1994 USGA Annual Report
USGA Executive Summary

Donald Y. Kobayashi and Bruce B. Clarke. Identification of Parasitic Bacteria as Biological Control Agents Against Summer Patch Disease. Rutgers University, Department of Plant Pathology, New Brunswick, NJ 08903.

Summer patch, caused by the ectotrophic, root-infecting fungus, *Magnaporthe poae*, is a devastating disease of cool season turfgrasses. Current control methods for summer patch, as well as other patch diseases caused by root-infecting fungi, rely heavily on the use of fungicides. In efforts to reduce the amount of fungicides used to control turfgrass diseases such as summer patch, we are investigating the potential use of beneficial bacteria as biological control agents for the disease. In previous studies, we isolated several bacteria by a fungal trapping method and by enrichment procedures that were capable of suppressing summer patch symptom development at significant levels under controlled environmental conditions. Characterization of these bacteria indicated that several isolates shared common features that included the expression of extracellular enzymes such as chitinases, glucanases, lipases or proteases. In addition, all bacteria identified as good suppressors were capable of colonizing the turfgrass rhizosphere at high concentrations. Few isolates were observed to produce antibiotic-like activity against *M. poae* in in vitro assays. Two bacteria, *Xanthomonas maltophilia* and *Serratia marcescens*, were further characterized for their suppressive abilities. These bacteria were found to consistently suppress summer patch symptom production at greater than 50% compared to untreated control plants over a 3 week period. Further characterization of these bacteria indicate that timing of application is important relative to the level of disease suppression that is achieved. In general, when bacteria were applied prior to fungal colonization of plant roots, less disease suppression was achieved. Dose level of bacteria also affected the level of disease suppression. A slight but significant difference was observed for the lowest and highest doses of *X. maltophilia*, ranging from 10^8 to 10^{10} cells/ml, in which disease suppression was greatest for plants treated with the highest dose. However, plants treated with similar doses of *S. marcescens* responded in drastically different fashion. Optimal suppression was observed with 10^9 cells/ml. The level of disease suppression decreased with either increasing or decreasing doses from this cell concentration. This data, correlated with root and soil populations of both bacteria, suggest that bacteria had a direct effect on the fungal pathogen inoculum density in the soil.

1994 USGA Annual Report

Dr. Donald Kobayashi, PI
Dr. Bruce Clarke, co-PI

Department of Plant Pathology
Rutgers, the State University of New Jersey
New Brunswick, NJ 08903-0231

Title: Identification of parasitic bacteria as biological control agents against summer patch disease.

Specific Research Objectives:

1) To isolate and identify bacteria which can colonize and parasitize the mycelia of *Magnaporthe poae*, the causal agent of summer patch disease.

2) To screen bacteria isolated in objective one for disease control potential using controlled growth chamber and field studies.

RESEARCH TO DATE

Fungal Baiting Method for Isolation of Potential Bacterial Antagonists. Several bacteria were isolated using an enrichment culture procedure described in the 1993 annual report. The enrichment procedure consisted of a minimal salts medium supplemented with *Magnaporthe poae* mycelium as a sole carbon source, inoculated with selected soils that served as initial bacterial inoculum sources. Inoculated media were incubated until growth was observed, as determined by turbidity in cultures. Small aliquots were then transferred to fresh media and these cultures were incubated until growth was observed. This process was continued for several transfers (up to 5 transfer cultures) over a total period of 5-10 weeks. With each transfer, samples of cultures were diluted and plated, and representative colonies were selected for disease suppression assays. Fourteen different soil sources were utilized in the enrichment procedure.

Over 1000 bacterial isolates selected by the enrichment procedure were screened in growth chamber assays. Several isolates were identified that suppressed summer patch symptom development on Kentucky bluegrass var. Baron at significant levels compared to untreated control plants (Table 1). Each isolate was further characterized for the ability to express extracellular enzymes that have been previously implicated in biocontrol mechanisms of bacteria against fungal pathogens. These enzymes include the following: glucanase (as detected by growth on laminarin as a sole carbon source); chitinase (as detected by clearing on agar supplemented with 2% colloidal chitin; protease (as detected by clearing on agar supplemented with casein); and lipase (as detected by the formation of precipitate on agar supplemented with 2% Tween 80). The majority of bacterial isolates obtained from enrichment cultures that

suppressed summer patch were found to produce more than one of these extracellular enzymes (Table 2). This observation, combined with the method for isolation of the bacteria (the ability to utilize *M. poae* mycelium a sole carbon source), provides suggestive evidence that the extracellular, degradative enzymes are important in the mechanism for biocontrol of *M. poae*.

Each isolate was further characterized by determining their ability to colonize the rhizosphere of Kentucky bluegrass var. Baron. Bacteria were inoculated one time onto the roots of 2 week old turfgrass plants. After 1 week of incubation, turfgrass roots were harvested and the number of bacteria existing in the rhizosphere were determined. Most isolates that suppressed summer patch at significant levels were found to colonize roots at high levels (Table 2). All bacteria were able to colonize the turfgrass rhizosphere between 10^4 colony forming units(cfu)/gram roots (fresh weight) and $>10^7$ cfu/ gm roots. Most bacteria that were found to colonize the rhizosphere at the lower values of 10^4 cfu/gm roots were Gram positive bacteria. The majority of bacteria that were capable of colonizing the turfgrass rhizosphere at high concentrations were *Serratia* spp. and fluorescent pseudomonads. Both *Serratia* spp. and fluorescent pseudomonads have been previously identified as good rhizosphere colonizers of other plant hosts. From our observations, it is apparent that these bacteria also colonize the turfgrass rhizosphere in high numbers.

