EVALUATION OF BACTERIAL STRAINS FOR CONTROL OF DOLLAR SPOT ON CREEPING BENTGRASS AND BROWN PATCH ON TALL FESCUE

by

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(ABSTRACT)

Sclerotinia homoeocarpa F.T. Bennett, causal agent of dollar spot on creeping bentgrass (*Agrostis palustris* Huds.); and *Rhizoctonia solani* Kühn, causal agent of brown patch on tall fescue (*Festuca arundinacea* Schreb.); are important pathogens of turfgrass. This research evaluated the ability of twenty bacterial strains of the genus *Pseudomonas* as potential biological agents for the control of these diseases. Year 1 dollar spot field trials resulted in the identification of five strains performing statistically as well as the recommended fungicide, chlorothalonil. Year 2 trials, using the top strains from Year 1, employed different application schedules and inclusion of a commercial spray adjuvant, Agri-Dex™. Results from Year 2 dollar spot trials indicated that eight strains performed statistically better than the non-treated control. Results from Year 1 and Year 2 of brown patch field trials provided three strains that performed statistically better than the non-treated control during Year 1, but only one strain during Year 2. Laboratory tests

performed with all strains and both pathogens showed that demonstration of agar-based inhibition is more difficult to obtain with *R. solani*. Six strains evaluated using Agri-Dex™ and *S. homoeocarpa* in laboratory and greenhouse tests, determined that application with Agri-Dex™ can be more effective than with bacteria alone. Supporting greenhouse tests using the top strains from the *S. homoeocarpa* field trials to evaluate application timing in controlling dollar spot, indicated less disease with earlier and more frequent applications. With further research, several strains have the potential for disease control on turf, particularly dollar spot, and may reduce the reliance on chemical fungicides.

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I. INTRODUCTION

This research project was designed to aid in the development of a biological control technology that could potentially replace and directly reduce the quantities of fungicides applied to turfgrass by evaluating twenty strains of bacteria with disease control potential. The diseases studied in this research were dollar spot on creeping bentgrass, caused by *Sclerotinia homoeocarpa*, and tall fescue blight (brown patch), caused by *Rhizoctonia solani*. These are two serious fungal diseases affecting turfgrasses in the Mid-Atlantic region which receive substantial amounts of chemical fungicides for control, depending upon disease severity and weather conditions. While there are prescribed chemical treatments for both of these diseases, the future of many current fungicides is uncertain do to increased regulatory pressures, and control of brown patch is difficult to achieve even with recommended fungicides.

Bacteria with disease control capabilities may offer an alternative to chemical fungicide application. Previously, applying microorganisms for disease control has not proven to be commercially viable because of many factors including lack of suitable application technology, loss of organism viability, and sporadic performance in field trials. Recent advances however, in genetic manipulation of bacteria and formulation technology could offer new solutions to some of these problems.

II. LITERATURE REVIEW

Importance and Uses of Turf

Turfgrasses have been widely used for many different purposes and, because of their importance, have been cultivated for nearly 2,000 years (Smiley et al., 1983). In the United States, where turf serves many roles such as recreational, sports, ornamental, erosion control, and other functional uses, current estimates of established turf are between 12 and 15 million hectares. Although it is difficult to economically judge the value of turf because most turf species are perennials and remain on location for many years, turf remains one of the largest and most economically productive segments of U.S. agriculture (Smiley et al., 1983).

Turf maintenance represents a considerable expense wherever turf quality and aesthetics are important, particularly at golf courses and country clubs (Couch, 1987). Turf quality includes stand thickness, health, and stress tolerance, while aesthetics include visual qualities of color, uniformity, and absence of stress symptoms such as disease lesions. Individuals responsible for turf maintenance, where the primary interest is the aesthetic quality of turf, feel compelled to use large amounts of pesticides annually to control disease symptoms that detract from such quality. Fungicides comprise a significant proportion of the pesticides that are necessary to maintain turf to visual and quality standards (Couch, 1987; Ayers and Gilmore, 1991).

Currently, annual fungicide applications on turf exceed 13,867,000 kg of product (Anonymous, 1987; Ayers and Gilmore, 1991), and pesticide marketing figures show that more fungicides were sold in the U.S. for use on turfgrass than any other crop commodity, including the various food crops (Anonymous, 1989). The largest proportion of fungicides used on turf in the U.S. is on golf courses, which includes approximately 9,000,000 kg/yr of product, or 65% of total recreational use (Ayers and Gilmore, 1991).

Concerns raised by public interest groups and regulatory agencies over pesticide residues in the environment (including fungicides) have provided an incentive to find alternative methods of controlling fungal diseases. Potentially, such alternatives can provide a more cost-effective way to control diseases at desired levels and reduce the quantities of chemical fungicides currently applied to turf.

Turfgrass Diseases

Sclerotinia homoeocarpa F.T. Bennett, which causes dollar spot on creeping bentgrass (*Agrostis palustris* Huds.), is recognized as a widespread and serious problem of turfgrass. *Sclerotinia homoeocarpa* also infects bahiagrass (*Paspalum notatum*), bermudagrass (*Cynodon dactylon*), centipedegrass (*Eremochloa ophiuroides*), fescue (*Festuca* spp.) and zoysia (*Zoysia* spp.) (Couch and Moore, 1960; Couch and Bloom, 1960).

Lesions caused by the disease are characterized by small, circular sunken patches on turf, especially evident on closely-mowed golf greens. Warm days and cool nights with

high relative humidity are particularly conducive to disease development (Smith et al., 1970). This pathogen is ideally adapted to the climate found in the Mid-Atlantic region of the U.S., including Virginia.

The pathogen, *S. homoeocarpa*, overwinters as sclerotia and as dormant mycelia in the crowns and roots of infested plants (Couch, 1974). Sclerotia and mycelia are firm masses of individual hyphae that usually have no spores within or around them (Ainsworth, 1971). When conditions become favorable (warm days, high humidity), sclerotia and mycelia can parasitically colonize grass leaves, and subsequently, produce visible lesions (Couch, 1974).

Rhizoctonia solani Kühn is likewise considered to be a major fungal foliar disease of turfgrass. *R. solani*, which causes brown patch on tall fescue (*Festuca arundinacea* Schreb.), also infects bluegrass (*Poa* spp.), centipedegrass (*Eremochloa ophiuroides*), ryegrass (*Lolium* spp.), and zoysia (*Zoysia* spp.) (Couch, 1974).

Rhizoctonia brown patch is characterized by small lesions, and colonized leaves develop a dark brown color when exposed to favorable conditions. This disease is particularly a problem in areas of the United States with high summer temperatures and humidity levels (Couch, 1974). These regions subsequently receive the greatest quantity of fungicides to provide disease control and eliminate undesirable visible symptoms (Ayers and Gilmore, 1991). The pathogen, *R. solani*, is adapted to hot, humid weather and can cause very substantial seasonal damage to turf stands, particularly at temperatures of 26°C

to 29°C (Couch, 1974). Such environmental conditions are frequently found in many areas of the eastern United States.

R. solani survives both in plant debris and on the surface of the soil, and is very tolerant to adverse conditions. The fungus survives as bulbils, small sclerotium-like structures, made up of a small number of cells (Ainsworth, 1971); as monilioid cells, cells that have swellings at regular intervals (Ainsworth, 1971); or as thick walled mycelia in infested debris (Couch, 1974).

Exposure and Environmental Concerns with Fungicides

Since turf is a non-food crop that is used primarily for recreational purposes, fungicide toxicity issues have not been as much of a concern as with crops meant for direct human consumption. Fungicides are usually applied at high rates, with frequent re-application, to hold disease development at such a level that undesirable symptoms do not appear (Ayers and Gilmore, 1991). This heavy use of fungicides has raised numerous issues about potential problems associated with long-term fungicide exposure by human applicators and environmental pollution from residues of water-soluble fungicide formulations. In 1990, as a result of a national water sampling survey conducted by the United States Environmental Protection Agency , it was reported that over 70 pesticides were detected in ground water in 38 states (Ritter, 1990). Such survey results have prompted regulatory agencies to direct efforts towards reducing both the number and rate

of pesticide applications, including fungicides, to lower the potential for environmental contamination by chemical residues (Farrell, 1993; Gup, 1991).

Conservation organizations, regulatory agencies, and the general public are aware of the need to characterize and assess potential adverse effects of pesticides (including fungicides) as a consequence of environmental exposure (Farrell, 1993; Gup, 1991). Some adverse effects caused by pesticides that have been reported in the literature include modifications in the carbohydrate metabolism of non-target organisms (Mazur and Hughes, 1975), undesirable alterations in plant tissue dry weight (Warren et al., 1974), reduced concentrations of nutrients in grass leaves and stolons (Warren et al., 1974), changes in the microbial composition of turf thatch (Smith et al., 1970), and reduction in the mineralization of nitrogen in the soil (Dubey and Rodriguez, 1970).

In most instances, the human health effects that have been reported due to pesticide exposure have been acute (Ayers and Gilmore, 1991). The majority of fungicides have low mammalian toxicity, however, and in themselves are rarely the cause of acute intoxication. Reported toxicity cases, where investigated thoroughly, have always been found to be caused by other types of pesticides. Such findings do not negate the possibility of deleterious effects such as cell mutation that could possibly result in cancer caused by long-term exposure to low concentrations of particular compounds, including fungicides.

Biological Disease Control

Naturally occurring bacteria may be useful for biological control of turfgrass diseases (Thomson, 1993). Appropriate indigenous organisms should be able to compete and persist under harsh environmental conditions, including moisture and temperature extremes, soil acidity, and salt concentrations (Becker, 1984; Liu and Baker, 1980; Martin et al., 1985). There are numerous reports of rhizobacteria (bacteria isolated from plant root-soil zones) that exert a beneficial effect on plant growth, although this has often been attributed to the displacement of more harmful microorganisms (Cook and Rovira, 1976; Gardner et al., 1984; Kloepper and Schroth, 1981; Kloepper and Schroth, 1981; Suslow and Schroth, 1982).

Inconsistent field results with microbial agents applied to control plant diseases demonstrate a continued need to inventory and evaluate microbial rhizosphere populations and to determine what factors are important in affecting the composition of these populations (Gardner et al., 1984; Howell and Stipanovic, 1979; Kloepper and Schroth 1981). Such research approaches may result in strategies that enhance the activities of beneficial microorganisms (Howell and Stipanovic, 1979; Kleeberger et al., 1983; Lambert et al., 1987; Ritter, 1990; Schroth and Hancock, 1981).

Biological control of turfgrass diseases takes many forms. Control of these diseases can be aided by cultural means, such as regulating nitrogen content in the soil and plant tissue, moisture on leaf surfaces, and pH levels. Dollar spot disease can be reduced with a high nitrogen fertility program as well as keeping the field well irrigated (Couch,

1962). Brown patch severity can be reduced by removal of free water that has accumulated on the surface of the leaves, a balanced fertility program, and less frequent mowing of stands (Couch, 1962). These practices are not capable of controlling Sclerotinia dollar spot or Rhizoctonia brown patch but they may be useful in reducing the amount of disease (Couch, 1962). Research in this area is directed toward the applications of top-dressings on the top of turf amended with organic fertilizers and composts. By applying a sand and cornmeal top-dressing with an organic component, Nelson and Craft (1992, 1991, 1990) were able to reduce the severity of dollar spot, brown patch, and typhula blight, presumably due to resident bacterial populations found in organic matter (Nelson and Craft, 1992a, 1992b; Nelson and Craft, 1991; Nelson and Craft, 1990a, 1990b, 1990c).

More aggressive and conventional means of biological control using other organisms is a growing area of research. Goodman and Burpee (1991) have investigated controlling dollar spot on creeping bentgrass with top-dressings amended with potential antagonists including fungi and bacteria isolated from turfgrass, thatch, or soil. Four of 24 isolates were found to suppress dollar spot in field and greenhouse tests from isolates screened in the laboratory, including unidentified bacteria and fungi.

Biological control of fungal diseases using bacteria as antagonists is an active area of research in turfgrasses and other plants with satisfactory results. *Pseudomonas* strains have been shown to control take-all of wheat due to phenazine antibiotic production in the rhizosphere (Mazzola et al., 1992). These fluorescent pseudomonads are thought to be in

large part responsible for the biological control of this disease and can be applied as seed treatments to wheat seeds. These antibiotic strains are thought to be able to compete with resident microflora because antibiotics they produce are effect against a wide range of bacteria and fungi and therefore allow them to persist in the rhizosphere (Mazzola et al., 1992). Fluorescent pseudomonads have also been shown to be responsible for control of *R. solani* on cotton seedlings from naturally occurring strains isolated from the rhizosphere (Hagedorn et al., 1989; Howell and Stipanovic, 1979). *Pseudomonas fluorescens* is produced under the trade name Dagger G™, produced by Ecogen, and used for the control of Rhizoctonia and Pythium damping-off in cotton (Cook, 1993).

There is evidence in the literature of a wide range of potential biological control organisms used to control a variety of diseases. Research has shown bacteria to provide biological control of *Pythium* by treating seeds with *Pseudomonas fluorescens*, *Gliocladium virens*, *Enterobacter cloacae*, *Erwinia herbicola*, or antibiotics produced by theses species (Nelson, 1988; Howell, 1982; Howell and Stipanovic, 1980; Howell and Stipanovic; 1983; Howie and Suslow; 1986; Loper 1986). *Typhula phacorrhiza* isolates have been shown to suppress Typhula gray snow mold on creeping bentgrass in field plots using isolates grown on grain, air dried, and hand spread on plots (Burpee et al., 1987; Lawton and Burpee, 1990). These isolates were also tested for antagonism and inhibition using assays where organisms were paired on culture medium (Burpee et al., 1987). *Bacillus cereus* UW85 has been shown to control alfalfa damping-off when used as a seed treatment (Handelsman et al., 1990).

Dollar spot on creeping bentgrass can be controlled to some extent using *Fusarium heterosporum* applied as a weekly top-dressing of infested cornmeal (Goodman and Burpee, 1992). Dollar spot has also been shown to be effectively controlled by applying top-dressings infested with *Enterobacter cloacae* (Nelson and Craft, 1991). These top-dressing methods of application could readily be adapted for commercial use because many turfgrass stands are already top-dressed with sand and organic matter (Sutton and Peng, 1993). *Gliocladium virens* was also shown to suppress dollar spot when applied at two week intervals for 12 weeks, though not as well as the fungicide chlorothalonil (Haygood and Mazur, 1990). This organism is already marketed by W.R. Grace and Co. under the trade name of GlioGard™ for use against seedling diseases of ornamental and bedding plants (Cook, 1993).

Non-pathogenic fungi have been shown to control *R. solani* in field plots (Sutker and Lucas, 1987; Burpee and Goulty, 1984). Bi-nucleate, non-pathogenic species of *Rhizoctonia* (as opposed to the multi-nucleate pathogenic forms) have been shown to reduce Rhizoctonia brown patch disease on creeping bentgrass and tall fescue in field tests where infested grain of both types of fungi was used as inoculum in the field. Results showed that these non-pathogenic isolates did not cause disease and were able to suppress turfgrass disease caused by *R. solani*.

Future exploration in biological control lies with the development of transgenic grasses using plants that have been regenerated from protoplasts (Terakawa et al., 1992) that could be transformed with the potential to introduce mechanisms for fighting diseases

or protection from herbicide application at the genetic level. Several grasses have now been transformed in the laboratory. Systems for transforming creeping bentgrass, tall fescue, and orchardgrass with electroporation or microprojectile bombardment have been developed using selectable marker genes for hygromycin resistance and β-glucuronidase (Hartman et al., 1994; Wang et al., 1992; Ha et al., 1992; Horn et al., 1988). Herbicide resistance has been successfully conveyed into creeping bentgrass using microprojectile bombardment (Hartman et al., 1994). These discoveries could enable useful genes from other organisms to be inserted in these grasses.

Origin of Bacterial Strains

Initial pathogen repression tests, including the collection of bacterial strains used in this project, were performed by scientists at Allied Chemical Corporation. Three-hundred and ten isolates, chosen from the over 12,000 that were screened, provided control of seedling disease on cotton equal to that of a fungicide. From these 310 strains, 28 were eventually selected after a variety of secondary evaluations conducted in field trials on cotton (Hagedorn et al., 1987). The results of these cotton studies were the basis for initiating this research on the control of fungal pathogens on turfgrass (Hagedorn et al., 1989).

This project evaluated twenty strains, previously identified as members of the bacterial genus *Pseudomonas*, on turfgrass field plots to determine their effectiveness in controlling Sclerotinia dollar spot and Rhizoctonia brown patch. The strains that provided

the best results in reducing disease were then tested in greenhouse and laboratory experiments.

III. FIELD STUDIES

INTRODUCTION

Field trials are a necessary step in evaluating disease control agents to be used on turfgrass. Field trials can have variable results from year to year due to environmental and disease conditions and therefore must be repeated a second year.

Objectives

Year 1: Determine, in replicated field trials, the ability of twenty bacterial strains, all identified as members of the genus *Pseudomonas* (Hagedorn, 1989), to control Sclerotinia dollar spot on creeping bentgrass and Rhizoctonia brown patch on tall fescue.

