant roots in greenhouse tests. When tested in the field, *B. subtilis* effectively increased cotton yield and decreased *Fusarium*-caused root disease. *T. harzianum* effectively increased lettuce yield and decreased *Phytophthora*-caused disease, and *S. griseoviridis* (Mycostop) decreased *Thielaviopsis basicola*-caused disease on citrange when applied as a drench to soil in pots. These results appear to support the results of this research in that different biological control agents are most effective on different host plants and in controlling different diseases. While some biological control agents may appear to have performed badly in these tests, they might easily outperform the others under different circumstances.

Another interesting observation was that of the growth of petunia under low disease pressure conditions, compared to its growth under moderate disease pressure. Under low disease pressure, it was only involved in two of the ten most effective treatments listed. Perhaps the efficacy of the biological control agents as pre-transplant treatments was directly related to the lower susceptibility of petunia. Once the disease pressure was lowered enough for the natural suppressiveness of the Sunshine Mix to be effective, it easily outperformed both the pre-transplant biological treatments that were so

effective under moderate disease pressure in PGC Mix, and it easily increased the health of the pansy and basil plants to the level of the less susceptible petunias.

The results of this research are valuable for at least two reasons. First, the pre-transplant chemical treatment was definitively more effective than a post-transplant chemical treatment, especially under moderate disease pressure, for all host plants in both PGC Mix and Sunshine Mix. Secondly, in a time in which biological control agents are still perceived to be much less effective than any chemical treatment, these results show that under certain circumstances, biological controls are equally, if not more, effective than chemical treatments. Additionally, all of the treatments were made only at the time of transplant and no subsequent treatments were made prior to rating and harvesting the plants after a period of growth equivalent to that of the time between commercial transplant and sale. Most disease control regimes require repeated applications, which may or may not, if applied to the use of these biological control agents, further increase the efficacies reflected here. The observation that one pre-transplant chemical drench of seedlings in a plug tray can be effective in increasing plant vigor and dry weight under moderate disease pressure, has much potential for application in a commercial setting.

The opportunities for expanding upon this research are practically endless. Potentially useful research projects include combining biological fungicides with chemical fungicides, the other biological and chemical pesticides used in production, different fertilizer regimes, other growing mixes, a wider range of host plants, etc. Any insight into the ecology of biological control agents and their environments would be useful to their eventual wider application and improved efficacy. That petunia reacted so differently from pansy and basil in this research (Table 5) is evidence that different plants

react unpredictably to different experimental factors. In order to obtain more specific efficacy data, research should be concentrated on as few factors as possible.

The biological treatments that were especially effective were the pre-transplant treatments into PGC Mix and the post-transplant treatments into Sunshine Mix. Under moderate disease pressure, some pre-transplant biological drenches were as effective as the pre-transplant chemical drenches. Under low disease pressure, the post-transplant drenches were more effective than the chemical pre-transplant drenches. Under normal greenhouse conditions, by maintaining strict sanitation and good cultural conditions, root rot caused by *R. solani* should be easily kept to a minimum. Disease pressure should be low and at most, equivalent to the low disease pressure created for this research. Good cultural conditions and the use of new growing media and sterile planting equipment are much more effective than indiscriminate preventative fungicide use (13). Under the strictly sanitary conditions provided in this research, these results show that disease pressure was lowered by simply growing plants in Sunshine Mix #1, as opposed to PGC Mix.

CONCLUSIONS

The recommendations based on this research are to first, maintain strict sanitation standards and good growing conditions to minimize plant stress and maximize health. Secondly, consider using a disease-suppressive growing medium, such as Sunshine Mix or another peat- or compost-based mix, but keep in mind that results may vary with the plants grown, and experimentation on a small scale should precede switching all growing to a new medium. If moderate disease pressure is encountered and a pasteurized growing medium used, consider using a preventative, pre-transplant drench of plugs with either a recommended chemical control agent or an effective biological control agent, such as Gliocladium J1446 powder (*G. catenulatum*), Deny Liquid-Biological Nematicide (*B. cepacia*), Mycostop (*S. griseoviridis*), or Deny Liquid-Biological Fungicide (*B. cepacia*). Although not among the most effective biological control agents under moderate disease pressure, RootShield Drench (*T. harzianum*) and RootShield Granules (*T. harzianum*) showed promise. If low disease pressure is encountered, consider using a post-transplant biological treatment, again keeping in mind that results may vary with growing media and plants. Biological control agents that were shown to be most effective under low disease pressure include Deny Liquid-Biological Fungicide (*B. cepacia*) and Mycostop (*S. griseoviridis*). Although not among the most effective biological control agents under

low disease pressure, Gliocladium J1446 powder (*G. cantenulatum*), Kodiak (*B. subtilis*), and RootShield Drench (*T. harzianum*) showed promise.

These results should at least stimulate grower interest in using biological control agents. Eventually, stricter environmental regulations will require closer monitoring of greenhouse effluents and the use of recirculating water systems in all areas of the United States. In adapting to new regulations, biological control agents may prove to be more useful than the traditional chemical controls either by themselves or in combination with chemicals in an integrated disease management program.

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