Rhizosphere colonization ability has been regarded as an important trait for biocontrol agents of soilborne diseases, primarily due to the ability of biocontrol agents to persist at the site where infection by the pathogen occurs. Consequently, the bacteria that have been identified in this study as superior rhizosphere colonizers will be further characterized and developed as biocontrol agents against summer patch disease. However, those bacteria that colonized the turfgrass rhizosphere at lower levels also warrant further investigation and should not be excluded. Lower colonization ability may not be as important a trait for turfgrass biocontrol, since intense management practices contribute to certain advantages of using biocontrol on turfgrass diseases. One such advantage is the development of biocontrol agents as biological pesticides that can be used in multiple applications. This approach is consistent with strategies for targeting bacteria to reduce fungal inoculum levels in the soil. Experiments directed to address mechanisms that contribute to biocontrol by inoculum reduction are described in later sections.

Improvement of Growth Chamber Assay: Studies involving pathogenesis of *M. poae* under controlled environmental conditions.

Root mass vs. foliar symptom development. Infection of turfgrass roots by the summer patch pathogen occurs when the fungus penetrates the root surface, grows through the cortex and colonizes the vascular tissue. Although infection occurs in the roots, summer patch disease is usually evaluated by ratings based on levels of foliar symptoms. Landschoot et al. (Phytopathology 80:520-526) previously reported on

the evaluation of turfgrass infection by *M. poae* using a root rot rating system; however, this study did not compare root rot ratings with foliar symptom development of summer patch disease. Direct ratings of turfgrass root rot is difficult to assess due to the fragile nature of roots, as well as the large number of roots that are involved within a given sample. However, we felt that a direct correlation between foliar symptoms and root ratings was necessary to demonstrate a relationship between typical evaluations for summer patch with the tissue involved with infection by *M. poae*. Therefore, root mass was chosen as a root evaluation for comparisons with foliar symptoms of summer patch. Root length was considered as an option; however, too many inconsistencies within a given container were observed due to roots of individual plants that escaped colonization and infection by *M. poae*. Typical containers of Kentucky bluegrass were set up with varied amounts of inoculum of two different isolates of *M. poae* (73-15 and NAV-A1) to insure a wide spectrum of disease symptom production. Plants were then allowed to incubate under normal conditions for the disease assay. After 4 weeks incubation under disease conducive conditions, disease ratings were recorded for diseased containers. Total roots were then recovered, washed thoroughly, and the dry weight was recorded. Regressions were performed between root weight and foliar disease symptom ratings. A linear relationship was observed for studies using two different *M. poae* isolates, indicating a direct correlation between foliar symptom production and root mass (Fig. 1). Equations of regression lines for both fungal isolates were similar, indicating the similarity of the correlation of disease between these isolates. These results indicated that a direct, inverse relationship existed between foliar symptom development and root mass.

We have used a previously developed growth chamber assay using Kentucky bluegrass var. Baron grown in 8 inch containers for summer patch disease. However, a major problem with the growth chamber assay has been the inability to obtain disease at consistent levels in all replications. Therefore, studies were designed to determine the effect of several factors on disease development in the growth chamber assay. These factors included: depth of fungal inoculum placement; level of seed density; the level of fungal inoculum density; and the age of fungal inoculum. Results of these studies have provided a much more consistent and reliable assay for summer patch. In addition, these results should be directly applicable for developing disease assays using other root-infecting turfgrass pathogens.

Fungal Inoculum Concentration. Two different isolates of *M. poae* were used to study the effect of fungal inoculum density on disease development in the growth chamber assay. Five different inoculum densities replicated 10 times were used in the study. Disease ratings were recorded on a weekly basis using a 10 point rating scale (0 = no disease, 9 = 100% foliar area diseased). For analysis of the data, regressions were performed. Slight to drastic differences in the rate of disease progress, as indicated by slopes of regression, were observed between fungal isolates and inoculum densities (Fig. 2). However, general trends in the relationship between

inoculum density and disease progress could be observed for both fungal isolates. Regression lines indicated little difference in disease between low inoculum densities of 1/4 and 1/2 agar plugs of mycelium for either fungal isolate. In comparison, higher inoculum densities of 1, 2 and 3 plugs demonstrated higher levels of disease. With the exception of 3 plugs of isolate NAV-A1 (Fig. 2B), little differences could be distinguished between disease progression resulting from the varied inoculum densities. These results indicate that as little as 1/4 agar plug of fungal inoculum is sufficient to obtain disease in the container assay. However, higher levels of disease can be obtained with higher inoculum densities. Furthermore, above a given inoculum density (the equivalent of 1 agar plug), little difference in disease intensity was achieved. This indicates that disease progress was potentially maximized at this level. Although results appeared obvious, one important conclusion that can be interpreted from this study is that it is necessary to establish the critical inoculum density for disease. Disease suppression of inoculum may not be detected when using high inoculum densities, since a reduction of even 50-66% of inoculum (the difference between 1-3 plugs) may not be detected in disease progression.

Depth of inoculum placement. Depth of inoculum placement appeared to have the greatest effect on disease development in container assays. In general, as the distance between fungal inoculum and seeds were shortened, the level of disease between replicates was more consistent, and disease appeared more rapidly (Fig. 3). When fungal inoculum was placed at depths in soil of 3 cm or greater, the consistency of disease between replications decreased, as did the intensity of disease. Regressions for disease ratings over time are shown for each depth of inoculum placement for *M. poae* isolate 73-15 (Fig. 3A) and NAV-A1 (Fig. 3C). In addition, the standard deviations for the mean values of the 10 replications at each depth for each time point were also plotted for isolate 73-15 (Fig. 3B) and NAV-A1 (Fig. 3D).

The results suggest that early colonization of roots (resulting from closer placement of fungal inoculum to seeds) strongly influences disease development. With increased depth of inoculum placement, the level of disease development was decreased. Disease development thus appeared to be dependent upon infection and root rot near the crown of plants. Colonization and rot of roots occurring at farther distances from the crown led to less foliar symptom development. These observations can be correlated with increased root mass and foliar symptom development, where root mass is directly correlated with root length in this case.