Year 2: Further test those bacterial strains that demonstrated disease control in Year 1 to (a) revalidate observed disease control, (b) examine application frequency, and (c) determine the effects of application with a spray adjuvant on control.

MATERIALS AND METHODS

1. Bacterial Strains

Each strain was routinely maintained for cultivation, colony isolation, and purity on King's medium B (KB) agar (Hagedorn et al., 1989), with incubation for 24 h at 28°C. For long-term storage, individual colonies were taken from KB, suspended in a 15%

glycerol solution (in sterile water), and frozen at -87°C. The twenty bacterial strains have been identified as members of the genus *Pseudomonas* (Hagedorn et al., 1989) because of their Gram reactions, fluorescent properties, colony morphologies, and microscopic appearance (Appendix, Table 19).

2. Preparation of Bacterial Strains for Field Application

Each of the twenty bacterial strains used in the field trials was grown in a modified rhizosphere medium (RSM) (Appendix, Table 20) [RSM is a medium developed for testing biological control agents and enhancing antibiotic production, (Buyer et al., 1989)]. Strains were cultivated in 2 L flasks (1 L RSM broth) held at 28°C for 48 h, with occasional shaking by hand. At the end of the incubation period, the strains were in the late log phase and average bacterial counts were approximately 10^9 colony forming units (cfu)/ml (Appendix, Table 21). The strains were applied in the field by spraying each as a suspension in RSM at a concentration of approximately 10^9 cfu/ml (Field Studies, Materials and Methods, Section 5). Average counts were determined by dilution plating on Trypticase Soy Agar (TSA) after incubation in RSM broth at 28°C as described above for field trials.

3. Sclerotinia Dollar Spot Trial

This trial evaluated the ability of candidate bacterial isolates to control Sclerotinia dollar spot on 'Penneagle' creeping bentgrass under putting green management. This trial

was located at the VPI&SU Turfgrass Research Center in Blacksburg, VA. The trial utilized indigenous populations of *S. homoeocarpa* because the chosen test site had historically provided high levels of disease under favorable environmental conditions.

Year 1: Treatments included: (a) a non-treated control, (b) bacterial, and (c) chlorothalonil applications. Chlorothalonil (tetrachloroisophthalonitrile) was used as the fungicide control because it is a standard recommended treatment for use in chemical control of dollar spot on bentgrass (Couch, 1993). The treatments were applied beginning June 1, 1992, and re-applied at roughly 14-day intervals for the duration of the disease season for a total of seven applications.

A second trial, established in the same field, was conducted during mid-summer of Year 1 (July 7, 1992), using the five strains that had demonstrated the lowest overall disease index rating at that point. The treatments for this trial included (a) the five strains applied separately, (b) a non-treated control, and (c) chlorothalonil. This trial was conducted to determine whether or not bacterial isolates could be applied at the peak of the disease season, which usually occurs in July, and produce the same level of control as those treatments initiated when disease severity was low at the beginning of the field season.

Year 2: The six strains with the lowest overall disease index rating, demonstrating some ability for control in Year 1, were further evaluated in Year 2. Treatments included a total of six applications beginning on June 4 for the 14-day application cycles.

Treatments 1-6: The six strains were applied on a 14-day cycle, exactly following strain applications in Year 1. These treatments were designed to validate, through repetition, the results obtained in Year 1.

Treatments 7-12: The six strains were applied on a 28 day cycle. These treatments determined if the strains could perform under conditions of less frequent application, on a schedule that is more consistent with routine disease control application practices in the turf industry.

Treatments 13-15: Culture filtrate, from the top three strains, prepared by 30 min. centrifugation at 2200 x *g* in a Beckman TJ-6 refrigerated centrifuge to remove cells was applied on a 14-day cycle. Only the three most effective strains were used. These treatments determined if there were compounds in the growth medium that have disease control potential. It may be possible to isolate and apply a biologically produced compound without requiring living cells, thus avoiding the difficulties associated with handling and applying a living inoculum.

Treatments 16-18: Cells from the three most effective strains in the previous treatments (#13-15), suspended in pH 7.0 phosphate buffer, without growth medium present, were applied on a 14-day cycle. These treatments determined if the washed cells alone had any disease control potential. If so, it might be possible to mass harvest cells and store them in a buffer until application.

Treatments 19-21: Controls were applied on a 14-day cycle. Treatment 19 was non-inoculated RSM as prepared before addition of strains, treatment 20 was the non-treated control, and treatment 21 was chlorothalonil.

Treatments 22-27: The six strains were applied on a 14-day cycle with a 2% v/v addition Agri-Dex™ (in accordance with the manufacturer suggested application rate) added at the time of application. Agri-Dex™ (Helena Chemical Co., Memphis, TN.) is a commercial additive that has been used for biological control methods as well as chemicals in the turf and agricultural industries, and offers advantages as a "sticker" and adhesive that may enable the bacteria to better colonize turf foliage and to be more resistant to rain wash-off. Agri-Dex™ is a mixture of heavy-range paraffin-based petroleum oil, polyol fatty acid esters, and their polyethoxylated derivatives that function as spreaders, stickers, and/or penetrants. Although there are many chemical additives available, Agri-Dex™ represented a reasonable first attempt at additive evaluation because the compound is widely used and accepted by the private sector, and is inexpensive.

Treatment 28: Agri-Dex™ alone was applied as a control on a 14-day application cycle.

4. Rhizoctonia Brown Patch Trial

This trial was conducted to determine the relative effectiveness of candidate bacteria to control Rhizoctonia brown patch on 'Rebel II' turf-type tall fescue under sod management. This trial was located at the Brookmeade Sod Farm in Ashland, VA, where

the field contained indigenous populations of *R. solani*. The area provided by the landowner had historically produced high levels of disease under favorable environmental conditions.

Year 1: The treatments included: (1) a non-treated control, (2) bacterial, and (3) chlorothalonil applications. Chlorothalonil was used as the fungicide control because it is a recommended treatment for chemical control of brown patch on tall fescue (Couch, 1993). The treatments were applied beginning June 3 and re-applied at roughly 14-day intervals for the duration of the field season for a total of six applications.

Year 2: The treatments included: (1) a non-treated control, (2) bacterial, (3) RSM medium alone, and (4) chlorothalonil applications. The treatments were applied beginning June 10 and re-applied at roughly 14-day intervals for the duration of the field season for a total of six applications. The same treatments were employed in Year 2, as an exact repetition of Year 1.

5. Application of Bacterial Strains

For all field trials, individual plots measured 60 by 120 cm, and each treatment was randomized with four replications. The strains were cultured in RSM broth and applied with a CO_2-pressurized sprayer equipped with a Tee-Jet™ 8002 even flat fan spray tip in a Unijet™ nozzle at a nozzle pressure of 276 kPa (40 psi) (Couch, 1991). The application rate for each strain was 15.2 liters of liquid (4 gallons) per 92.5 m^2 (1000 ft^2). Each individual plot received 121.12 ml of media containing approximately 2.5×10^9

colony forming units per ml (an average of all twenty strains). Spray pressure did not affect the number of colony forming units per ml applied to plots. The fungicide treatment (positive control) was chlorothalonil, applied at the preventative rate of 7.6 L per 92.5 m^2, and a non-treated control (negative control) was included in each trial. Frequency of application generally followed a 14-day application schedule (adjusted as described in Section 3, Year 2, for dollar spot).

6. Evaluation of Dollar Spot Disease

Disease index rating for the dollar spot trial was based on a visual estimate of percent blighted foliage in each plot, conducted at 14-day intervals, as specified in the treatment procedures for the two field seasons. The disease index that accounted for both incidence and severity of dollar spot disease was a simplified version of standard visual assessment (Couch, 1991) of 0 to 5, where 0 = no blighted foliage (bf), 1 = 1 to 20% bf, 2 = 21% to 40% bf, 3 = 41% to 60% bf, 4 = 61% to 80% bf, and 5 = 81% to 100% bf in the plot area. Each field plot was numbered sequentially, without treatment numbers, so that the evaluator was not aware of the treatment type when rating disease (Appendix, Tables 22, 24).

7. Evaluation of Brown Patch Disease

Leaf samples were collected as leaf clippings, using a hand held clipper, and taken from three different areas of each plot. The disease index rating was a rating of the

incidence of disease for each plot and was determined by recording the number of leaves having any Rhizoctonia-induced lesions from a group of 100 randomly selected leaves (Couch and Smith, 1991). Rather than a visual assessment of disease, as was done with Sclerotinia dollar spot, leaf counting provides a precise number for the proportion of leaves that actually had lesions. Each field plot was sequentially numbered as above (Field Studies, Materials and Methods, Section 6) so that the evaluator was not aware of the treatment type when collecting leaf samples and rating for disease (Appendix, Tables 26, 28).

8. Analysis of Dollar Spot and Brown Patch Data

Data from each field trial were subjected to analysis of variance (ANOVA), and treatment means were compared with Duncan's Multiple Range Test at the ($P \leq 0.05$) level of significance. Mean disease evaluations were subsequently converted to percent disease control where the level of disease in the non-treated control plots served as the base point for comparison.

RESULTS AND DISCUSSION

Field Trials - Sclerotinia Dollar Spot

Year 1 (1992) Results

At the beginning of the trial there was virtually no occurrence of dollar spot in the plot area, however, by the June 23 application, dollar spot had developed extensively over

the plot area, demonstrated by a disease index rating of 3.8 on the non-treated plot (Table 1). The disease severity fluctuated with weather, being more prevalent when hot and humid conditions prevailed (6/23-7/22, and 8/14-9/11), and diminishing with cooler temperatures and cloudy conditions (7/22-8/14). Disease severity was high over the entire season as indicated by an average disease index rating of 3.5 out of 5 in the non-treated control (Table 2).

For the first evaluation date (6/12/92), only the fungicide chlorothalonil repressed the small amount of disease that was present (Table 1). By 6/23/92 (high disease index rating, 3.8 non-treated control), seven of the bacterial strains and the fungicide treatment were superior to the non-treated control. For the 7/7/92 evaluation date (high disease index rating, 3.8 non-treated control), five bacterial strains and the fungicide treatment were superior to the non-treated control. By 7/22/92 (slightly lower disease index rating, 3.5 non-treated control), 18 of the bacterial strains and fungicide were superior to the non-treated control. The large number of bacterial strains that were effective on 7/22/92 appeared to be related to weather conditions that reduced disease severity over the plots during the first two weeks of July. By 8/14/92, disease severity was lower (3.3 non-treated control), and only two bacterial strains and the fungicide were superior to the non-treated control, possibly due to variation among the repetitions. By the last evaluation date, 9/11/92, disease severity was higher again (3.8 disease index rating, non-treated control) and 10 strains plus the fungicide were superior to the non-treated control.

Table 1. Disease Index Ratings: *Sclerotinia homoeocarpa* 1992

Treatment:		Disease Index Rating[1]					
Number	Name	6/12/92	6/23/92	7/7/92	7/22/92	8/14/92	9/11/92
1	L-849	2.8[2] a[3]	1.5 c	1.5 b	1.3 bc	1.8 b-d	1.3 f
2	L-850	3.3 a	1.5 c	1.5 b	1.3 bc	1.0 d	1.3 f
3	L-851	3.3 a	2.5 a-c	2.3 ab	1.8 bc	2.3 a-d	2.0 d-f
4	L-852	3.5 a	2.5 a-c	2.5 ab	2.3 bc	2.8 a-c	2.8 a-e
5	L-853	4.0 a	2.3 a-c	2.5 ab	2.5 ab	2.3 a-d	2.5 b-e
6	L-854	3.0 a	2.0 bc	3.0 ab	2.3 bc	2.5 a-d	2.3 c-f
7	L-855	3.3 a	2.0 bc	1.5 b	1.5 bc	1.5 cd	2.3 c-f
8	L-856	3.3 a	2.0 bc	3.0 ab	2.5 ab	2.8 a-c	3.3 a-c
9	L-890	3.0 a	3.5 ab	3.3 ab	2.0 bc	2.8 a-c	3.5 ab
10	L-886	3.0 a	3.0 a-c	3.0 ab	2.0 bc	3.3 ab	2.8 a-e
11	L-891	3.0 a	2.3 a-c	1.5 b	1.3 bc	2.0 a-d	2.0 d-f
12	L-892	3.3 a	3.0 a-c	2.5 ab	1.8 bc	2.5 a-d	2.8 a-e
13	AC4-52	3.0 a	2.0 bc	2.5 ab	1.5 bc	2.5 a-d	2.9 a-d
14	AD4-34	3.8 a	2.8 a-c	3.0 ab	2.0 bc	2.0 a-d	3.0 a-d
15	G-226	2.8 a	3.0 a-c	2.8 ab	1.8 bc	2.8 a-c	3.3 a-c
16	M17(D)	3.5 a	2.3 a-c	3.3 ab	2.3 bc	3.3 ab	2.5 b-e
17	31-12	3.3 a	1.5 c	1.5 b	1.0 c	1.8 b-d	1.8 ef
18	WSB15134	3.0 a	2.5 a-c	2.5 ab	2.0 bc	2.3 a-d	2.3 c-f
19	RAL3	2.8 a	2.3 a-c	2.8 ab	2.3 bc	3.5 a	3.3 a-c
20	TR-21	3.0 a	3.0 a-c	3.0 ab	2.3 bc	2.8 a-c	3.0 a-d
21	Daconil™[4]	1.0 b	1.5 c	1.5 b	1.8 bc	1.3 cd	2.0 d-f
22	Non-treated[5]	2.8 a	3.8 a	3.8 a	3.5 a	3.3 ab	3.8 a

[1]Disease index based on a scale of 1 (1% to 20% visible disease)
to 5 (81% to 100% visible disease),a 0 represents no visible disease
[2]Means represent an average of four repetitions
[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)
[4]Chemical fungicide chlorothalonil
[5]No treatment

Table 2. Season Disease Index Ratings: *Sclerotinia homoeocarpa* 1992

Treatment: Number	Name	Application Schedule	Disease Index Rating[1] Average	% Control[2]
21	Daconil™[3]	14-Day	1.5[4] a[5]	57%
2	L-850	14-Day	1.7 a	36%
1	L-849	14-Day	1.7 a	36%
17	31-12	14-Day	1.8 a	34%
11	L-891	14-Day	2.0 ab	30%
7	L-855	14-Day	2.0 ab	30%
3	L-851	14-Day	2.4 bc	22%
13	AC4-52	14-Day	2.4 bc	22%
18	WSB15134	14-Day	2.4 b-d	22%
6	L-854	14-Day	2.5 b-d	20%
12	L-892	14-Day	2.7 cd	16%
5	L-853	14-Day	2.7 cd	16%
4	L-852	14-Day	2.7 cd	16%
15	G-226	14-Day	2.8 cd	14%
14	AD4-34	14-Day	2.8 cd	14%
8	L-856	14-Day	2.8 cd	14%
19	RAL3	14-Day	2.8 cd	14%
20	TR-21	14-Day	2.9 cd	12%
10	L-886	14-Day	2.9 cd	12%
16	M17(D)	14-Day	2.9 cd	12%
9	L-890	14-Day	3.0 de	10%
22	Non-treated[6]	14-Day	3.5 e	0%

[1]Disease index based on a scale of 1 (1% to 20% visible disease)
to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Numbers represent the percentage of disease control based on the non-treated season average

[3]Chemical fungicide chlorothalonil

[4]Means represent averages of disease index ratings for the 1992 field season (six dates, four repetitions each)

[5]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[6]No treatment

To identify the best strains over the season, results for each strain were statistically compared against all other strains, the non-treated control, and the fungicide. Nineteen strains were identified by this procedure as better than the non-treated control and five strains statistically performed as well as the chemical control. Combined season results for these (disease index rating and percent control) are shown in Table 2. The five best bacterial strains (L-850, L-849, 31-12, L-891, and L-855) were equivalent to the chlorothalonil (57% control) and provided control that ranged from 30% to 36%. The remaining strains that were statistically better than the non-treated plot (0% control) provided control that ranged from 12% to 22%. Plot number assignments and weather data for dollar spot 1992 are provided in the Appendix, Tables 22 and 23.

By comparing the means of each disease evaluation date for the entire field season (Table 1) to the non-treated control and the chemical fungicide, the better bacterial strains were easily identified. For example, strain L-850 produced a mean disease severity of 3.3, 1.5, 1.5, 1.3, 1.0, and 1.3. The best five strains were equivalent to the fungicide for five of the six disease evaluation dates, while two other strains (L-855 and L-891) were equal to the fungicide for four out of six and three out of six evaluation dates, respectively. Figure 1 shows the top three strains from the 1992 season in relation to the fungicide and the non-treated control. Such a trend of better pathogen repression over time may indicate that repeated applications are necessary for at least some of the bacterial strains prior to successful disease control.

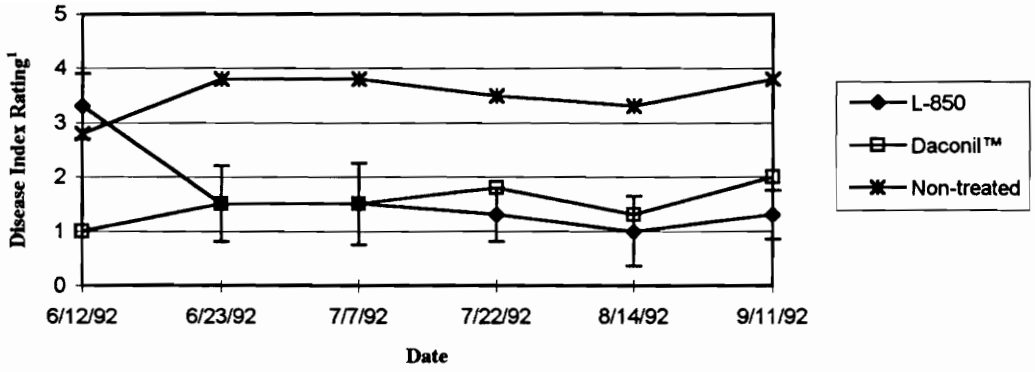

Figure 1.a

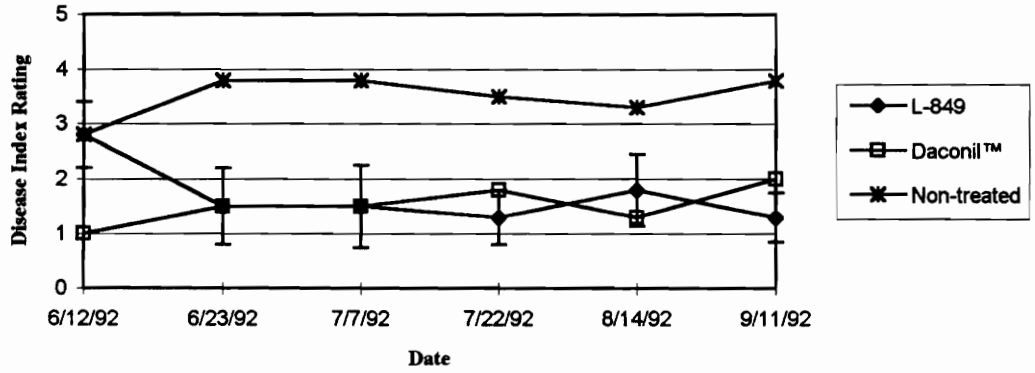

Figure 1.b

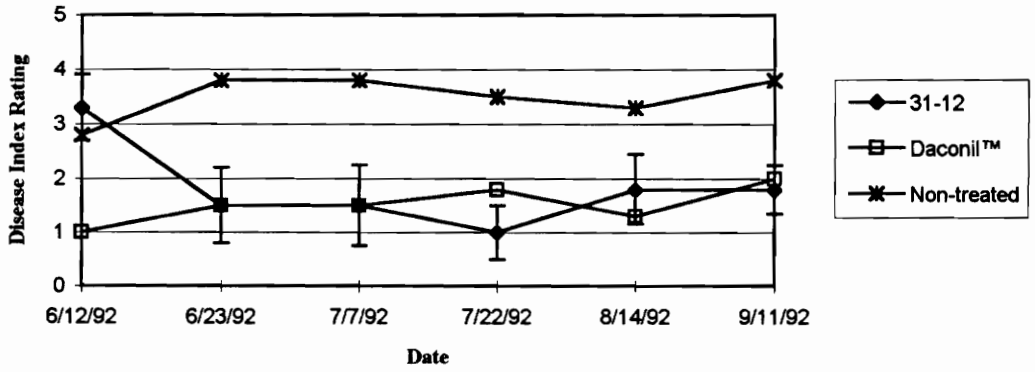

Figure 1.c

Figure 1. Dollar Spot 1992

[1]Disease index based on a scale of 1 (1% to 20% visible disease)
to 5 (81% to 100% visible disease),a rating of 0 represents no visible disease,
error bars represent the least significant difference between values for each evaluation date

Based on the 7/7/92 results, four of the best strains (L-850, L-849, 31-12, and L-891 from Table 2) were applied as a separate (second) field trial at the same location (Table 3). The first of three applications occurred on 7/7/92 and, by the first evaluation date (second application) on 7/22/92, only one strain (strain 31-12) was superior to the non-treated control. By the second evaluation date (third application) on 8/14/92, no strains were superior to the non-treated control. Further inspections of the plots indicated that none of the strains reduced disease severity, and this second trial was discontinued. An interpretation of the results from this second trial would indicate that the bacterial strains do not perform as well when used as a mid-season curative treatment when high disease severity is already present.

Year 1 (1992) Discussion

The field trials on Sclerotinia dollar spot indicated that there were five strains capable of repressing the disease at levels equivalent to the fungicide, and were worthy of further consideration, plus several that had some potential, but were not able to repress the disease at fungicide levels. This single result satisfied Objectives Year 1, and led into Objectives Year 2 (second year of this project) to optimize performance of the best strains through evaluation of application frequency, and application with a spray adjuvant to enhance performance. The best six strains were chosen from the overall field results of 1992 to further study in Objectives Year 2. Five of these strains statistically performed as

Table 3. *Sclerotinia homoeocarpa* Small Field Trial 1992

Treatment:		Disease Index Rating[1]	
Number	**Name**	**7/22/92**	**8/14/92**
1	L-849	3.8[2] a[3]	3.0 a
2	Daconil™[4]	1.3 b	1.5 b
3	L-891	4.0 a	2.0 ab
4	31-12	1.1 b	2.8 a
5	L-850	3.8 a	2.3 ab
6	Non-treated[5]	4.1 a	2.5 ab

[1]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Means represent an average of four repetitions

[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[4]Chemical fungicide chlorothalonil

[5]No treatment

well as the chemical fungicide; the sixth performed better than the non-treated control and was included because it represented a group of strains that provided 22% control.

Year 2 (1993) Results

The bacterial strains were first applied on June 4 and were re-applied five additional times based on the schedule described above under Field Studies, Materials and Methods, Section 3. Evaluations began on 6/29/93, and were conducted every two weeks for a total of five evaluation dates (Table 4). At the beginning of the trial and through the first evaluation date the severity of dollar spot was low in the plot area but, by July 15, dollar spot had developed at moderately high levels over the plot area (3.3 disease index rating for the non-treated control). The disease severity fluctuated with weather, reaching the highest levels when hot and humid conditions prevailed, beginning on 7/15 and continuing through the 8/12 evaluation. Disease had again declined by September 9 due to cooler temperatures. Due to lack of moisture, plots were regularly watered to maintain growing and productive stands of grass. Early applications of the bacterial strains, prior to onset of high disease severity, allowed the strains to become established in the plots and have a greater potential for controlling Sclerotinia dollar spot.

On the first evaluation date, there was light to moderate disease (2.3 disease index rating for the non-treated control) (Table 4), and only the fungicide and 31-12 plus Agri-Dex™ repressed the moderate amount of disease that was present. The 6/29 evaluation was the only time that 31-12 plus Agri-Dex™ was superior to the non-treated control. By

Table 4. Disease Index Ratings: *Sclerotinia homoeocarpa* 1993

Treatment:			Disease Index Rating[1]				
Number	Name		6/29/93	7/15/93	7/30/93	8/12/93	9/1/93
1	L-850		2.5[2] a-c[3]	3.3 ab	2.0 c-e	2.5 b-e	1.5 de
2	L-849		2.5 a-c	2.8 a-c	2.8 a-d	3.0 a-c	1.8 cd
3	31-12		2.3 a-d	3.3 ab	1.5 ef	2.0 c-f	1.8 cd
4	L-891		2.0 b-d	2.5 b-d	1.8 de	3.0 a-c	2.5 a-d
5	L-855		2.5 a-c	3.5 ab	2.8 a-d	3.0 a-c	2.5 a-d
6	L-851		2.3 a-d	3.0 a-c	3.0 a-c	3.5 ab	2.5 a-d
7	L-850	28-Day	3.0 ab	2.8 a-c	2.8 a-d	3.5 ab	2.3 a-d
8	L-849	28-Day	2.8 a-c	3.0 a-c	2.8 a-d	3.3 ab	2.5 a-d
9	31-12	28-Day	3.0 ab	2.8 a-c	3.0 a-c	3.5 ab	2.8 a-c
10	L-891	28-Day	3.3 a	2.8 a-c	3.0 a-c	3.0 a-c	2.8 a-c
11	L-855	28-Day	2.0 b-d	3.0 a-c	2.3 b-e	3.0 a-c	3.3 a
12	L-851	28-Day	3.0 ab	3.8 a	2.8 a-d	4.0 a	3.0 ab
13	L-850 Culture filtrate		2.8 a-c	2.8 a-c	2.5 a-e	2.8 b-d	3.0 ab
14	L-849 Culture filtrate		2.8 a-c	3.3 ab	3.3 ab	3.3 ab	2.5 a-d
15	31-12 Culture filtrate		2.5 a-c	2.8 a-c	2.0 c-e	2.8 b-d	2.8 a-c
16	L-891	Cells[4]	2.5 a-c	3.0 a-c	2.0 c-e	3.0 a-c	2.5 a-d
17	L-855	Cells	2.5 a-c	3.0 a-c	2.5 a-e	3.0 a-c	3.3 a
18	L-851	Cells	2.3 a-c	2.5 b-d	3.3 ab	3.5 ab	2.8 a-c
19	Medium[5]		3.3 a	3.5 ab	3.3 ab	3.5 ab	1.8 cd
20	Non-treated[6]		2.3 a-d	3.3 ab	3.5 a	3.3 ab	2.3 a-d
21	Daconil™[7]		0.5 f	0.5 f	0.5 f	0.0 h	0.5 e
22	L-850	Agri-Dex™[8]	1.8 c-e	2.5 b-d	1.5 ef	1.8 d-g	2.0 b-d
23	L-849	Agri-Dex™	2.3 a-d	2.0 c-e	1.8 de	1.5 e-g	2.3 a-d
24	31-12	Agri-Dex™	0.8 ef	2.5 b-d	2.8 a-d	2.5 b-e	1.8 cd
25	L-891	Agri-Dex™	2.0 b-d	1.3 ef	1.8 de	0.8 gh	1.5 de
26	L-855	Agri-Dex™	1.8 c-e	1.5 d-f	1.5 ef	1.3 fg	2.0 b-d
27	L-851	Agri-Dex™	1.3 d-f	2.0 c-e	1.8 de	2.0 c-f	2.3 a-d
28	Agri-Dex™[9]		1.8 c-e	2.0 c-e	1.8 de	2.5 b-e	2.8 a-c

[1]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Means represent an average of four repetitions

[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[4]Cells removed from medium and resuspended in phosphate buffer pH 7

[5]Medium only

[6]No treatment

[7]Chemical fungicide chlorothalonil

[8]Agri-Dex™ added to cell culture at 2% v/v

[9]Agri-Dex™ in water at 2% v/v

7/15 (high disease index rating, 3.3 non-treated control), two of the treatments (L-891 plus Agri-Dex™ and L-855 plus Agri-Dex™) and the fungicide were superior to the non-treated control. For the 7/30 evaluation (high disease index rating, 3.5 non-treated control), 11 treatments and the fungicide were superior to the non-treated control. Those treatments were L-850, 31-12, L-891, culture filtrate of 31-12, resuspended cells of L-850, and all strains plus Agri-Dex™, except 31-12 plus Agri-Dex™. Agri-Dex™ alone was also superior to the non-treated control. By the 8/12 evaluation (high disease index rating, 3.3 non-treated control), six of the treatments and the fungicide were superior to the non-treated control. Those were 31-12, and five of the six treatments plus Agri-Dex™, except 31-12 plus Agri-Dex™. Agri-Dex™ alone was not different from the non-treated control.

The average season evaluations of the 1993 dollar spot field trial indicate that while no strains performed statistically as well as the fungicide (Table 5), eight treatments did perform better than the non-treated control. Two of the best three strains (L-891 with Agri-Dex™ and L-851 with Agri-Dex™) with Agri-Dex™ were better than the non-treated control for four out of five evaluations and the third strain (L-855 with Agri-Dex™) was better than the non-treated control for three of the five evaluation dates (Figure 2). Those treatments include all strains plus Agri-Dex™, Agri-Dex™ alone, and 31-12 applied on a 14-day schedule. Those treatments provided control that ranged from

Table 5. Season Disease Index Ratings: *Sclerotinia homoeocarpa* 1993

Treatment: Number	Name	Application Schedule	Disease Index Rating[1] Average	% Control[2]
21	Daconil™[3]	14-Day	0.4[4] a[5]	87%
25	L-891 Agri-Dex™[6]	14-Day	1.5 b	50%
26	L-855 Agri-Dex™	14-Day	1.6 bc	47%
27	L-851 Agri-Dex™	14-Day	1.9 b-d	37%
22	L-850 Agri-Dex™	14-Day	1.9 b-d	37%
23	L-849 Agri-Dex™	14-Day	2.0 b-e	33%
24	31-12 Agri-Dex™	14-Day	2.1 b-f	30%
28	Agri-Dex™[7]	14-Day	2.2 c-g	27%
3	31-12	14-Day	2.2 c-g	27%
1	L-850	14-Day	2.4 d-h	20%
4	L-891	14-Day	2.4 d-h	20%
15	31-12 Culture Filtrate	14-Day	2.6 e-i	13%
2	L-849	14-Day	2.6 e-i	13%
16	L-891 Cells[8]	14-Day	2.6 e-i	13%
11	L-855	28-Day	2.7 f-j	10%
13	L-850 Culture Filtrate	14-Day	2.8 g-j	7%
6	L-851	14-Day	2.9 h-j	3%
17	L-855 Cells	14-Day	2.9 h-j	3%
5	L-855	14-Day	2.9 h-j	3%
18	L-851 Cells	14-Day	2.9 h-j	3%
8	L-849	28-Day	2.9 h-j	3%
7	L-850	28-Day	2.9 h-j	3%
20	Non-treated[9]	14-Day	3.0 h-j	0%
10	L-891	28-Day	3.0 h-j	0%
9	31-12	28-Day	3.0 h-j	0%
14	L-849 Culture Filtrate	14-Day	3.0 ij	0%
19	Medium[10]	14-Day	3.1 ij	-3%
12	L-851	28-Day	3.3 j	-10%

[1]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Numbers represent the percentage of disease control based on the non-treated season average

[3]Chemical fungicide chlorothalonil

[4]Means represent averages of disease index ratings for the 1993 field season (five dates, four repetitions each)

[5]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[6]Agri-Dex™ added to cell culture at 2% v/v

[7]Agri-Dex™ in water at 2% v/v

[8]Cells removed from medium and resuspended in phosphate buffer pH 7

[9]No treatment

[10]Medium only

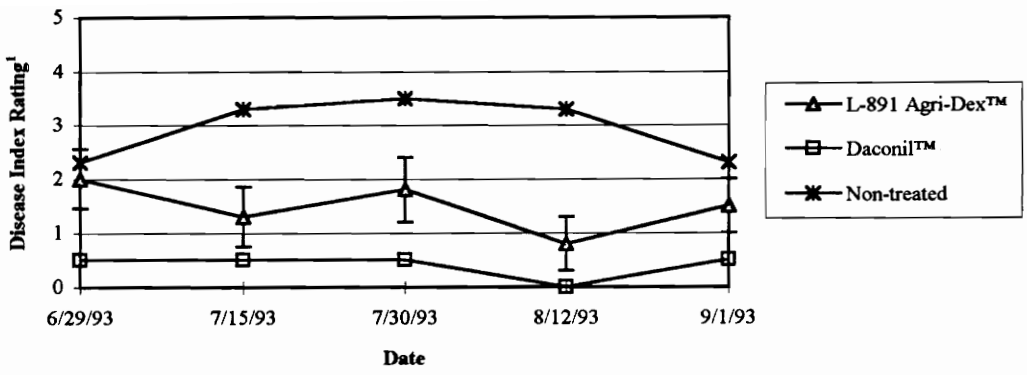

Figure 2.a

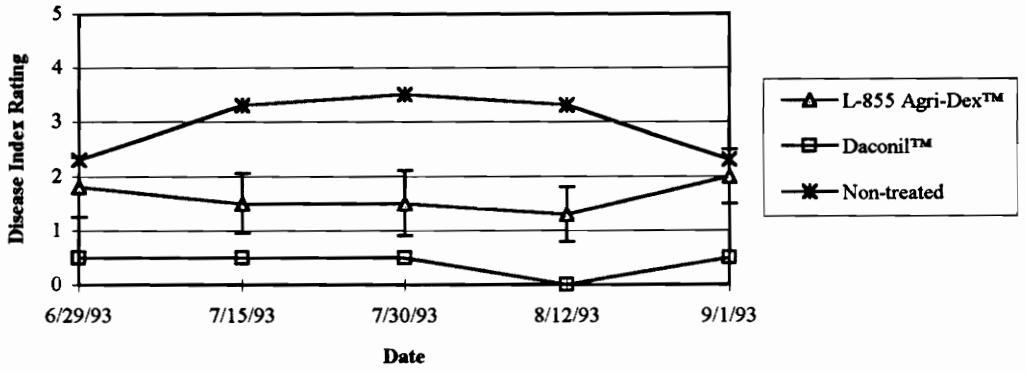

Figure 2.b

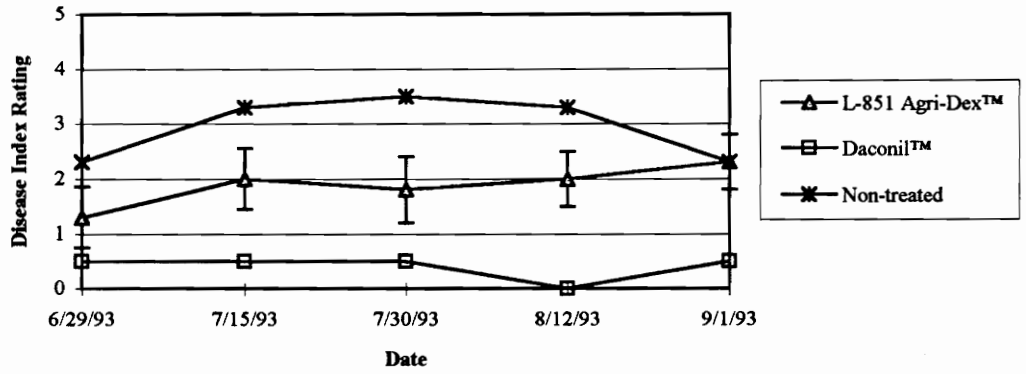

Figure 2.c

Figure 2. Dollar Spot 1993

[1]Disease index based on a scale of 1 (1% to 20% visible disease)
to 5 (81% to 100% visible disease),a rating of 0 represents no visible disease,
error bars represent the least significant difference between values for each evaluation date

27% to 50%. The chemical fungicide provided 87% control and the non-treated control provided 0% control. Plot number assignments and weather data are available in the Appendix, Tables 24 and 25, for the 1993 dollar spot field trial.