Seed density. Seed density was a second factor that appeared to influence disease development in the growth chamber assay. In general, disease appeared more consistently and rapidly in replications that received higher seed densities (Fig. 4). Although disease in 0.05 g seed/container was not statistically significant from disease in 0.025 g seed/container, more disease was consistently seen at the higher seed density. Less disease was observed at the lowest seed density of 0.0125 g seed/container. At least two interpretations can be made from these observations.

Common for soilborne diseases, increased root densities (resulting from higher seed densities) within a given area of soil may increases the probability that fungal inoculum within the soil will contact roots, allowing increased colonization and thus infection to occur. At lower seed densities, there is less chance for roots to come into contact with fungal inoculum. A second, less likely interpretation is that higher seed densities may contribute to increased root masses within containers that contributes to increased root stress that enhances disease.

Age of fungal inoculum. The age of fungal inoculum was also studied to determine if there was an effect on disease intensity. However, no differences in disease progression or intensity were observed between fungal inoculum originating from 3 day old, or 1, 2, 3 or 4 week old inoculum (data not shown).

Further characterization of *Serratia marcescens* 9M5 and *Xanthomonas maltophilia* 34S1. *S. marcescens* 9M5 and *X. maltophilia* 34S1 are two previously identified bacterial isolates that suppressed summer patch symptom development at significant levels in growthchamber studies. To further characterize these isolates, the effect of timing of application of bacteria and bacterial inoculum concentration on disease suppression were studied. In addition, the population levels at which these bacteria colonized turfgrass roots and survived in the soil were determined.

Bacterial timing. The effect of timing of application of bacteria was studied using the standard growth chamber assay. Two bacteria, which included *X. maltophilia* 34S1 and *S. marcescens* 9M5, were used in the study. Bacteria were applied as a single application of 25 ml of 10^9 colony forming units (CFU)/ml. Application times were altered on a weekly basis, beginning at the time of planting of Kentucky bluegrass seeds (time 0). The last treatments were applied four weeks after planting, 24 hours prior to transfer of plants into the growthchamber. No significant difference was observed with plants treated with *X. maltophilia* 34S1 at the time of planting compared with untreated control plants. However, significant suppression was observed for all other applications times (Fig. 5A). Levels of disease suppression for plants treated with *S. marcescens* 9M5 3 and 4 weeks after planting (times 4 and 5) were significantly different from untreated control plants (Fig 5B). These observations indicated that disease suppression was highest when bacteria were applied to plants after turfgrass seeds had germinated. Based on the ectotrophic nature of *M. poae*, it was assumed that at times 2 to 4 weeks after planting, the fungus was actively growing and colonizing the surface of roots. Prior to active growth, the fungus, in a dormant state, may be less susceptible to the antagonistic activity of the bacteria. *M. poae* mycelium is highly melanized in the dormant state. A function of melanin in fungi is to provide cell wall resistance to degradative enzymes produced by microbes. Since the primary mechanism of activity by these bacteria is thought to be by destructive parasitism (through the production of degradative enzymes), the lack of disease suppression at early time points may be due to fungal resistance to the activity of the bacteria.

Bacterial dose studies. Plant were treated with various doses of *X. maltophilia* 34S1 and *S. marcescens* 9M5 to determine the effect of bacterial concentration on disease suppression. Five doses were tested for both bacteria, ranging from 10^8 to 10^{10} CFU/ml. A total of 10 replications were used for each treatment, and disease ratings were recorded over a 5 week period. For each isolate, data from all 5 doses were pooled and analyzed. Regression analysis indicated a quadratic relationship with disease ratings over time for plants treated with *X. maltophilia* 34S1 (Fig 6). A significant difference was only observed between regression lines for plants treated with *X. maltophilia* 34S1 at 10^{10} CFU/ml, and 10^8 CFU/ml, where the higher dose of bacteria provided the greatest suppression.

Plants treated with *S. marcescens* 9M5 responded differently compared to plants treated with *X. maltophilia* 34S1. Regression analysis indicated no significant quadratic relationship for disease over time; however significant linear relationships were observed. The slopes for regression lines (indicating rates of disease progression) were the same for plants treated with all five doses of bacteria. Disease suppression in plants treated with all five doses were significantly different from untreated control plants ($P<0.05$). However, the optimal concentration of *S. marcescens* 9M5 that provided the greatest level of disease suppression was not the highest concentration of cells used. Instead, the concentration of 10^9 CFU/ml provided the greatest level of disease suppression. Lower concentrations of $5x10^8$ and 10^8 CFU/ml resulted in less disease suppression. As concentrations of bacteria increased above 10^9 CFU/ml, levels of disease suppression also decreased.

Population dynamics of bacteria. Populations of *X. maltophilia* and *S. marcescens* were monitored in the soil and on roots of turfgrass plants treated with three different concentrations of bacteria, (10^8, 10^9 and 10^{10} CFU/ml). Soil populations were found to steadily decrease over time for both bacteria. Mean population levels were statistically different between the highest and lowest concentrations of *X. maltophilia* in the soil over a thirty five day period, reaching levels between $4\log_{10}$ and $5\log_{10}$ (Fig. 7). Populations on roots were measured over a three week period, beginning three weeks after planting when root masses were large enough for sampling. A significant different was observed between the highest dose and the lower doses at the first time point. However, populations leveled to ca. $4\log_{10}$ at the fourth week, with no significant differences observed between the doses.

Soil populations of *S. marcescens* followed a similar trend to that observed with *X. maltophilia*. Significant differences in soil populations were detected between soils inoculated with the highest and lowest doses (Fig 7B). Populations did not decrease as rapidly as *X. maltophilia*, reaching final population levels between $5\log_{10}$ and $6\log_{10}$ after 35 days. Rhizosphere populations of *S. marcescens* also followed a similar trend as that observed for *X. maltophilia* 34S1. Populations declined between the first and second sampling, but then stabilized to a level $5\log_{10}$ and $6\log_{10}$ (Fig. 7D). No significant differences were detected between any of the three doses at any of the

three time points.