Year 2 (1993) Discussion

The field trials on Sclerotinia dollar spot indicated that the bacterial strains applied alone did not perform as well as they did during Year 1. None of the treatments applied on a 28 day cycle performed better than the non-treated control on any evaluation date, indicating that 28 days is too long a period between applications (Table 4). For the 14-day application cycle, only strain 31-12 performed well and was superior to the non-treated control on only two evaluation dates. The culture filtrate and the cells alone did not provide any control. The combination treatments of Agri-Dex™ plus bacterial strains appeared to provide better control than the strains in medium applied alone, with the exception of Agri-Dex™ plus 31-12. Treatments L-891 plus Agri-Dex™ and L-855 plus Agri-Dex™ were superior to the non-treated control for three of the five evaluation dates. Agri-Dex™ alone appears to provide some disease repression, as evidenced by consistently lower disease index ratings than the non-treated control, but were only statistically significant at the 7/30 evaluation (Table 4). There does appear to be an additive effect of disease repression by the bacteria plus Agri-Dex™ combination. Strain L-855 showed the most striking example of this. Strain L-855 had the second lowest overall disease index rating (Table 5) when applied in conjunction with Agri-Dex™

(disease index rating of 1.6), however when this strain was applied alone on a 14-day application schedule it resulted in an overall disease index rating of 2.9 and performed no better than the non-treated control. This could be due to the chemical itself or the addition with the bacteria that would allow the bacteria a greater chance of sticking to the leaf surface.

Field Trials - Rhizoctonia Brown Patch

Year 1 (1992) Results

Applications of the strains at two-week intervals began on 6/3/92 and were discontinued when it became apparent that little disease would occur at the test location. This first site received the first two applications. In the first trial location at the Brookmeade Sod Farm, the incidence of disease was light and did not progress to high levels when weather conditions should have been favorable for disease development. The trial was then duplicated, beginning on 7/1/92, in another part of the sod field where the incidence of disease was higher. This trial received the remaining four applications and was continued as long as the disease was detected. Grass samples were collected on 8/5/92 and 9/5/92 from the second trial, disease lesions were counted, and results are presented in Table 6. The 8/5/92 evaluation indicated that the incidence of disease was moderate (48.3% disease incidence for non-treated control), the fungicide treatment did not control disease (40.5% disease incidence), and only one strain, (L-850), was superior to the non-treated control (Table 6).

Table 6. Incidence of Disease[1]: *Rhizoctonia solani* 1992

Treatment:		Leaves with Lesions per 100 Leaves	
Number	Name	8/5/92	9/2/92
1	L-849	47.5[2] bc[3]	24.3 b-d
2	L-850	38.5 d	28.0 a-d
3	L-851	46.3 b-d	22.3 cd
4	L-852	44.8 cd	26.8 b-d
5	L-853	55.5 a	27.5 b-d
6	L-854	53.3 ab	29.5 a-d
7	L-855	43.8 cd	24.3 b-d
8	L-856	46.8 b-d	29.8 a-c
9	L-890	46.3 b-d	32.5 ab
10	L-886	44.8 cd	24.5 b-d
11	L-891	44.3 cd	22.0 cd
12	L-892	46.8 b-d	27.5 b-d
13	AC4-52	48.8 a-c	22.8 cd
14	AD4-34	40.3 cd	30.3 a-c
15	G-226	42.3 cd	26.0 b-d
16	M17(D)	44.0 cd	25.8 b-d
17	31-12	41.0 cd	30.0 a-c
18	WSB15134	46.5 b-d	29.3 a-d
19	RAL3	42.8 cd	23.8 b-d
20	TR-21	45.5 b-d	24.3 b-d
21	Daconil™[4]	40.5 cd	20.8 d
22	Non-treated[5]	48.3 a-c	36.3 a

[1]Incidence of disease based on the number of leaves that have one or more lesions in a 100 leaf sample

[2]Means represent an average of four repetitions

[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[4]Chemical fungicide chlorothalonil

[5]No treatment

The 9/2/92 evaluation indicated that the incidence of disease was low (36.3% incidence of disease in the non-treated control) and eleven strains plus the fungicide treatment were superior to the non-treated control. The fungicide controlled the disease with a 20.8% incidence of disease. After 9/2/92, the intensity of brown patch disease continued to decline due to unfavorable weather (brown patch is a typical summer disease that abates rapidly in the fall). These last results demonstrated that there were some strains that could repress Rhizoctonia brown patch, and indicated the importance of repeating the entire trial in 1993. Plot number assignments, and weather data are included in the Appendix, Tables 26 and 27 for brown patch 1992.

When both evaluations were combined into mean seasonal incidence for brown patch during 1992, only three strains performed better than the non-treated control, though seventeen strains performed as well as the chemical fungicide treatment due to a relatively small difference between the counts for the chemical fungicide and the non-treated control (Table 7). Those three strains, L-891, L-850, and RAL 3, provided levels of control at 21% to 22%. The remaining 14 strains were statistically the same as the fungicide, though also the same as the non-treated control, and provided levels of control from 9% to 19%. The chemical fungicide provided a control level of 27% based on the non-treated control.

Table 7. Season Incidence of Disease[1]: *Rhizoctonia solani* 1992

Treatment:		Application	Leaves with Lesions per 100 Leaves	%
Number	Name	Schedule	Average	Control[2]
21	Daconil™[3]	14-Day	30.7[4] a[5]	27%
11	L-891	14-Day	33.2 ab	22%
2	L-850	14-Day	33.3 ab	21%
19	RAL3	14-Day	33.3 ab	21%
7	L-855	14-Day	34.1 a-c	19%
15	G-226	14-Day	34.2 a-c	19%
3	L-851	14-Day	34.3 a-c	19%
10	L-886	14-Day	34.7 a-c	18%
20	TR-21	14-Day	34.9 a-c	17%
16	M17(D)	14-Day	34.9 a-c	17%
14	AD4-34	14-Day	35.3 a-c	17%
17	31-12	14-Day	35.5 a-c	16%
13	AC4-52	14-Day	35.8 a-c	15%
4	L-852	14-Day	35.8 a-c	15%
1	L-849	14-Day	35.9 a-c	15%
12	L-892	14-Day	37.2 a-c	12%
18	WSB15134	14-Day	37.9 a-c	10%
8	L-856	14-Day	38.3 a-c	9%
9	L-890	14-Day	39.4 bc	7%
6	L-854	14-Day	41.4 bc	2%
5	L-853	14-Day	41.5 bc	2%
22	Non-treated[6]	14-Day	42.3 c	0%

[1]Incidence of disease based on the number of leaves that have one or more lesions in a 100 leaf sample
[2]Numbers represent the percentage of disease control based on the non-treated season average
[3]Chemical fungicide chlorothalonil
[4]Means represent averages of disease incidence for the 1992 field season (two dates, four repetitions each)
[5]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)
[6]No treatment

Year 1 Discussion

Results from the second part of the 1992 field season (at the second site that was started late) indicated that there were some strains able to repress *Rhizoctonia solani* and reduce brown patch disease (Table 7). These results however were repeated as the trial was started late in the season due to low incidence of disease at the original site. This new trial was conducted at a location on the sod farm in 1993 where there was a higher incidence of disease.

Year 2 (1993) Results

Applications of strains at two-week intervals began on June 10, 1993, and first grass clippings were taken and evaluated on 7/13/93, when moderate disease incidence was present (Table 8). Additional clippings were taken on 8/18/93 for a second evaluation. The 7/13/93 lesion counts indicated that disease incidence was moderate (49.3% disease incidence in the non-treated control plot), the fungicide treatment successfully reduced disease incidence (27.5% diseased leaves), and six strains were superior to the non-treated control. These were M-17, L-890, L-855, L-856, L-854, and L-853. While the six best bacterial treatments were statistically equivalent to the fungicide, only L-854 produced an incidence of disease that was numerically equivalent to the fungicide.

The second evaluation from clippings taken on August 18, 1993 showed that overall disease had decreased dramatically due a change to cooler weather that was less

Table 8. Incidence of Disease[1]: *Rhizoctonia solani* 1993

Treatment:		Leaves with Lesions per 100 Leaves	
Number	Name	7/13/93	8/18/93
1	31-12	37.3[2] a-e[3]	28.0 ab
2	L-852	38.8 a-e	33.0 ab
3	AC4-52	42.0 a-d	28.0 ab
4	L-886	45.0 a-c	29.3 ab
5	TR-21	49.0 a	28.0 ab
6	RAL-3	39.0 a-e	33.0 ab
7	WSB15134	38.5 a-e	29.5 ab
8	L-891	35.8 a-e	26.3 ab
9	AD4-34	46.8 ab	24.3 ab
10	L-850	38.0 a-e	24.0 ab
11	G226	43.8 a-c	25.0 ab
12	M17(D)	33.3 b-e	28.0 ab
13	L-890	33.5 b-e	27.0 ab
14	L-855	33.8 b-e	31.5 ab
15	L-849	36.3 a-e	28.3 ab
16	L-851	46.3 ab	25.3 ab
17	L-856	31.0 c-e	28.8 ab
18	L-854	27.8 de	29.3 ab
19	L-853	34.5 b-e	35.5 ab
20	L-892	44.5 a-c	28.3 ab
21	Medium[4]	37.5 a-e	34.3 ab
22	Daconil™[5]	27.5 e	27.5 ab
23	Non-treated[6]	49.3 a	34.5 ab

[1]Incidence of disease based on the number of leaves that have one or more lesions in a 100 leaf sample
[2]Means represent an average of four repetitions
[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)
[4]Medium only
[5]Chemical fungicide chlorothalonil
[6]No treatment

favorable to disease development. No significant difference was obtained between the non-treated control (34.5% diseased leaves) and the chemical fungicide (27.5% diseased leaves). Because of this, no individual strains were identified as performing better than these controls.

The combined season results (Table 9) showed only one strain as performing better than the non-treated control and as well as the chemical fungicide, L-854, providing 32% disease control as compared to 34% disease control for the chemical fungicide. Nineteen strains and the non-inoculated medium alone were not different from the non-treated control while twenty strains and the non-inoculated medium were not different from the fungicide.

Weather conditions at the field site for the majority of the season, particularly during the first half, were very hot and dry and Hanover County, where the plots were located, was awarded Federal disaster relief for drought. The trial site was maintained by irrigation, and the hot conditions contributed to development of brown patch disease. Plot number assignments and weather data for brown patch 1993 are provided in the Appendix, Tables 28 and 29, respectively.

Year 2 Discussion

The level of control obtained from Year 1 was not convincing. Results from Year 2 were not much more encouraging (Tables 8 and 9) and have demonstrated that there was only one strain with some ability to repress brown patch disease. This strain, L-

Table 9. Season Incidence of Disease[1]: *Rhizoctonia solani* 1993

Treatment:		Application	Leaves with Lesions per 100 Leaves	%
Number	Name	Schedule	Average	Control[2]
22	Daconil™[3]	14-Day	27.5[4] a[5]	34%
18	L-854	14-Day	28.6 a	32%
17	L-856	14-Day	29.9 ab	29%
13	L-890	14-Day	30.3 ab	28%
12	M17(D)	14-Day	30.7 ab	27%
10	L-850	14-Day	31.0 ab	26%
8	L-891	14-Day	31.1 ab	26%
15	L-849	14-Day	32.3 ab	23%
1	31-12	14-Day	32.7 ab	22%
14	L-855	14-Day	32.7 ab	22%
7	WSB15134	14-Day	34.0 ab	19%
11	G226	14-Day	34.4 ab	18%
3	AC4-52	14-Day	35.0 ab	16%
19	L-853	14-Day	35.0 ab	16%
9	AD4-34	14-Day	35.6 ab	15%
16	L-851	14-Day	35.8 ab	15%
21	Medium[6]	14-Day	35.9 ab	14%
2	L-852	14-Day	35.9 ab	14%
6	RAL-3	14-Day	36.0 ab	14%
20	L-892	14-Day	36.4 ab	13%
4	L-886	14-Day	37.2 ab	11%
5	TR-21	14-Day	38.5 ab	8%
23	Non-treated[7]	14-Day	41.9 b	0%

[1]Incidence of disease based on the number of leaves that have one or more lesions in a 100 leaf sample
[2]Numbers represent the percentage of disease control based on the non-treated season average
[3]Chemical fungicide chlorothalonil
[4]Means represent averages of disease incidence for the 1993 field season (two dates, four repetitions each)
[5]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)
[6]Medium only
[7]No treatment

854, was among the lowest performing strains in the 1992 field season, indicating that other factors, such as weather or plot location, may have played a role in disease control and this strain may not be suitable for consideration as an independent biological control agent.

IV. LABORATORY EXPERIMENTS

A. INHIBITION STUDIES

INTRODUCTION

There are many agar-based assays available to test the ability of one microorganism to act as an antagonist and inhibit the growth of a second organism. It was necessary to assess several methods of applying the organisms and times of inoculation to find a way to provide the most reliable results with the selected test organisms. It was possible that an appropriate method and time of inoculation for both the fungus and the bacterium would not be appropriate for the two fungal pathogens, *S. homoeocarpa* and *R. solani*. This would depend on the medium, the growth rate of both the bacterium and the fungi on the medium, the method of inoculation for both organisms, and the diffusion rate of any compounds produced by the bacterium.

A number of different bacterial inoculation methods were evaluated to find a procedure that could be used to compare the twenty *Pseudomonas* strains against the selected pathogens for inhibition of fungal growth. The bacterium and the fungus were inoculated in three different ways on agar plates to determine which was most effective. First, bacteria were inoculated at four points around the edge of the plate using an inoculating loop of bacterial suspension and the fungus inoculated at the center of the plate. Second, bacteria were applied on one side over approximately one half of the plate, and the fungal plug was applied opposite. Third, bacteria were applied as a pie-shaped

wedge extending from the edge of the plate covering approximately one quarter of the plate, and the fungal plug applied opposite the point. The bacteria and fungus were then allowed to spread towards each other.

The distance between the bacteria and the fungus and the time between the inoculation of the bacteria and the fungus depended on their growth rates on agar surfaces. Plates were first inoculated with bacterial strains and fungi individually in order to evaluate rates of growth. The fungi grew much more rapidly than the bacteria. Prior inoculation provides time for the bacterial inoculant to become established before inoculation with the faster spreading fungus.

Each method and inoculation time was initially evaluated using a small selection of bacterial strains incubated on RSM agar. Strains used were chosen for there characteristic growth rates, one with a slow rate of growth, one with a faster rate of growth, and one strain that appeared to be intermediate. This was done to obtain a range where all the bacterial strains would grow under one set of conditions. Once a suitable method and inoculation time for each of the fungi were found, all twenty isolates were evaluated against each of the two fungal pathogens. While inhibition assays can be useful in determining modes of action and as a method for experimenting with multiple treatments, if the results of laboratory inhibition are not comparable to the situation one is attempting to simulate, the results may not necessarily indicate how a particular organism will perform in the field or other situation. These assays can still be useful in planning further experimentation.

Objective

Laboratory studies were conducted to determine the potential of twenty *Pseudomonas* isolates to inhibit growth of the fungal pathogens *R. solani* and *S. homoeocarpa* in agar-based assays.