Conclusions. Based on bacterial populations and the effects of timing of application and concentration of bacterial inoculum, arguments can be made that both *S. marcescens* 9M5 and *X. maltophilia* suppressed disease by reducing fungal inoculum in the soil, compared to other mechanisms of biocontrol such as competition with the pathogen for nutrients or space. As stated above, time of application was most effective in disease suppression when the fungus was most likely to be actively growing on the surface of turfgrass roots. At this time, the fungal pathogen would be most susceptible to degradative enzymes produced by the bacteria. Further supporting evidence for fungal inoculum reduction includes the results of concentration studies with *X. maltophilia*. In this case, the highest level of disease control was observed with the highest concentration of 10^{10} CFU/ml, and the lowest level of disease control was observed with the lowest concentration of 10^{8} CFU/ml. These results, correlated with population studies, in which population levels of *X. maltophilia* 34S1 observed in nonrhizosphere soil (in which differences were observed between inoculum concentrations) and to rhizosphere populations (which plays an important role for mechanisms of disease control involving competition, and where no differences were observed), provide further supporting evidence that the bacteria acted directly to reduce fungal inoculum.

Similar arguments can be made for *S. marcescens* 9M5, in which similar results were observed. In contrast to *X. maltophilia*, however, the optimal level of control was not observed with the highest concentration of bacterial inoculum. This result may be a reflection of deleterious activity of the bacterium on turfgrass roots, resulting in enhanced colonization and disease caused by the fungal pathogen. Studies are currently in progress to determine what factors may be involved in decreased disease suppression at high bacterial concentrations.

Field Studies.

Field inoculations of *X. maltophilia* 34S1, *Serratia marcescens* 9M5 and *Serratia* sp. N2-4 were performed during the summer of 1994. These studies were designed to determine efficacy of disease suppression in the field, and to monitor survival of the bacteria in the field throughout the summer. In addition, studies that included the timing of inoculation and the concentration of inoculum of *X. maltophilia* 34S1 were also included in field studies. Timing of inoculation experiments included treatment application of *X. maltophilia* on plots every two weeks and every 4 weeks throughout the summer months. Inoculum for timing experiments consisted of 1 liter of bacteria at a concentration of $5x10^{8}$ CFU/ml, as determined by OD A595 = 0.3 applied to each plot. Concentration of bacteria utilized bacteria at three different concentrations of $5x10^{8}$; $5x10^{7}$ and $5x10^{6}$ CFU/ml. A complete randomized block design was used, with six replications. Plot design involved a 3 x 3 foot plot with a 2 foot boarder. Each plot (uninoculated controls excluded) was inoculated at 4 different positions with

either 20 cc (2 spots) or 40 cc (2 spots) of barley seeds colonized with *M. poae*. Cleary's 1991 and Banner at recommended rates were applied as treatments for use as fungicide controls. Bacterial populations were monitored at the highest concentration of inoculum for *X. maltophilia* and *S. marcescens* every two weeks throughout the summer months. Collection of field data has recently be completed; however, the data has not be analyzed and therefore is not included in this report.

Additional Relevant Studies.

Field trials. A second summer of field trials for 10 bacterial isolates selected as biocontrol agents using root colonization ability as a preselected screen was performed. Data is currently being analyzed.

Determination of role of hydrolytic enzymes. Most of the bacteria selected from the strategy based on funding for this project express at least one of the four extracellular enzymes, B-1,3-glucanase (laminarinase), chitinase, lipase and protease (see Table 2), suggesting that these enzymes function in the observed suppression of summer patch by these bacteria. Both glucanases and chitinases produced by bacteria have been previously implicated to have significant roles in biocontrol of plant diseases. In addition, lipases and proteases have been suggested to contribute to the competitive ability of bacteria in the rhizosphere. In order to determine the role of these enzymes in biocontrol ability of the various organisms, two genetic approaches have been taken. The first was to clone the genes for the enzymes. The second approach was to mutate the genes in the wildtype organism, and assess the ability of these organisms to suppress the disease. The chitinase from *S. marcescens* 9M5 and from *X. maltophilia* have both been cloned. Both chitinase genes were transferred into *Pseudomonas* isolate BF92-14 (a previously identified, superior root-colonizing bacterium with summer patch suppressive ability). The resulting transconjugant containing the *chiA* gene from *S. marcescens* resulted in enhanced disease suppression ability of this organism (data not shown). However, the resulting transconjugant containing the chitinase gene from *X. maltophilia* did not express the gene product at high levels. This was interpreted as a cellular processing problem, in which the gene product appeared not to be effectively secreted from the bacterial cell. Mutagenesis of the chitinase in *X. maltophilia*, however, indicated that little difference in disease suppression was observed between the wildtype *X. maltophilia* and the mutant that no longer expressed chitinase activity, suggesting that the chitinase gene in *X. maltophilia* has no significant role in the observed suppression by this organism. Construction of mutants of the chitinase gene in *S. marcescens* is currently in progress. These mutants will provide further evidence to determine the role of chitinase for the biocontrol by this organism.

Similar projects are currently underway to study the role of lipase and protease in biocontrol ability and competitiveness of bacteria in the soil environment. These studies will provide further insight into the potential for using these bacteria as

biological pesticides, and will further determine the mode of action and therefore the factors (e.g. environmental) that may effect the efficacy of the bacteria against *M. poae*.