MATERIALS AND METHODS

1. Assay

The fungi had no difficulty growing on the RSM agar, therefore the medium for evaluation was the modified RSM agar described in Section 2 of Field Studies. Plates were filled with 15 ml of RSM agar. The bacterial strains were inoculated on the outer edge of the plate and the agar plug of fungal pathogen was placed in the center of the plate. The bacterial strains were inoculated first and allowed to grow for 36 hours in the case of *Sclerotinia homoeocarpa* and for 60 hours in the case of *Rhizoctonia solani*. This was necessary in order to give the bacterial strains time to become established before pathogen introduction because of the rapid growth of these two fungi on such a rich medium. Plates were then inoculated with the appropriate pathogen by applying a 7 mm agar plug removed from a one week-old PDA plate of fungus to the middle of the RSM plate. Each plate was incubated at 28°C and measured at 12 h intervals beginning at the time of fungal inoculation for growth of the respective organisms.

2. Measurement of Inhibition

Antagonism between the two organisms can be observed and measured in several ways. There may be clear zones around the bacterial lawn where the fungus has not grown, indicating the production of some type of anti-fungal water soluble compound that inhibits the fungal pathogen. Alternatively, the fungus may no longer grow when it comes in direct contact with the bacterium, or it may stop growing and the mycelium may actually lyse. The distance between the edge of the fungal hyphae and the bacterial lawn was measured at 12 h intervals. Results were analyzed using the average measurement from the edge of the fungus to the edge of the bacterial lawn for all times (14 measurements for *S. homoeocarpa* and 12 measurements for *R. solani*) with three repetitions. Controls for these experiments included agar plates with fungal inoculation and no bacteria, agar plates with bacterial inoculation and no fungus, agar plates with fungus and chlorothalonil, and agar plates containing the medium only as a check for contamination during incubation.

3. Analysis of Data

The data generated from these experiments were analyzed using the statistical software program, Costat™ (Co-Hort Software; Berkeley, CA). Analysis of variance (ANOVA) of treatment means was used and mean separations were obtained with the Duncan's Multiple Range test when the overall F test is significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Sclerotinia homoeocarpa

Results

The results of the inhibition assay with the twenty bacterial strains and *Sclerotinia homoeocarpa* are presented in Table 10. Seven strains were statistically better than Daconil™, having the greatest average distance between the bacteria and the fungus. These seven strains were 31-12, L-852, AD4-34, AC4-52, L-886, G226, and M17(D); with ranges of 20.0 to 15.5 mm average distances. The Daconil™ had an average measurement of 7.8 mm. The remaining 14 strains, with higher degrees of variation (Appendix, Table 30), were at least equivalent to the Daconil™ with a range of 15.0 mm for L-850 to 7.5 mm for L-849.

Discussion

The individual strain performance does not seem to be directly connected to any ability towards success in the field. This is possible or even likely because the conditions in the lab can never be exactly like those in the field, and are much more simplistic as no plant surface to colonize, no competing microorganisms, and no environmental stress was present. Of the three top performers in the field trials and subsequent greenhouse experiments, strain L-849, did not inhibit fungal growth of *S. homoeocarpa* in the laboratory at all and was the only strain to inhibit fungal growth less than the chemical

Table 10. *Sclerotinia homoeocarpa* Inhibition in an Agar-Based Assay[1]

Strain Name	Measurement Average (mm)
31-12	20.0[2] a[3]
L-852	16.8 ab
AD4-34	16.8 ab
AC4-52	16.0 a-c
L-886	15.8 a-c
G226	15.5 a-c
M17(D)	15.5 a-c
L-850	15.0 a-d
L-856	12.8 a-d
L-853	12.7 a-d
WSB15134	12.7 a-d
L-855	12.4 b-d
TR-21	11.5 b-d
RAL-3	11.5 b-d
L-854	11.4 b-d
L-891	11.2 b-d
L-851	11.0 b-d
L-892	9.3 b-d
L-890	9.0 cd
Daconil™[4]	7.8 d
L-849	7.5 d

[1]Inhibition performed on agar plates using RSM agar
[2]Means represent averages of all measurements in mm (14 measurements, three repetitions each)
[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)
[4]Chemical fungicide chlorothalonil

fungicide. Strain L-850 provided moderate inhibition of the fungus in the laboratory, and strain 31-12 provided the most inhibition in the laboratory.

It is possible that Daconil™ had a low measurement of inhibition, even though it is the recommended fungicide, because the active ingredients are not water soluble (Budavari, 1989). This means the compound cannot diffuse through the medium, while in the case of the bacterial inoculants, compounds may be able to diffuse through the medium which is evidenced by a ring of inhibition around the bacteria. Since the chemical fungicide has to be in contact with the fungus to have any effect, the fungus did not grow over the top of the Daconil™ application area, though it was able to grow up to the edge of the Daconil™.

Rhizoctonia solani

Results

Two strains were statistically better than the Daconil™ chemical fungicide (Table 11). These two strains, L-886 and 31-12, were also in the top strains for the greatest inhibition of dollar spot in the field and had inhibition distance averages of 14.7 and 13.5 mm respectively. The remaining eighteen strains were statistically equal to Daconil™ although only nine had numerically greater measurement averages (Appendix, Table 31) ranging from L-852 with 11.0 mm to WSB15134 with 8.7 mm. Daconil™ had a measurement of 8.6 mm.

Table 11. *Rhizoctonia solani* Inhibition in an Agar-Based Assay[1]

Strain Name	Measurement Average (mm)
L-886	14.7[2] a[3]
31-12	13.5 a
L-852	11.0 b
L-856	10.4 bc
L-850	10.0 bc
L-855	10.0 bc
L-851	10.0 bc
AD4-34	9.0 b-d
L-854	8.8 b-d
WSB15134	8.7 b-d
Daconil™[4]	8.6 b-d
L-853	8.4 b-d
AC4-52	8.2 cd
M17(D)	8.0 cd
L-849	8.0 cd
G226	7.8 cd
TR-21	7.6 cd
L-890	7.6 cd
L-891	6.9 d
RAL-3	6.8 d
L-892	6.5 d

[1]Inhibition performed on agar plates using RSM agar

[2]Means represent averages of all measurements in mm (12 measurements, three repetitions each)

[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[4]Chemical fungicide chlorothalonil

Discussion

The inhibition measurements were generally lower for *Rhizoctonia solani* than *Sclerotinia homoeocarpa*. This indicates that the bacterial strains were less able to inhibit growth of *R. solani* because the average measurement of the distance between the edge of the fungus and the bacteria was less. This is another indication that *R. solani* is more difficult to control even with the prescribed chemical fungicides available on the market. Daconil™ had a higher numerical average than 10 strains as compared with the *S. homoeocarpa* inhibition results in which Daconil™ was numerically higher than only one strain. As with the *S. homoeocarpa*, there does not seem to be a direct relationship between field performance and laboratory inhibition. The top laboratory strain, L-886, was the seventh best field strain of 1992, and the nineteenth best strain of 1993.

B. AGRI-DEX™ STUDIES

INTRODUCTION

The purpose of Field Studies Objective 2 was to determine if Agri-Dex™ affects the growth of any of the six bacterial strains from the 1993 dollar spot trials or *Sclerotinia homoeocarpa* individually. This research is an effort to explain field observations where Agri-Dex™ appeared to make some contribution to disease control in the 1993 Sclerotinia dollar spot field studies. Such biological control could occur either by enhancing bacterial growth and therefore biological control activity, by repressing fungal growth, or by some combination of both. Knowing how Agri-Dex™ affects these organisms could be

important to determine whether an adjuvant, such as Agri-Dex™, could be useful as an independent or combination disease control agent with the bacterial strains.

Objective

Measure the effects on fungal growth with the adjuvant Agri-Dex™, and growth for the six bacterial strains applied with Agri-Dex™ in the field trials during the 1993 field season.

MATERIALS AND METHODS

1. Bacterial Strains

The six strains that were applied in conjunction with Agri-Dex™ in the field in the 1993 trials were used in these experiments: 31-12, L-849, L-850, L-851, L-855, and L-891. Agri-Dex™, at three different concentrations, was mixed with an RSM broth culture of each of the six bacterial strains. The Agri-Dex™ concentrations were 2% v/v (field rate), 4% v/v (2X field rate of) Agri-Dex™, and 1% (1/2X field rate) of Agri-Dex™. The two concentrations not used in the field (1/2X and 2X) were tested in the laboratory to determine if increased or decreased concentrations of Agri-Dex™ have additive or subtractive effects on bacterial or fungal growth. Flasks with 50 ml of RSM broth with and without Agri-Dex™ that had been individually inoculated with the six bacterial strains were incubated for 14 hours at 28°C. The broth was then plated on TSA to determine the number of cfu/ml of broth.

2. *Sclerotinia homoeocarpa*

For testing the sensitivity of *Sclerotinia homoeocarpa* to Agri-Dex™, the fungal culture was grown using PDA plates to which Agri-Dex™ had been added at the same concentrations as applied in the field (2% v/v). Additional concentrations of 2X the field rate of Agri-Dex™, and 1/2X the field rate of Agri-Dex™ were also tested. The plates were then poured (15 ml) and allowed to solidify. A 7 mm PDA plug of fungus from a fresh PDA-grown culture was placed in the center of each plate using a sterile cork borer and followed by incubation at 28°C. Plates were measured for growth at 24 hour intervals for four days. These measurements were compared to control plates with no Agri-Dex™.

As an alternative to adding Agri-Dex™ directly to the PDA agar, the effects on *S. homoeocarpa* growth was evaluated using a different method of Agri-Dex™ application. The Agri-Dex™ was applied over the freshly inoculated fungus on PDA plates to simulate spraying. The adjuvant was applied at the 2% v/v field rate, 2X field rate, and 1/2X field rate. The adjuvant was applied by mixing the appropriate amount with water and pipetting 1.0 ml onto the surface of the plates. The delivery for the Agri-Dex™ application was based on the actual amount delivered per square inch as calculated for the 1993 field trials.

A third method of applying Agri-Dex™ was also tested using a CO_2-pressurized sprayer with an 8002 flat fan nozzle as was used in the field, at the concentrations above. The plates were spread out on a 60 by 120 cm flat surface and sprayed with the a concentration of Agri-Dex™ for 10 seconds (15.14 mls/square foot/10 seconds = 1.0 mls

per petri plate). The plates were then measured for growth at 24 hour intervals for four days.

3. Analysis of Data

Data were analyzed as described in Laboratory Studies, Inhibition, Materials and Methods, Section 3. Each bacterial strain was analyzed individually and separately from the fungal pathogen with the Agri-Dex™ concentrations as the main effects.

RESULTS AND DISCUSSION

Bacterial Strains

Results

The overall results of the numbers of viable bacteria present when incubated with Agri-Dex™ produced variable results. Bacteria incubated with 4% and 0% Agri-Dex™ produced the highest cfu/ml while the two intermediate concentrations produced slightly lower cfu/ml (Table 12).

Individually, the results of each bacteria strain differed. Strain L-849 followed the pattern of the overall results. The two intermediate concentrations of Agri-Dex™ increased cell growth. Agri-Dex™ had no apparent effect on strain L-855 indicated by the relatively constant numbers for all the concentrations. Strain L-891 had a decreased cell count for the 1% concentration while the other concentrations produced the same number of cfu/ml. Strain 31-12 had the most dramatic results with a direct reduction in the

Table 12. Effect of Agri-Dex™ on Bacterial Growth

Strain Name	Agri-Dex™ Concentration[1]	Average Cell Count[2]	Duncan Grouping[3]
Bacteria	4% Agri-Dex™	1.6×10^9	a
Analyzed	No Agri-Dex™	1.5×10^9	a
Together	1% Agri-Dex™	1.2×10^9	b
	2% Agri-Dex™	1.1×10^9	b
L-849	No Agri-Dex™	2.6×10^9	a
	4% Agri-Dex™	2.3×10^9	a
	1% Agri-Dex™	1.2×10^9	b
	2% Agri-Dex™	1.0×10^9	b
L-855	1% Agri-Dex™	1.9×10^9	a
	4% Agri-Dex™	1.4×10^9	a
	2% Agri-Dex™	1.3×10^9	a
	No Agri-Dex™	1.2×10^9	a
L-891	4% Agri-Dex™	2.3×10^9	a
	2% Agri-Dex™	1.6×10^9	a
	No Agri-Dex™	1.5×10^9	ab
	1% Agri-Dex™	8.5×10^8	b
31-12	No Agri-Dex™	1.3×10^9	a
	1% Agri-Dex™	5.1×10^8	b
	2% Agri-Dex™	4.3×10^8	b
	4% Agri-Dex™	1.4×10^8	c
L-850	4% Agri-Dex™	2.1×10^9	a
	2% Agri-Dex™	1.4×10^9	ab
	1% Agri-Dex™	1.3×10^9	ab
	No Agri-Dex™	1.2×10^9	b
L-851	No Agri-Dex™	1.4×10^9	a
	2% Agri-Dex™	1.4×10^9	a
	4% Agri-Dex™	1.2×10^9	a
	1% Agri-Dex™	1.1×10^9	a

[1]Percentage of Agri-Dex™ v/v in RSM liquid medium

[2]Means represent an average of the number of colonies per ml (three repetitions)

[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

number of viable cells as the concentration of Agri-Dex™ increased. Strain L-850 produced the greatest number of bacteria when incubated with the highest concentration of Agri-Dex™ and the remainder of concentrations were relatively constant. Strain L-851 had no changes with the addition any concentration of Agri-Dex™.

Discussion

The maximum recommended concentration of Agri-Dex™ in application is 2.5%. It is expected that higher concentrations, in this case 4%, could lower the number of bacteria present in a culture. The opposite is indicated in the overall analysis in which 4% has the highest cell count. The overall analysis also indicates that two concentrations, 1% and 2%, were lower from those cultures that received no Agri-Dex™.

The results of the experiment do not support any specific conclusion and results seem too variable to credit with a particular field performance. Inoculation procedures may have not have been reliable enough to produce dependable results. The field application rate may have slightly lowered the number of bacteria actually applied, though those strains did perform well with the addition of the 2% concentration Agri-Dex™. This could be possible because the potential beneficial aspects of applying Agri-Dex™ could outweigh any possible detrimental effects on the bacterial strains such as its slightly harmful effect on the fungus and possibly by allowing the bacteria to remain on the surface of the leaf longer.

Fungus

Results

Fungal growth was affected by application of Agri-Dex™ to the fungal plugs, though the concentration of Agri-Dex™ did not seem to affect fungal growth (Table 13). Any amount of Agri-Dex™ added, including the rate applied in the field reduced fungal growth. Growth with no addition was an average of 56.9 mm, while fungus with 1%, 2%, and 4% had measurements of 45.8 mm, 45.5 mm, and 44.8 mm, respectively.

The method used for the experiment was the method of pipetting the Agri-Dex™ directly on to the fungal plug and agar plate. The alternative two methods of applying Agri-Dex™ did not seem appropriate. The Agri-Dex™ that was mixed with the agar would be of lesser concentration than actually believed because it is mixed throughout the 15 ml of agar so the fungus does not come into contact with the full amount. The plates with the Agri-Dex™ that had been applied with the backpack sprayer became too contaminated to read within 24 hours.

Discussion

The results indicate that the Agri-Dex™ alone has some effect in inhibiting growth of *S. homoeocarpa*. This is an implication of why those strains that were applied in conjunction with the Agri-Dex™ had a greater ability to reduce disease in field results, producing an additive effect and improving strain performance for five of the six strains applied with Agri-Dex™.

Table 13. Effect of Agri-Dex™ on Growth of *Sclerotinia homoeocarpa*

Agri-Dex™ Concentration[1]	Average Measurement
No Agri-Dex™	56.9[2] a[3]
1% Agri-Dex™	45.8 b
2% Agri-Dex™	45.5 b
4% Agri-Dex™	44.8 b

[1]Percentage of Agri-Dex™ v/v in RSM liquid medium

[2]Means represent the average diameter of fungus in mm (three repetitions)

[3]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

V. GREENHOUSE EXPERIMENTS

A. TIMING STUDIES

INTRODUCTION

Timing is important for the application of any biological disease control agent or fungicide formulation to achieve control. Knowing the correct application time can influence the degree of success or failure of any disease control treatment. In many cases, a product must be applied at a specific time in the disease cycle of a pathogen to obtain the desired level of disease control. The following greenhouse experiments were useful in determining the optimal time to apply selected biological control strains to obtain levels of disease control equivalent to those observed in the field studies.

To evaluate application timing, the three best bacterial strains from the Year 1 dollar spot data (L-850, L-849, and 31-12) were applied at specific intervals prior to, together with, and subsequent to inoculation with the dollar spot pathogen, *S. homoeocarpa*. Only the three best strains were chosen because it was desirable to apply more treatments which necessitated using a fewer number of isolates due to limited greenhouse space.

Objective

Evaluate the application timing of selected bacterial strains for control of Sclerotinia dollar spot on creeping bentgrass.