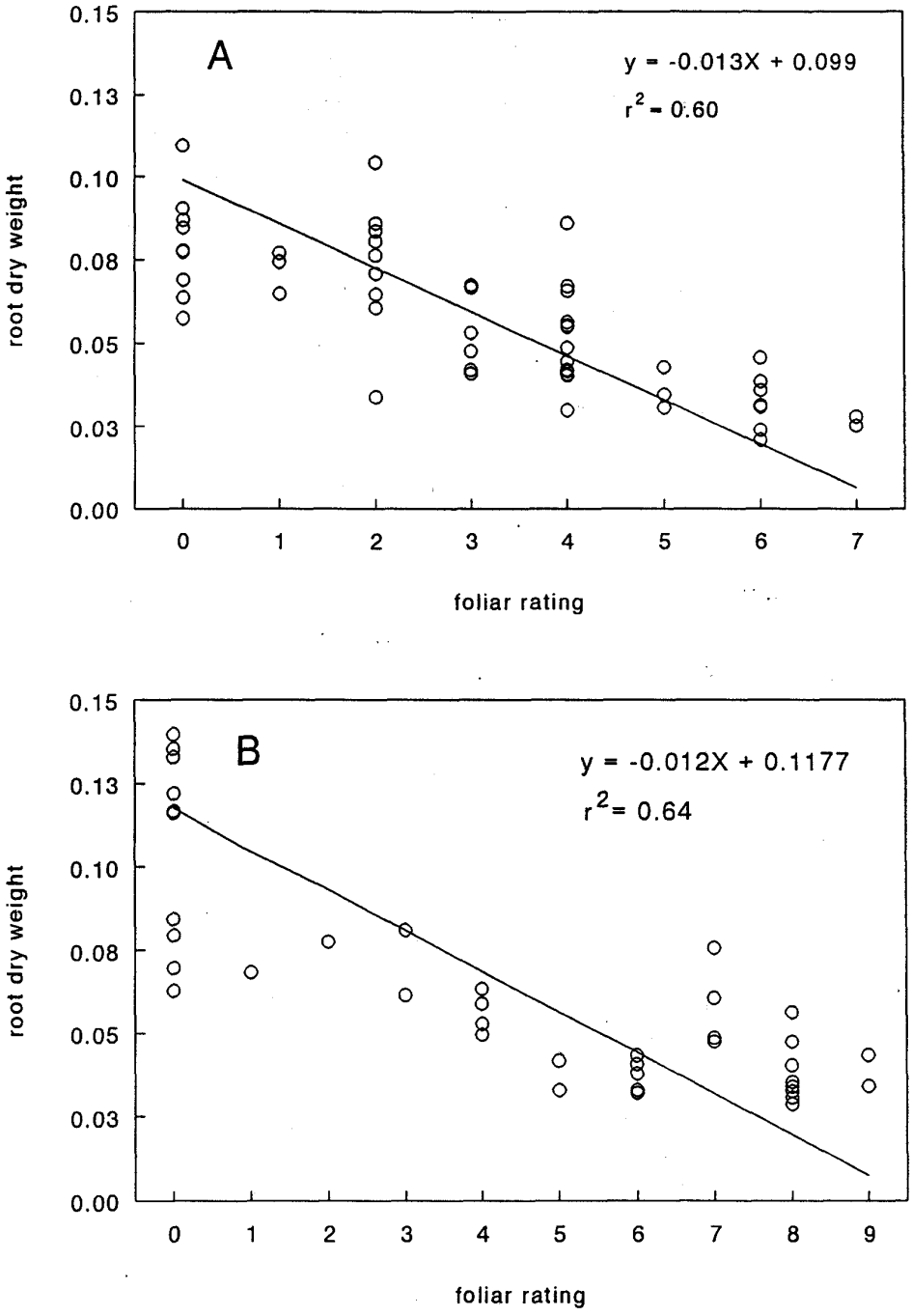

Figure 1. Scatter plot diagram showing the relationship between foliar rating of summer patch disease and the root dry weight on Kentucky bluegrass var. Baron. Regressions, represented by lines within the graph, are given for each fungal isolate. A) *Magnaporthe poae* isolate 73-15. B) *Magnaporthe poae* isolate NAV-A1.