MATERIALS AND METHODS

1. Experimental Method

To evaluate application timing, periods for adding the bacterial inoculants were selected at six, four, and two days pre- and post-fungal (pathogen) inoculation, and inoculation together (time zero). Creeping bentgrass was planted in Metro-Mix 360™ (Grace-Sierra) potting media in 10 cm by 10 cm plastic pots and approximately 0.07 g of seed (≈984 seeds) was planted and covered by a thin layer of soil to provide adequate soil contact and cover for germination. The grass was grown in a greenhouse with humidity levels maintained at approximately 80-90%, using individual dew chamber pot covers (Carolina Biological Supply), and at a temperature between 24°C and 30°C in order to facilitate disease development (Couch and Smith, 1991). These dew chambers included a plastic top that helped to maintain high moisture and humidity levels, and these conditions encouraged disease development when coupled with warm temperatures.

The pots were watered by placing in trays of water until the leaves were just visible. The pots were then removed from the trays and watered as needed. The grass was fertilized weekly with 11 g (1 tablespoon) of Peter's™ general purpose fertilizer per 3.785 L (1 gallon) of water beginning when the pots were removed from the trays and every week thereafter.

2. Fungal Inoculation of Bentgrass

A preliminary experiment was run to determine the best fungal application method to achieve disease within a few days of application. A spray suspension and/or a ground millet powder infested with the fungus were applied to pots in different combinations. Through trial and error it was determined that to achieve sufficient disease for the study on bentgrass, two types of application were needed: (1) a fungal suspension sprayed until runoff occurred [2 liters for 150 pots] and, (2) approximately 0.12 g of ground millet/fungus mixture shaken onto each pot.

The fungal suspension was prepared in potato dextrose broth using an agar plug of *S. homoeocarpa* to inoculate 1 liter of broth. The broth was incubated at 28°C for 2-3 weeks with occasional shaking by hand, then blended in a Waring™ commercial blender for 3 to 5 seconds and poured through two layers of cotton cheesecloth to remove large mycelium pieces that may clog the spray apparatus. The strained fungal inoculum containing small pieces of mycelium was sprayed on the turf pots using a CO_2-pressurized sprayer equipped with an 8002 flat fan nozzle, as described for the Field Studies in Materials and Methods, Section 5.

The pathogen was also applied as a solid inoculum prepared in a granular form on ground millet where 50 g of millet seed was autoclaved with 50 ml of water. The millet was placed in a 1 liter flask to which several agar plugs of *S. homoeocarpa* were then added. The fungus was allowed to colonize the seed for 10 days at 28°C with occasional tapping to ensure distribution throughout the flask. The entire flask of millet was then

spread on a 23 cm by 32 cm metal tray, covered with 1 layer of 38 cm by 43 cm tissue paper, and allowed to dry for 2-3 days. The dried millet was then ground to a powder for 20 seconds in a Tekmar™ (Tekmar Company; Cincinnati, OH) IKA-A 10 analytical mill and tapped through a Mw 1000 mm sieve evenly onto the pots (Couch and Smith, 1991).

3. Preparation of Bacterial Strains

In order to evaluate as many times as possible, only the three best strains (having the lowest disease index ratings over the disease season) from the 1992 Field Studies were tested. Each bacterial strain, L-850, L-849, and 31-12, was cultured in RSM broth as was done for the Field Studies, Section 2. The strains were grown in 500 ml amounts for 24 h at 28°C prior to use.

4. Bacterial Applications to Bentgrass

Each bacterial strain was sprayed 10 seconds for each 60 cm by 120 cm area of pots to mimic field applications. Spraying was performed with a CO_2-pressurized sprayer equipped with an 8002 even flat fan spray tip at 276 kPa (40 psi), see Field Studies, Materials and Methods, Section 5. The strains were applied to different sets of pots at different times (Table 14). The strains that were applied at -6 days were re-applied at -4 days, -2 days, day 0, +2 days, +4 days, and +6 Days. Those applied at -4 days were re-applied at subsequent time intervals etc., until all the grass died, ending the trial. The pathogen was applied at day 0 (time zero). These times were chosen in order to provide a

Table 14. Application of Bacterial Strains in Greenhouse Timing Trials

Application Time[1]:	-6 Day Treatment	-4 Day Treatment	-2 Day Treatment	Day 0 Treatment	+2 Day Treatment	+4 Day Treatment	+6 Day Treatment
-6 Days	X[2]						
-4 Days	X	X					
-2 Days	X	X	X				
Time 0	X	X	X	X			
+2 Days	X	X	X	X	X		
+4 Days	X	X	X	X	X	X	
+6 Days	X	X	X	X	X	X	X

[1]Bacterial applications are in reference to fungal inoculation at time 0

[2]X Denotes application of bacteria

window of treatment around the inoculation of the fungus in order to more closely determine the most appropriate treatment application time.

There was also a treatment where five pots of each strain received only one bacterial application at -6 days and fungal inoculation at time 0 to determine if repeated applications of bacterial strains were necessary to maintain disease control. Controls for this experiment include five pots of potting mix with pathogen inoculation at time 0 and no bacteria, five pots (per strain) with bacterial inoculation at -6 days and no pathogen, five pots with no bacterial or fungal application, and five pots with fungal inoculation and chlorothalonil applied as a chemical fungicide 24 h prior to inoculation.

The pots were evaluated for disease symptoms during the application of the fungus and evaluations were conducted every two days until +10 days after fungal inoculation. Each time designation and all control treatments had five pots per strain.

5. Disease Evaluation

The pots were evaluated using a disease index of based on a scale of 0 to 5 as was done in the field trials. Evaluations were based on twenty percent increments of visible disease symptoms, blighted foliage (bf), where 0 is no visible symptoms, 1 is 1-20% bf, 2 is 21-40% bf, 3 is 41-60% bf, 4 is 61-80% bf, and 5 is 81-100% bf.

6. Data Analysis

Data were analyzed as described in Laboratory Studies, Materials and Methods, Section 3. Each bacterial strain was analyzed individually.

RESULTS AND DISCUSSION

Timing Trial #1

Results

The results for the greenhouse timing trial #1 were compared against those pots that received no treatment but did receive fungus at time 0 (Table 15). Strain L-849 showed a very clear correlation between time of application and disease control. Treatments that began six days prior to fungal inoculation had the lowest average disease index rating (0.1) as compared to treatments that were begun six days after fungal inoculation with an average disease index rating of 1.8. The average disease index rating of those pots with no treatment and fungal inoculation was 1.3. This number seems low, but it is an average of all evaluations at each treatment time which includes zeros for the first two evaluations (Appendix, Table 32).

Although not quite as distinct, the other two strains, L-850 and 31-12, also showed a clear relationship between time of application and fungus control. In these two cases the disease index rating for pots that received treatment six days after fungal inoculation was actually higher (more disease) than pots with no bacterial treatment, though only statistically the same for 31-12.

Table 15. Greenhouse Timing Trial #1

Strain Name	Treatment Time[1]	Disease Index Rating[2] Average	Duncan Grouping
31-12	No Fungus[3]	0.0[4]	a[5]
	Non-treated	0.0	a
	-6	0.0	a
	-4	0.1	a
	-2	0.2	a
	0	0.5	b
	+2	0.6	b
	+4	1.0	c
	Fungus Only[6]	1.3	c
	+6	1.8	d
L-850	No Fungus	0.0	a
	Non-treated	0.0	a
	-4	0.0	a
	-2	0.0	a
	-6	0.1	a
	0	0.4	b
	+2	1.1	c
	Fungus Only	1.3	cd
	+4	1.4	cd
	+6	1.5	d
L-849	No Fungus	0.0	a
	Non-treated	0.0	a
	-6	0.1	a
	-4	0.2	ab
	-2	0.4	a-c
	0	0.5	bc
	+2	0.7	c
	+6	1.1	d
	+4	1.3	d
	Fungus Only	1.3	d

[1]Bacterial application times refer to the number of days before and after fungal application

[2]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[3]Bacterial application with no fungal application

[4]Number represents an average of all times with five repetitions

[5]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[6]Fungal application at time 0, no bacteria

Discussion

Timing of application is clearly an important factor in controlling disease. It is critical for treatment to begin early in the disease season for these bacterial strains to be able to control the fungus and prevent disease. Statistically, application at times of 6, 4, and, 2 days before fungal application had the same disease index ratings, though numerically the earlier the application the lower the disease index rating. The grass in the pots receiving applications of bacterial strains at six days after fungal inoculation died from the fungus before they were able to receive more than one application. The bacterial application does not seem to be curative and is unable to reduce disease that has already become established. Fungus did not develop in those pots that received no treatments or applications nor the pots that received no fungal inoculation. Average disease index ratings at each evaluation time are in the Appendix, Table 32.

Timing Trial #2

Results

The results of the second timing of application trial were similar to the first trial (Table 16). This trial included the chemical fungicide Daconil™ and pots that received only one application of bacterial strains at six days before fungal application. The Daconil™ completely controlled the disease. The pots that received only the one application of bacterial strains did have fungus develop. One treatment did not prevent the

Table 16. Greenhouse Timing Trial #2

Strain Name	Treatment Time[1]	Disease Index Rating[2] Average	Duncan Grouping
31-12	No Fungus[3]	0.0[4]	a[5]
	Non-treated	0.0	a
	Daconil™[6]	0.0	a
	-6	0.0	a
	-4	0.0	a
	-2	0.1	a
	+2	0.4	b
	0	1.1	c
	+4	1.2	cd
	+6	1.3	cd
	Fungus Only[7]	1.4	d
	-6 w/ Fungus[8]	1.4	d
L-850	No Fungus	0.0	a
	Non-treated	0.0	a
	Daconil™	0.0	a
	-6	0.1	a
	-4	0.1	a
	-2	0.3	a
	0	0.3	a
	+2	0.8	b
	+4	1.1	c
	Fungus Only	1.2	c
	+6	1.3	c
	-6 w/ Fungus	1.4	c
L-849	No Fungus	0.0	a
	Non-treated	0.0	a
	Daconil™	0.0	a
	-6	0.0	a
	-4	0.3	a
	-2	0.4	a
	+2	0.8	b
	0	0.9	bc
	+4	1.1	bc
	+6	1.2	bc
	Fungus Only	1.2	bc
	-6 w/ Fungus	1.4	c

[1]Bacterial application times referring to the number of days before and after fungal application
[2]Disease index based on a scale of 1 (1% to 20% visible disease)
to 5 (81% to 100% visible disease),a 0 represents no visible disease
[3]Bacterial application with no fungal application
[4]Number represents an average of all times with five repetitions
[5]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)
[6]Chemical fungicide chlorothalonil
[7]Fungal application at time 0, no bacteria
[8]Bacterial application at time -6 only with fungal application at time 0

fungus from becoming established as is evident from the high disease index rating of 1.4, which was the same as the fungus only control.

Pots that received bacterial application before fungal inoculation; 6, 4, and, 2 days before fungal inoculation; all have the same statistical disease index rating. Applications of bacterial strains at time 0 were generally the first treatments that had results that were statistically not as high as the earlier application times.

Discussion

These trials were duplicated to ensure that the results from the first trial were reproducible. The same pattern was evident for both timing trials (Discussion, Timing Trial #1). The earlier the treatment was initiated and repeated, the better the control. Treatments applied after fungal inoculation were not able to decrease disease development (Appendix, Table 33). Daconil™ was able to control disease and had a disease index rating statistically the same as bacterial application at 6, 4, and 2 days before fungal inoculation for all three strains.

B. AGRI-DEX™ STUDIES

INTRODUCTION

Agri-Dex™ alone demonstrated some ability to control Sclerotinia dollar spot in the field trials during the 1993 field season and had a slight effect on the fungus alone in the laboratory. This observation was tested under more controlled conditions in the

greenhouse using the three best strains from the timing trials and the three concentrations in the laboratory experiments (Laboratory Experiments, Agri-Dex™ Studies, Materials and Methods, Section 1).

Objective

To determine in a greenhouse experiment whether Agri-Dex™ in three different concentrations has any controlling effect alone on Sclerotinia dollar spot disease or when used in conjunction with bacterial application.

MATERIALS AND METHODS

1. Experimental Method

Bacterial inoculants were applied six days before fungal inoculation because that time had the greatest capability for disease control based on the results in Table 15. This time was chosen to simplify the experiment. Bacterial application of the best three strains from the dollar spot field trials (31-12, L-849, L-850) occurred at two day intervals after the initial application until completion of the experiment at six days after fungal inoculation for a total of seven applications. Agri-Dex™ was added to the medium in three different concentrations before application. These concentrations were 1%, 2% (field concentration), and 4%. There were five replications of each treatment. Treatments included bacterial strains only (31-12, L-849, L-850), bacterial strains with each concentration of Agri-Dex™, Agri-Dex™ in the three concentrations alone with fungus,

and Agri-Dex™ at the 4% concentration with no fungus. There were also control treatments with Daconil™, fungus only, and no treatment.

2. Bacterial Strains

Bacterial strains (above) were cultured and prepared as in Greenhouse Experiments, Timing Trials, Materials and Methods, Section 3.

3. Fungal Inoculation

Fungal inoculation was applied as in Greenhouse Experiments, Timing Trials, Materials and Methods, Section 2 above.

4. Bacterial Application to Bentgrass

Bacterial application followed procedures outlined in Greenhouse Experiments, Timing Trials, Materials and Methods, Section 4.

5. Evaluation and Data Analysis

Evaluation and data analysis was performed as above in Greenhouse Experiments, Timing Trials, Materials and Methods, Section 5.

RESULTS AND DISCUSSION

Agri-Dex™ Trial #1

Results

The results for all three strains were the same (Table 17). Some disease developed when Agri-Dex™ was the treatment, though the Agri-Dex™ alone also had a tendency to kill the grass, especially at the higher concentrations. No disease developed in any pots that received bacterial application with no Agri-Dex™ or any of the pots that received bacterial strains with any concentration of Agri-Dex™, though again, Agri-Dex™ at the higher concentrations tended to kill the grass. It is difficult to tell if the Agri-Dex™ had any additive disease controlling effects because the bacterial strains alone controlled disease as well as those applications with Agri-Dex™.

Discussion

Agri-Dex™ alone is able to moderately control Sclerotinia dollar spot as compared to the pathogen alone control, though doing so may risk the health of the grass. Agri-Dex™ also controls disease when applied in conjunction with the bacterial strains and produces numerically lower disease index ratings (less disease) than the Agri-Dex™ alone and at the lowest concentration will not harm the grass itself. The ability of the bacterial strains to control dollar spot can be enhanced by the addition of Agri-Dex™ at low rates (1%). The bacterial strains alone also reduced disease without the addition of Agri-

Table 17. Greenhouse Agri-Dex™ Trial #1

Strain Name	Agri-Dex™ Concentration[1]	Disease Index Rating[2] Average	Duncan Grouping
31-12	0% w/ Fungus, w/ bacteria	0.0[3]	a[4]
	Non-treated	0.0	a
	Daconil™[5]	0.0	a
	4% no Fungus	0.0	a
	4% w/ Fungus, w/ bacteria[6]	0.0	a
	1% w/ Fungus, w/ bacteria	0.0	a
	2% w/ Fungus, w/ bacteria	0.0	a
	4% w/ Fungus, no bacteria[7]	0.2	a
	1% w/ Fungus, no bacteria	0.3	a
	2% w/ Fungus, no bacteria	0.3	a
	Fungus	1.3	b
L-850	0% w/ Fungus, w/ bacteria	0.0	a
	Non-treated	0.0	a
	Daconil™	0.0	a
	4% no Fungus	0.0	a
	4% w/ Fungus, w/ bacteria	0.0	a
	1% w/ Fungus, w/ bacteria	0.0	a
	2% w/ Fungus, w/ bacteria	0.0	a
	4% w/ Fungus, no bacteria	0.2	a
	1% w/ Fungus, no bacteria	0.3	a
	2% w/ Fungus, no bacteria	0.3	a
	Fungus	1.3	b
L-849	0% w/ Fungus, w/ bacteria	0.0	a
	Non-treated	0.0	a
	Daconil™	0.0	a
	4% no Fungus	0.0	a
	4% w/ Fungus, w/ bacteria	0.0	a
	1% w/ Fungus, w/ bacteria	0.0	a
	2% w/ Fungus, w/ bacteria	0.0	a
	4% w/ Fungus, no bacteria	0.2	a
	1% w/ Fungus, no bacteria	0.3	a
	2% w/ Fungus, no bacteria	0.3	a
	Fungus	1.3	b

[1]Agri-Dex™ concentration in medium (v/v)

[2]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[3]Number represents an average of all times with five repetitions

[4]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[5]Chemical fungicide chlorothalonil

[6]Agri-Dex™ applied with bacteria and fungal inoculation at time 0

[7]Fungus and Agri-Dex™ with no bacterial application

Dex™. Individual assessments of disease severity for each evaluation date are available in the Appendix, Table 34.

Agri-Dex™ Trial #2

Results

The results of the second Agri-Dex™ trial were very similar to the first trial (Greenhouse Experiments, Agri-Dex™ Trial #2, Results and Discussion) with slightly more variability among treatments (Table 18). The second trial differed only in that the disease index rating was slightly higher (+0.1) for all evaluations possibly due to slightly cooler temperatures in the greenhouse that were more conducive to disease development, as the heat wave during the first trial raised the temperature in the greenhouse to 38°C+.