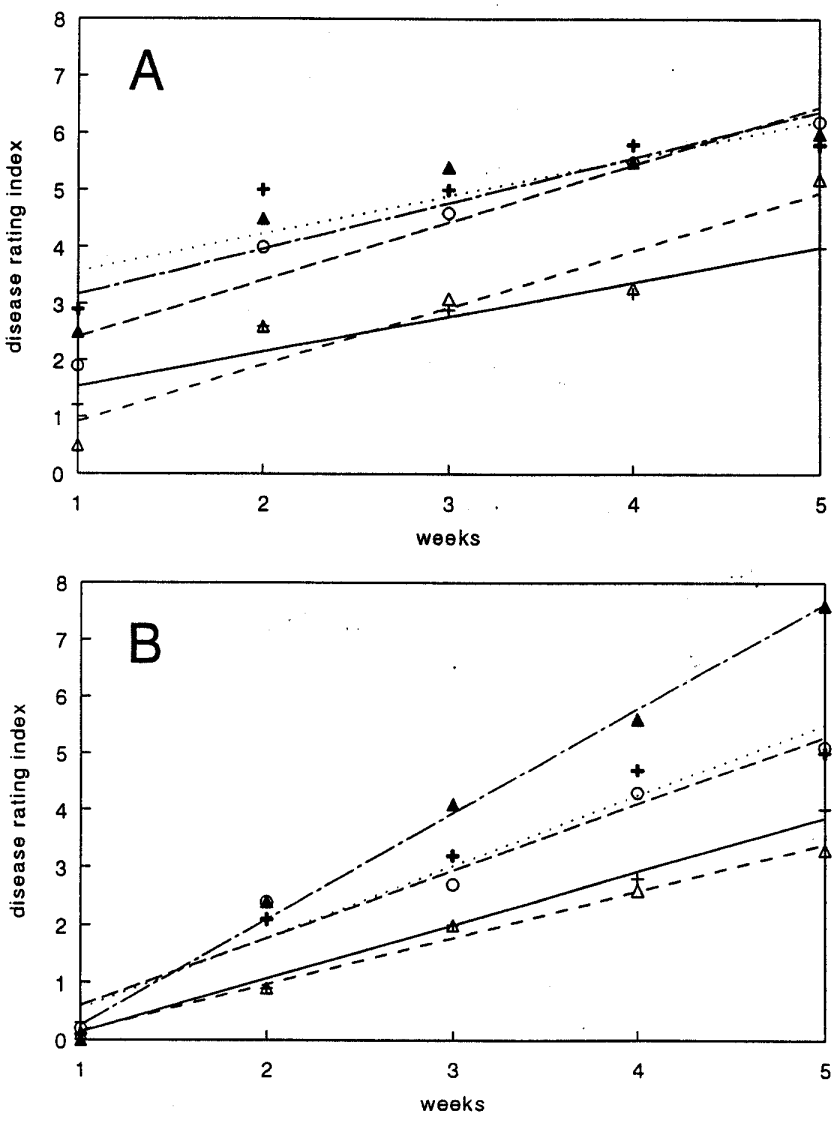

Figure 2. The effect of fungal inoculum concentration on summer patch disease. Kentucky bluegrass var. Baron was inoculated with the equivalent of 1/4 4mm agar plug (straight line, normal cross), 1/2 agar plug (small dashed line, open triangle), 1 plug (large dashed line, open circle), 2 plugs (dotted line, bold cross) or 3 plugs (dash-dotted line, closed triangle) of *Magnaporthe poae* grown on potato dextrose agar. Individual plots represent the mean value of 10 replications. Disease was recorded once a week for five weeks, beginning 1 week after moving plants into the growthchamber. A) *M. poae* isolate 73-15; B) *M. poae* isolate NAV-A1.

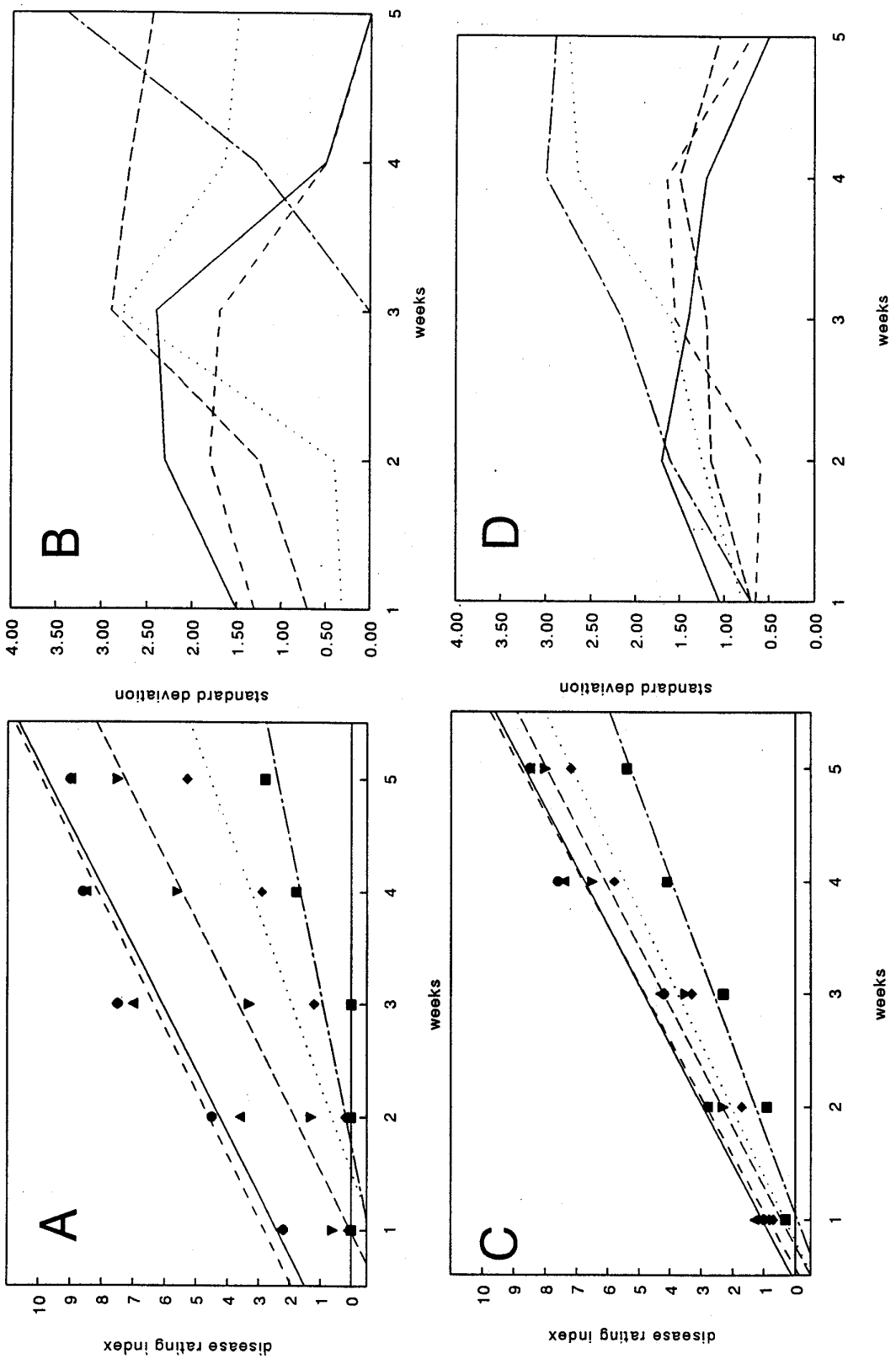
A
B
C
D
disease rating index
standard deviation
weeks
0
1
2
3
4
5
6
7
8
9
10
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00

Figure 3 (Previous page). The effect of depth of placement of fungal inoculum on summer patch symptom development. A standard inoculum density of the equivalent of 1 plug of *Magnaporthe poae* grown on potato dextrose agar was placed at a depth of 0 cm (straight line, upright triangles), 1.5 cm (small dashed lines, circles), 3 cm (large dashed lines, inverted triangles), 4.5 cm (dotted lines, diamonds), or 6 cm (dashed-dotted line, squares) from the level at which Kentucky bluegrass seeds were sown. Plotted values are the means of disease ratings for 10 replications for *M. poae* 73-15 (A) and NAV-A1 (C), taken over 5 weeks, and the regression for values of each depth over a 5 week period. Values of standard deviations for each mean at each sample time were plotted and compared for isolate 73-15(B) and NAV-A1 (D).

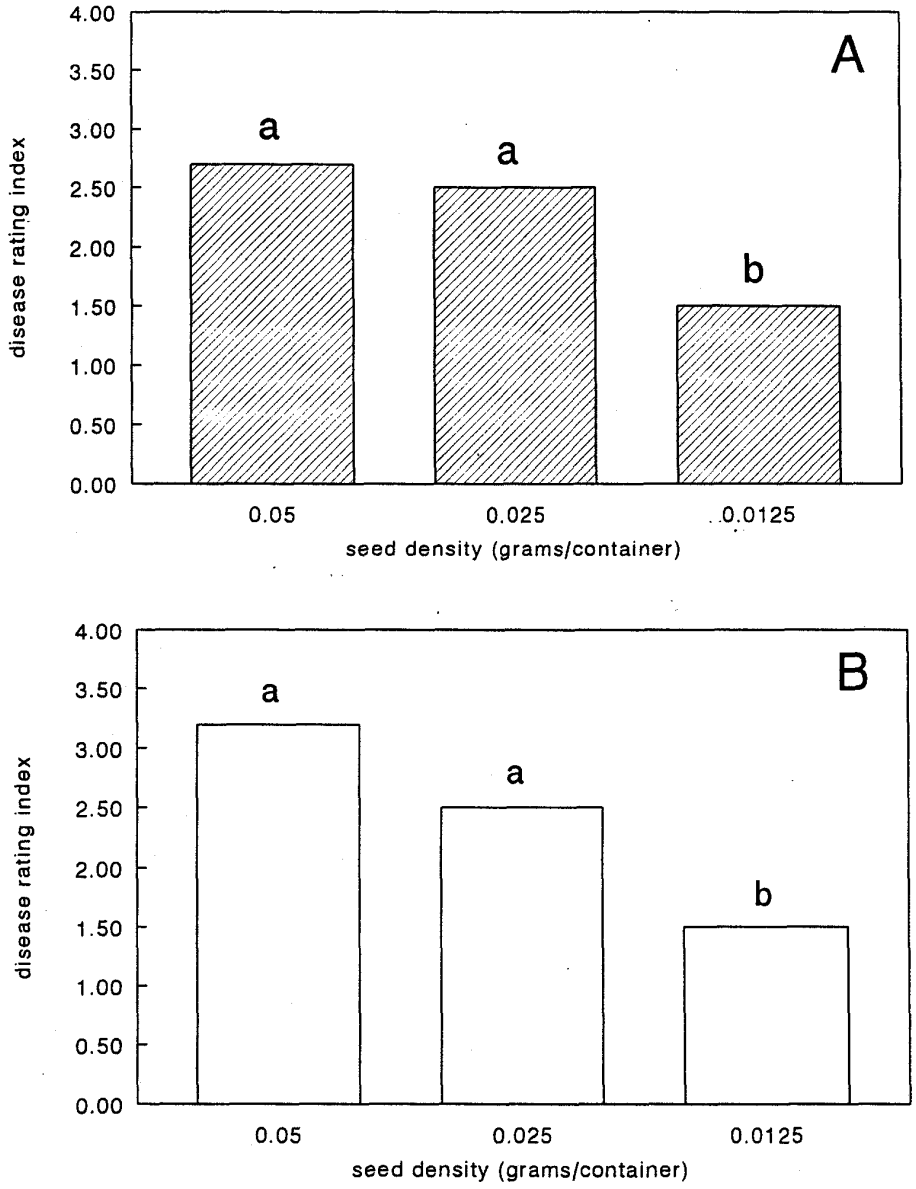

Figure 4. The effect of density of Kentucky bluegrass var. Baron seeds on summer patch symptom development. Disease was recorded, using a 10 point logarithmic rating scale, three weeks after transferring plants to the growthchamber. Bar values with different letters represent statistically significant differences according to the Waller-Duncan K-ratio T test. A) *Magnaporthe poae* isolate 73-15; B) *M. poae* isolate NAV-A1.