Discussion

The repeat of the trial showed the same conclusions as the first trial (Discussion, Agri-Dex™ Trial #1 above). Individual disease index ratings for each evaluation date are available in the Appendix, Table 35.

Table 18. Greenhouse Agri-Dex™ Trial #2

Strain Name	Agri-Dex™ Concentration[1]	Disease Index Rating[2] Average	Duncan Grouping
31-12	0% w/ Fungus, w/ bacteria	0.0[3]	a[4]
	Non-treated	0.0	a
	Daconil™[5]	0.0	a
	4% no Fungus	0.0	a
	4% w/ Fungus, w/ bacteria[6]	0.0	a
	1% w/ Fungus, w/ bacteria	0.0	a
	2% w/ Fungus, w/ bacteria	0.0	a
	4% w/ Fungus, no bacteria[7]	0.3	ab
	1% w/ Fungus, no bacteria	0.3	b
	2% w/ Fungus, no bacteria	0.4	b
	Fungus	1.4	c
L-850	0% w/ Fungus, w/ bacteria	0.0	a
	Non-treated	0.0	a
	Daconil™	0.0	a
	4% no Fungus	0.0	a
	4% w/ Fungus, w/ bacteria	0.0	a
	1% w/ Fungus, w/ bacteria	0.0	a
	2% w/ Fungus, w/ bacteria	0.0	a
	4% w/ Fungus, no bacteria	0.3	ab
	1% w/ Fungus, no bacteria	0.3	b
	2% w/ Fungus, no bacteria	0.4	b
	Fungus	1.4	c
L-849	0% w/ Fungus, w/ bacteria	0.0	a
	Non-treated	0.0	a
	Daconil™	0.0	a
	4% no Fungus	0.0	a
	4% w/ Fungus, w/ bacteria	0.0	a
	1% w/ Fungus, w/ bacteria	0.0	a
	2% w/ Fungus, w/ bacteria	0.0	a
	4% w/ Fungus, no bacteria	0.3	ab
	1% w/ Fungus, no bacteria	0.3	b
	2% w/ Fungus, no bacteria	0.4	b
	Fungus	1.4	c

[1]Agri-Dex™ concentration in medium (v/v)

[2]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[3]Number represents an average of all times with five repetitions

[4]Means followed by the same letter do not significantly differ (Duncan's MRT, $P \leq 0.05$)

[5]Chemical fungicide chlorothalonil

[6]Agri-Dex™ applied with bacteria and fungal inoculation at time 0

[7]Fungus and Agri-Dex™ with no bacterial application

VI. SUMMARY

The 1992 season of dollar spot had the best results of all the field seasons for both diseases. Five strains performed statistically as well as the chemical fungicide. Nineteen strains performed statistically better than the non-treated control. The six top strains were used in the second field season with different application schedules and the addition of Agri-Dex™ as a spray adjuvant. The results from 1993 season indicate, that while no strains were statistically as good as the chemical fungicide, eight strains , including those applied with Agri-Dex™, did perform statistically better than the non-treated control.

The brown patch field trials provided slightly less optimistic results. The 1992 season resulted in three strains that performed statistically better than the non-treated control. There were, however, fifteen strains that performed statistically as well as the chemical control. This is due to low incidence of disease for all treatments due to lack of favorable conditions. The 1993 season resulted in only one strain that performed statistically better than the non-treated control and the remaining nineteen strains performed statistically as well as the chemical fungicide but not statistically better than the non-treated control. This is due to a decline in the incidence of disease and draught conditions at the end of the field season and the fact that brown patch is hard to control with chemicals.

For laboratory experiments, inhibition was greater in general when the strains were tested against *Sclerotinia homoeocarpa*. Nineteen strains were able to numerically inhibit

fungal growth greater than the chemical fungicide. The one strain that did not do as well as the fungicide did perform well in the field and greenhouse experiments. *Rhizoctonia solani* is more difficult to control in the field and this was evident when only 10 strains were able to provide numerically more inhibition than the fungicide.

The addition of Agri-Dex™ to bacterial cultures produced variable results among bacterial strains for each concentration. Overall, the 1% and 2% (field concentration) concentrations of Agri-Dex™ had slightly less growth than the 4% and 0% concentrations. Fungal growth was decreased when grown with Agri-Dex™, though the concentration did not have any effect.

Timing of application in relation to inoculation with the pathogen proved to be an important aspect in disease control in the greenhouse experiments. The earlier the bacterial strains were applied the greater the disease control. It does not seem to be important when the bacterial strains were applied, as long as applications were begun before fungal inoculation (or before significant disease development in the field) and repeated. The single applications were less satisfactory. Agri-Dex™ does seem to have some ability to control disease, though it can harm the grass in high concentrations.

VII. CONCLUSIONS

The purpose of the field studies (Year 1) was to determine the ability of twenty bacterial strains that have previously shown biological control capabilities on seedling disease of cotton to control Sclerotinia dollar spot on creeping bentgrass and Rhizoctonia brown patch on tall fescue in replicated field trials. Year 2 was an effort to further test the top six strains from Year 1 by examining application frequency and the effects of application with a spray adjuvant, Agri-Dex™. These goals were accomplished over two field seasons and showed encouraging results, particularly in controlling Sclerotinia dollar spot.

The results of the field trials led to some questions concerning the addition of Agri-Dex™. Agri-Dex™ appeared to increase the ability of the six strains to control Sclerotinia dollar spot and even had some controlling effect when applied alone. In an attempt to explain these results, further tests were conducted in the laboratory on the bacterial strains and *S. homoeocarpa* separately and in the greenhouse, under more controlled and environmentally stable conditions. Laboratory bio-assay inhibition experiments were also performed for both fungi and all twenty bacterial isolates to determine if there was any correlation between field performance and inhibition in an agar assay and to develop an assay that could be used in the future to further study the mechanisms by which these isolates work.

Laboratory inhibition experiments showed no direct correlation between field performance and inhibitory ability on agar. The laboratory experiments that were performed to explain the effects of Agri-Dex™ showed that Agri-Dex™ did slightly slow fungal growth by itself which could have resulted in the increased field performance seen in 1993. The bacterial strains were generally not affected by the Agri-Dex™. There were slight variations in the amount of growth noticed with the varying concentrations, though they seemed too varied to attribute to increased field performance. Agri-Dex™ could have also worked by merely keeping the bacteria on the leaf surface for a longer period of time.

The timing of application trials in the greenhouse, repeated twice to ensure reproducibility, had more dramatic results than for the top three strains and the *S. homoeocarpa* used. These results were most likely due to more controlled conditions and an increase in application frequency. The key to disease control seems to be repeated application early in the field season before significant disease development. The Agri-Dex™ alone in the greenhouse did not completely control disease as the bacteria did in those trials. This is most likely due to repeated application that proved toxic to the grass and therefore probably the fungus also. The greenhouse experiments were performed using only Sclerotinia dollar spot because of poor field performance from the Rhizoctonia field trials.

Strains 31-12, L-850, and L-849 have some potential to control disease, especially dollar spot. Further testing would be necessary to study them more in depth. The goal of

this research was to lay a groundwork for future research with strains that have demonstrated some ability. The successful identification of strains that repress dollar spot also opens several lines of more basic investigations to determine the mechanisms involved in repression of Sclerotinia dollar spot by these strains, and to genetically alter these strains to increase performance.

These experiments also form the basis for possibly producing a future biological control agent for the control of Sclerotinia dollar spot on turf. Though these studies were basic and simple, they are a necessary part in the development of products used for biological control. Future research could focus toward several directions: further field testing, mechanism studies, and genetic manipulation.

Field testing should next focus on combining strains to achieve optimum protection and more consistent field results. Mechanism studies are an important part of developing a useful product and can help in questions of formulation and development. Genetic manipulation could possibly provide a superior strain that has all of the desired capabilities of several strains of bacteria. These studies are all necessary and important to the future of any biological control agent that could someday reduce chemical pesticide use.

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APPENDIX

Table 19. Strain Characteristics of All Bacterial Strains Used

Strain Name	Gram Reaction	KB[1] Fluorescence	Colony Morphology	Microscopic Appearance
31-12	negative	high	round, shiny, mucoid	rods
L-852	negative	low	round, shiny	rods
AC4-52	negative	low	round, shiny	rods
L-886	negative	low	round, shiny, mucoid	rods
TR-21	negative	high	round, shiny	rods
RAL3	negative	low	round, shiny	rods
WSB15134	negative	high	round, shiny, mucoid	rods
L-891	negative	high	round, shiny, mucoid	rods
AD4-34	negative	low	round, shiny	rods
L-850	negative	high	round, shiny	rods
G226	negative	medium	round, shiny, mucoid	rods
M17(D)	negative	high	round, shiny	rods
L-890	negative	high	round, shiny	rods
L-855	negative	low	round, shiny	rods
L-849	negative	high	round, shiny	rods
L-851	negative	high	round, shiny	rods
L-856	negative	low	round, shiny	rods
L-854	negative	medium	round, shiny	rods
L-853	negative	medium	round, shiny	rods
L-892	negative	medium	round, shiny, mucoid	rods

[1]KB = Kings medium B (Hagedorn et al., 1989)

Table 20. RSM[1] Medium Used for Bacterial Growth

Ingredients per 1 Liter:		
	$CaNo_3 \cdot 4\ H_20$	0.75 g
	$MgSo_4$	0.502 g
	ACES (buffer)	18.24 g
	NaOH	2.0 g
	Distilled H_20	852 ml
	Agar (optional)	15.0 g
After autoclaving add:		
	KH_2PO_4 (1M, pH 7.0)	1.0 ml
	$ZnSO_4 \cdot 7H_2O$ (7×10^{-4}M)	
	+ $MnSO_4 \cdot 4H_2O$ (9×10^{-4}M)	1.0 ml
	Casamino acids (5%)	100 ml
	Glucose (30%)	33.3 ml
	$FeCl_3$ (100mM)	1.0 ml
Final pH is 6.8		

[1]RSM = Rhizosphere medium (Buyer et al., 1989)

Table 21. Approximate Bacterial Counts For All Field Applications

Strain Number	Strain Name	Colony Number[1]
1	31-12	1.1×10^9
2	L-852	2.5×10^9
3	AC4-52	3.2×10^9
4	L-886	1.2×10^9
5	TR-21	1.3×10^9
6	RAL-3	2.2×10^9
7	WSB15134	2.8×10^9
8	L-891	1.3×10^9
9	AD4-34	2.5×10^9
10	L-850	2.5×10^9
11	G226	5.9×10^9
12	M17(D)	4.4×10^9
13	L-890	4.4×10^9
14	L-855	1.4×10^9
15	L-849	1.2×10^9
16	L-851	1.4×10^9
17	L-856	2.2×10^9
18	L-854	1.5×10^9
19	L-853	4.3×10^9
20	L-892	2.5×10^9

[1]Number represents the average number of colony forming units per ml in culture of RSM

Table 22. Spray Sheet: *Sclerotinia homoeocarpa* 1992

Treatment:		Application	Plot number by repetition number:			
Number	Name	Schedule	1	2	3	4
1	L-849	14-Day	101	221	317	421
2	L-850	14-Day	102	205	301	403
3	L-851	14-Day	103	212	319	415
4	L-852	14-Day	104	208	321	408
5	L-853	14-Day	105	210	320	407
6	L-854	14-Day	106	211	309	406
7	L-855	14-Day	107	201	303	401
8	L-856	14-Day	108	219	316	404
9	L-890	14-Day	109	215	322	405
10	L-886	14-Day	110	214	313	412
11	L-891	14-Day	111	204	305	414
12	L-892	14-Day	112	208	302	409
13	AC4-52	14-Day	113	207	311	420
14	AD4-34	14-Day	114	202	304	411
15	G-226	14-Day	115	220	310	422
16	M17(D)	14-Day	116	203	312	413
17	31-12	14-Day	117	217	314	417
18	WSB15134	14-Day	118	216	308	416
19	RAL3	14-Day	119	222	318	419
20	TR-21	14-Day	120	206	307	402
21	Daconil™[1]	14-Day	121	209	306	418
22	Non-treated[2]	14-Day	122	213	315	410

[1]Chemical fungicide chlorothalonil
[2]No treatment

Table 23. Weather Data: *Sclerotinia homoeocarpa* 1992

	Spray #1	Spray #2	Spray #3	Spray #4	Spray #5	Spray #6	Spray #7
Spray date	6/1/92	6/11/92	6/23/92	7/8/92	7/22/92	8/11/92	9/1/92
Time	11:00 AM	2:30 PM	3:00 PM	3:00 PM	2:30 PM	10:00 AM	11:30 AM
Air temperature	18° C	20° C	25° C	27° C	27° C	23° C	26° C
Relative humidity	60%	95%	60%	85%	70%	75%	60%
Wind speed	8 KM/H	4.8 KM/H	8 KM/H	4.8 KM/H	6.4 KM/H	0 KM/H	4.8 KM/H
Wind direction	WEST	WEST	WEST	WEST	EAST	NONE	SOUTH
Dew presence	NO	YES	NO	NO	NO	YES	YES
Leaf temperature	19° C	18° C	28° C	31° C	31° C	33° C	31° C
Soil moisture	HIGH	HIGH	MOD	MOD	MOD	MOD	HIGH
Cloud cover	95%	90%	70%	70%	70%	0%	5%
Treatments applied	1-22	1-22	1-22	1-22	1-22	1-22	1-22

Table 24. Spray Sheet: *Sclerotinia homoeocarpa* 1993

Treatment:			Application	Plot number by repetition number:			
Number	Name		Schedule	1	2	3	4
1	L-850		14-Day	101	214	325	408
2	L-849		14-Day	102	206	305	426
3	31-12		14-Day	103	201	317	415
4	L-891		14-Day	104	203	321	419
5	L-855		14-Day	105	222	319	404
6	L-851		14-Day	106	218	309	410
7	L-850		28-Day	107	228	313	414
8	L-849		28-Day	108	208	310	423
9	31-12		28-Day	109	219	316	422
10	L-891		28-Day	110	224	315	421
11	L-855		28-Day	111	225	312	406
12	L-851		28-Day	112	204	327	407
13	L-850 Culture Filtrate		14-Day	113	215	303	412
14	L-849 Culture Filtrate		14-Day	114	211	307	403
15	31-12 Culture Filtrate		14-Day	115	216	328	427
16	L-891	Cells[1]	14-Day	116	205	326	420
17	L-855	Cells	14-Day	117	221	323	413
18	L-851	Cells	14-Day	118	213	318	428
19	Medium[2]		14-Day	119	226	314	401
20	Non-treated[3]		14-Day	120	220	308	416
21	Daconil™[4]		14-Day	121	209	301	418
22	L-850	Agri-Dex™[5]	14-Day	122	210	322	411
23	L-849	Agri-Dex™	14-Day	123	223	306	424
24	31-12	Agri-Dex™	14-Day	124	202	304	417
25	L-891	Agri-Dex™	14-Day	125	227	311	425
26	L-855	Agri-Dex™	14-Day	126	207	320	402
27	L-851	Agri-Dex™	14-Day	127	217	302	405
28	Control	Agri-Dex™[6]	14-Day	128	212	324	409

[1]Cells removed from medium and resuspended in phosphate buffer pH 7

[2]Medium only

[3]No treatment

[4]Chemical fungicide chlorothalonil

[5]Agri-Dex™ added to cell culture at 2% v/v

[6]Agri-Dex™ in water at 2% v/v

Table 25. Weather Data: *Sclerotinia homoeocarpa* 1993

	Spray #1	Spray #2	Spray #3	Spray #4	Spray #5	Spray #6
Spray date	6/4/93	6/18/93	7/2/93	7/15/93	7/30/93	8/12/93
Time	9:15 AM	9:00 AM	1:00 PM	2:30 PM	11:00 AM	10:00 AM
Air temperature	21° C	24° C	29° C	31° C	29° C	23° C
Relative humidity	76%	70%	55%	70%	70%	55%
Wind speed	1.6 KM/H	3.2 KM/H	8 KM/H	O KM/H	16 KM/H	1.6 KM/H
Wind direction	WEST	WEST	WEST	NONE	WEST	WEST
Dew presence	YES	YES	NO	NO	NO	YES
Soil temperature	21° C	22° C	29° C	27° C	27° C	23° C
Soil moisture	MOD	MOD	HIGH	HIGH	LOW	MOD
Cloud cover	75%	0%	85%	70%	85%	10%
Leaf temperature	27° C	30° C	32° C	28° C	29° C	27° C
Treatments applied	1-28	1-6,13-28	1-28	1-6,13-28	1-28	1-6,13-28

Table 26. Spray Sheet: *Rhizoctonia solani* 1992

Treatment:		Application	Plot number by repetition number:			
Number	Name	Schedule	1	2	3	4
1	L-849	14-Day	101	221	317	421
2	L-850	14-Day	102	205	301	403
3	L-851	14-Day	103	212	319	415
4	L-852	14-Day	104	208	321	408
5	L-853	14-Day	105	210	320	407
6	L-854	14-Day	106	211	309	406
7	L-855	14-Day	107	201	303	401
8	L-856	14-Day	108	219	316	404
9	L-890	14-Day	109	215	322	405
10	L-886	14-Day	110	214	313	412
11	L-891	14-Day	111	204	305	414
12	L-892	14-Day	112	2018	302	409
13	AC4-52	14-Day	113	207	311	420
14	AD4-34	14-Day	114	202	304	411
15	G-226	14-Day	115	220	310	422
16	M17(D)	14-Day	116	203	312	413
17	31-12	14-Day	117	217	314	417
18	WSB15134	14-Day	118	216	308	416
19	RAL3	14-Day	119	222	318	419
20	TR-21	14-Day	120	206	307	402
21	Daconil™[1]	14-Day	121	209	306	418
22	Non-treated[2]	14-Day	122	213	315	410

[1]Chemical fungicide chlorothalonil
[2]No treatment

Table 27. Weather Data: *Rhizoctonia solani* 1992

	Spray #1	Spray #2	Spray #3	Spray #4	Spray #5	Spray #6
Spray date	5/13/92	6/3/92	6/16/92	7/1/92	8/5/92	8/19/92
Time	1:30 PM	12:00 PM	11:00 AM	12:30 PM	5:30 PM	11:30 AM
Air temperature	23° C	24° C	24° C	28° C	27° C	28° C
Relative humidity	65%	75%	80%	85%	60%	70%
Wind speed	4.8 KM/H	4.8 KM/H	8 KM/H	8 KM/H	0 KM/H	3.2 KM/H
Wind direction	WEST	WEST	EAST	WEST	NONE	WEST
Dew presence	NO	NO	NO	NO	NO	YES
Leaf temperature	25° C	28° C	27° C	29° C	29° C	34° C
Soil moisture	DRY	MOD	MOD	MOD	MOD	HIGH
Cloud cover	60%	50%	95%	95%	60%	0%
Treatments applied	1-22	1-22	1-22	1-22	1-22	1-22

Table 28. Spray Sheet: *Rhizoctonia solani* 1993

Treatment:		Application	Plot number by repetition number:			
Number	Name	Schedule	1	2	3	4
1	31-12	14-Day	101	202	313	405
2	L-852	14-Day	102	215	305	404
3	AC4-52	14-Day	103	216	314	418
4	L-886	14 Day	104	201	310	415
5	TR-21	14-Day	105	217	312	403
6	RAL-3	14-Day	106	203	309	406
7	WSB15134	14-Day	107	206	308	422
8	L-891	14-Day	108	220	319	411
9	AD4-34	14-Day	109	219	318	412
10	L-850	14-Day	110	205	315	414
11	G226	14-Day	111	210	316	402
12	M17(D)	14-Day	112	213	302	416
13	L-890	14-Day	113	207	321	410
14	L-855	14-Day	114	222	320	421
15	L-849	14-Day	115	204	311	417
16	L-851	14-Day	116	218	303	419
17	L-856	14-Day	117	208	322	413
18	L-854	14-Day	118	209	304	407
19	L-853	14-Day	119	221	301	409
20	L-892	14-Day	120	211	317	420
21	Medium[1]	14-Day	121	212	307	408
22	Daconil™[2]	14-Day	122	214	306	401
23	Non-treated[3]	14-Day	123	223	323	423

[1]Medium only
[2]Chemical fungicide chlorothalonil
[3]No treatment

Table 29. Weather Data: *Rhizoctonia solani* 1993

	Spray #1	Spray #2	Spray #3	Spray #4	Spray #5	Spray #6
Spray date	6/10/93	6/23/93	7/8/93	7/21/93	8/4/93	8/17/93
Time	2:00 PM	2:00 PM	12:00 PM	12:00 PM	12:00 PM	10:45 AM
Air temperature	34° C	33° C	37° C	34° C	32° C	25° C
Relative humidity	38%	19%	45%	31%	56%	60%
Wind speed	8 KM/H	6.4 KM/H	8 KM/H	4.8 KM/H	4.8 KM/H	16 KM/H
Wind direction	WEST	WEST	EAST	EAST	EAST	WEST
Dew presence	NO	NO	NO	NO	NO	NO
Soil temperature	32° C	31° C	29° C	29° C	27° C	24° C
Soil moisture	MOD	MOD	MOD	MOD	DRY-MOD	MOD
Cloud cover	0%	0%	0%	10%	20%	100%
Leaf temperature	37° C	31° C	43° C	37° C	33° C	26° C
Treatments applied	1-23	1-23	1-23	1-23	1-23	1-23

Table 30. *Sclerotinia homoeocarpa* Inhibition[1] Among Repetitions

Assigned Number	Strain Name	Repetition 1 Average	Repetition 2 Average	Repetition 3 Average
1	31-12	20.5[2]	20.7	18.7
2	L-852	9.5	19.9	20.9
3	AC4-52	12.9	19.3	15.8
4	L-886	13.5	14.4	19.4
5	TR-21	7.1	16.0	11.6
6	RAL-3	8.6	16.0	9.8
7	WSB15134	13.5	12.1	12.4
8	L-891	10.6	12.5	10.4
9	AD4-34	17.7	15.2	17.4
10	L-850	16.6	11.1	17.1
11	G226	20.2	8.4	17.9
12	M17(D)	19.4	13.0	14.1
13	L-890	9.4	8.5	9.1
14	L-855	19.8	7.7	9.6
15	L-849	8.6	7.1	6.6
16	L-851	16.2	7.3	9.4
17	L-856	11.1	12.4	14.9
18	L-854	9.2	9.1	15.8
19	L-853	11.0	6.1	21.1
20	L-892	11.6	8.9	7.3
21	Daconil™[3]	9.1	7.2	7.2

[1]Inhibition performed on agar plates using RSM agar
[2]Means represent an average of all 14 measurements
[3]Chemical fungicide chlorothalonil

Table 31. *Rhizoctonia solani* Inhibition[1] Among Repetitions

Assigned Number	Strain Name	Repetition 1 Average	Repetition 2 Average	Repetition 3 Average
1	31-12	14.1[2]	12.6	13.7
2	L-852	12.2	9.5	11.4
3	AC4-52	6.2	9.4	9.0
4	L-886	16.1	15.5	12.4
5	TR-21	7.9	6.4	8.6
6	RAL-3	6.2	6.7	7.6
7	WSB15134	7.4	8.3	10.4
8	L-891	6.9	5.4	8.3
9	AD4-34	10.8	8.3	8.0
10	L-850	12.2	9.0	8.7
11	G226	9.6	5.8	8.1
12	M17(D)	10.5	5.0	8.6
13	L-890	7.9	7.4	7.5
14	L-855	7.6	10.8	11.3
15	L-849	7.4	8.0	8.6
16	L-851	10.2	9.1	10.4
17	L-856	10.3	11.3	9.5
18	L-854	7.6	8.3	10.5
19	L-853	8.7	7.8	8.6
20	L-892	7.6	6.6	5.3
21	Daconil™[3]	8.1	8.5	9.1

[1]Inhibition performed on agar plates using RSM agar
[2]Means represent an average of all 12 measurements
[3]Chemical fungicide chlorothalonil

Table 32. Greenhouse Timing Trial #1: Disease Index Ratings

Strain Name	Treatment Time[2]	Disease Index Rating[1]					
		Day +0[3]	Day +2	Day +4	Day +6	Day +8	Day +10
31-12	No Fungus[4]	0.0 [5]	0.0	0.0	0.0	0.0	0.0
	-6	0.0	0.0	0.0	0.0	0.0	0.2
	-4	0.0	0.0	0.2	0.0	0.0	0.6
	-2	0.0	0.0	0.2	0.0	0.2	0.6
	0	0.0	0.0	1.0	1.0	0.4	0.6
	+2	0.0	0.0	1.4	1.2	0.6	0.4
	+4	0.0	0.0	1.4	2.0	1.2	1.2
	+6	0.0	0.0	2.6	3.0	2.4	2.8
L-850	No Fungus	0.0	0.0	0.0	0.0	0.0	0.0
	-6	0.0	0.0	0.0	0.0	0.0	0.4
	-4	0.0	0.0	0.0	0.0	0.0	0.0
	-2	0.0	0.0	0.0	0.0	0.0	0.2
	0	0.0	0.0	0.2	0.2	0.4	1.8
	+2	0.0	0.0	1.4	1.4	0.4	3.2
	+4	0.0	0.0	2.0	2.2	1.4	2.4
	+6	0.0	0.0	1.8	2.2	1.8	3.0
L-849	No Fungus	0.0	0.0	0.0	0.0	0.0	0.0
	-6	0.0	0.0	0.2	0.2	0.0	0.0
	-4	0.0	0.0	0.0	0.0	0.0	1.0
	-2	0.0	0.0	0.6	0.4	0.2	1.0
	0	0.0	0.0	0.8	0.8	0.4	1.0
	+2	0.0	0.0	0.8	0.6	1.2	1.6
	+4	0.0	0.0	1.6	1.6	1.8	2.8
	+6	0.0	0.0	1.4	1.2	1.8	2.0
Controls	Non-treated	0.0	0.0	0.0	0.0	0.0	0.0
	Fungus Only[6]	0.0	0.0	1.4	1.6	2.4	2.0

[1]Disease index based on a scale of 1 (1% to 20% visible disease)
to 5 (81% to 100% visible disease),a 0 represents no visible disease
[2]Bacterial application times referring to the number of days before and after fungal application
[3]Times refer to the days after fungal application
[4]Bacterial application with no fungal application
[5]Number represents an average of five repetitions
[6]Fungal application at time 0, no bacteria

Table 33. Greenhouse Timing Trial #2: Disease Index Ratings

Strain Name	Treatment Time[2]	Disease Index Rating[1]					
		Day +0[3]	Day +2	Day +4	Day +6	Day +8	Day +10
31-12	No Fungus[4]	0.0[5]	0.0	0.0	0.0	0.0	0.0
	-6 w/ Fungus[6]	0.0	0.0	1.8	1.8	2.0	2.8
	-6	0.0	0.0	0.0	0.0	0.0	0.0
	-4	0.0	0.0	0.2	0.0	0.0	0.0
	-2	0.0	0.0	0.4	0.0	0.0	0.2
	0	0.0	0.0	1.0	0.6	0.4	0.4
	+2	0.0	0.0	1.8	2.0	1.4	1.2
	+4	0.0	0.0	2.0	2.0	1.6	2.0
	+6	0.0	0.0	1.8	1.8	2.2	2.8
L-850	No Fungus	0.0	0.0	0.0	0.0	0.0	0.0
	-6 w/ Fungus	0.0	0.0	1.8	1.6	2.6	2.6
	-6	0.0	0.0	0.2	0.0	0.6	0.0
	-4	0.0	0.0	0.2	0.0	0.2	0.2
	-2	0.0	0.0	1.0	0.2	0.2	0.4
	0	0.0	0.0	0.8	0.4	0.2	0.4
	+2	0.0	0.0	1.2	1.4	0.8	1.2
	+4	0.0	0.0	1.6	1.6	1.6	1.8
	+6	0.0	0.0	1.6	1.4	2.2	2.6
L-849	No Fungus	0.0	0.0	0.0	0.0	0.0	0.0
	-6 w/ Fungus	0.0	0.0	1.6	2.0	2.2	2.8
	-6	0.0	0.0	0.0	0.0	0.0	0.0
	-4	0.0	0.0	0.2	0.4	1.0	0.2
	-2	0.0	0.0	0.4	0.6	0.6	0.6
	0	0.0	0.0	0.8	1.0	2.2	1.6
	+2	0.0	0.0	0.8	1.0	1.4	1.6
	+4	0.0	0.0	1.4	1.8	1.4	1.8
	+6	0.0	0.0	1.6	1.4	1.8	2.6
Controls	Daconil™[7]	0.0	0.0	0.0	0.0	0.0	0.0
	Non-treated	0.0	0.0	0.0	0.0	0.0	0.0
	Fungus Only[8]	0.0	0.0	1.6	1.4	2.0	2.4

[1]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Bacterial application times referring to the number of days before and after fungal application

[3]Times refer to the days after fungal application

[4]Bacterial application with no fungal application

[5]Number represents an average of five repetitions

[6]Bacterial application at time -6 only with fungal application at time 0

[7]Chemical fungicide chlorothalonil, applied once 24 hours prior to fungal inoculation

[8]Fungal application at time 0, no bacteria

Table 34. Greenhouse Agri-Dex™ Trial #1: Disease Index Ratings

Strain Name	Agri-Dex™ Concentration[2]	Disease Index Rating[1]					
		Day +0[3]	Day +2	Day +4	Day +6	Day +8	Day +10
31-12[4]	No Agri-Dex™	0.0[5]	0.0	0.0	0.0	0.0	0.0
	1%	0.0	0.0	0.0	0.0	0.0	0.0
	2%	0.0	0.0	0.0	0.0	0.0	0.0
	4%[6]	0.0	0.0	0.0	0.0	0.0	0.0
L-850	No Agri-Dex™	0.0	0.0	0.0	0.0	0.0	0.0
	1%	0.0	0.0	0.0	0.0	0.0	0.0
	2%	0.0	0.0	0.0	0.0	0.0	0.0
	4%[6]	0.0	0.0	0.0	0.0	0.0	0.0
L-849	No Agri-Dex™	0.0	0.0	0.0	0.0	0.0	0.0
	1%	0.0	0.0	0.0	0.0	0.0	0.0
	2%	0.0	0.0	0.0	0.0	0.0	0.0
	4%[6]	0.0	0.0	0.0	0.0	0.0	0.0
Controls	1% w/ Fungus[6]	0.0	0.0	0.4	0.4	0.4	0.4
	2% w/ Fungus	0.0	0.0	0.6	0.6	0.4	0.2
	4% w/ Fungus	0.0	0.0	0.4	0.4	0.2	0.0
	4% no Fungus	0.0	0.0	0.0	0.0	0.0	0.0
	Daconil™[7]	0.0	0.0	0.0	0.0	0.0	0.0
	No Treatment	0.0	0.0	0.0	0.0	0.0	0.0
	Fungus	0.0	0.0	2.0	2.0	1.8	1.8

[1]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Agri-Dex™ concentration in medium (v/v)

[3]Times refer to the days after fungal application

[4]Agri-Dex™ applied with bacteria and fungal inoculation at time 0

[5]Number represents an average of five repetitions

[6]Fungus and Agri-Dex™ with no bacterial application

[7]Chemical fungicide chlorothalonil, applied once 24 hours prior to fungal inoculation

Table 35. Greenhouse Agri-Dex™ Trial #2: Disease Index Ratings

Strain Name	Agri-Dex™ Concentration[2]	Disease Index Rating[1]					
		Day +0[3]	Day +2	Day +4	Day +6	Day +8	Day +10
31-12[4]	No Agri-Dex™	0.0[5]	0.0	0.0	0.0	0.0	0.0
	1%	0.0	0.0	0.0	0.0	0.0	0.0
	2%	0.0	0.0	0.0	0.0	0.0	0.0
	4%[6]	0.0	0.0	0.0	0.0	0.0	0.0
L-850	No Agri-Dex™	0.0	0.0	0.0	0.0	0.0	0.0
	1%	0.0	0.0	0.0	0.0	0.0	0.0
	2%	0.0	0.0	0.0	0.0	0.0	0.0
	4%[6]	0.0	0.0	0.0	0.0	0.0	0.0
L-849	No Agri-Dex™	0.0	0.0	0.0	0.0	0.0	0.0
	1%	0.0	0.0	0.0	0.0	0.0	0.0
	2%	0.0	0.0	0.0	0.0	0.0	0.0
	4%[6]	0.0	0.0	0.0	0.0	0.0	0.0
Controls	1% w/ Fungus[6]	0.0	0.0	0.6	0.6	0.4	0.2
	2% w/ Fungus	0.0	0.0	0.4	0.8	0.6	0.4
	4% w/ Fungus	0.0	0.0	0.4	0.6	0.6	0.0
	4% no Fungus	0.0	0.0	0.0	0.0	0.0	0.0
	Daconil™[7]	0.0	0.0	0.0	0.0	0.0	0.0
	Non-treated	0.0	0.0	0.0	0.0	0.0	0.0
	Fungus	0.0	0.0	2.2	2.4	2.0	2.0

[1]Disease index based on a scale of 1 (1% to 20% visible disease) to 5 (81% to 100% visible disease),a 0 represents no visible disease

[2]Agri-Dex™ concentration in medium (v/v)

[3]Times refer to the days after fungal application

[4]Agri-Dex™ applied with bacteria and fungal inoculation at time 0

[5]Number represents an average of five repetitions

[6]Fungus and Agri-Dex™ with no bacterial application

[7]Chemical fungicide chlorothalonil, applied once 24 hours prior to fungal inoculation

VITA

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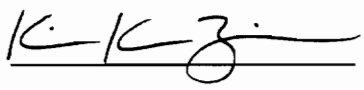

Krista Kaye Zimmerman