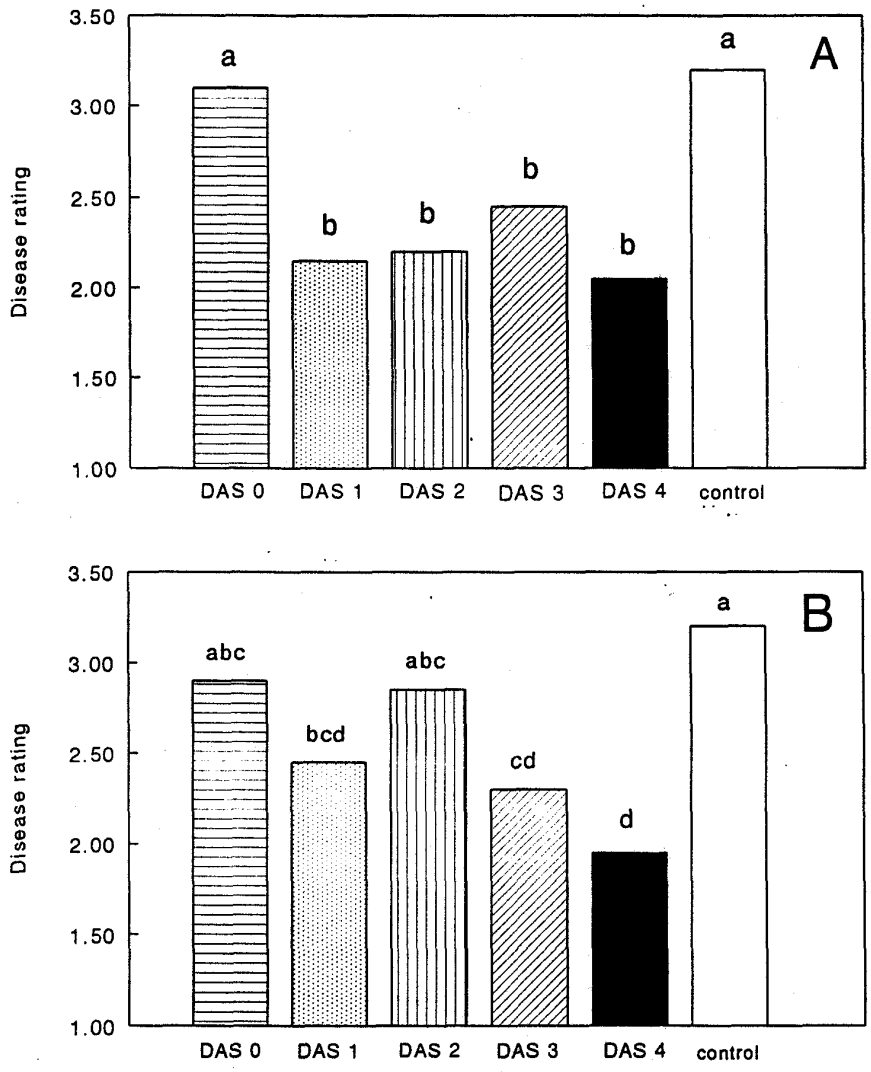

Figure 5. Effect of timing of bacterial application on the suppression of summer patch disease. Bacteria were applied one time as a soil drench at a rate of 10^9 CFUs ml^{-1}. Application times differed by seven day intervals, over a four week period, beginning at the time of sowing Kentucky bluegrass seeds (Time 0) to 24 h prior to transfer of plants into the growth chambers to induce disease (Time 4). Plants were scored based on percentage of dead foliar area using a 9 point logarithmic scale where 0.5 = no disease and 5.0 = 100% disease. A) *Xanthomonas maltophilia* 34S1; B) *Serratia marcescens* 9M5. Values with common letters represent no significant differences according to Duncan's multiple range test (P=0.05).

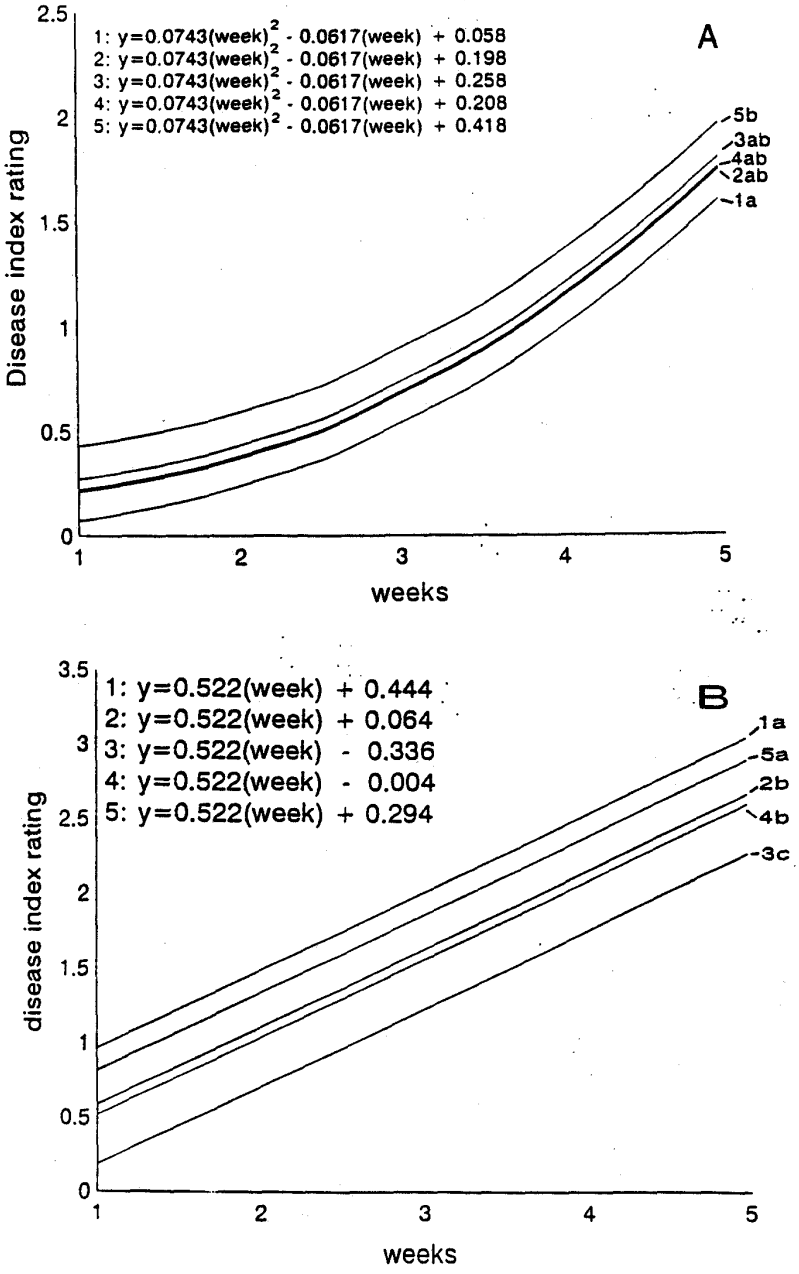

Figure 6. Effect of bacterial concentration on summer patch disease progress in Kentucky bluegrass. Plants were transferred to growth chambers to induce disease 4 weeks after incubation in growth chambers. A) Plants were treated with *Xanthomonas maltophilia* 34S1. B) Plants were treated with *Serratia marcescens* 9M5. $1 = 10^{10}$ cfu ml^{-1}; $2 = 5 \times 10^{9}$ cfu ml^{-1}; $3 = 1 \times 10^{9}$ cfu ml^{-1}; $4 = 5 \times 10^{8}$ cfu ml^{-1}; $5 = 1 \times 10^{8}$ cfu ml^{-1}.

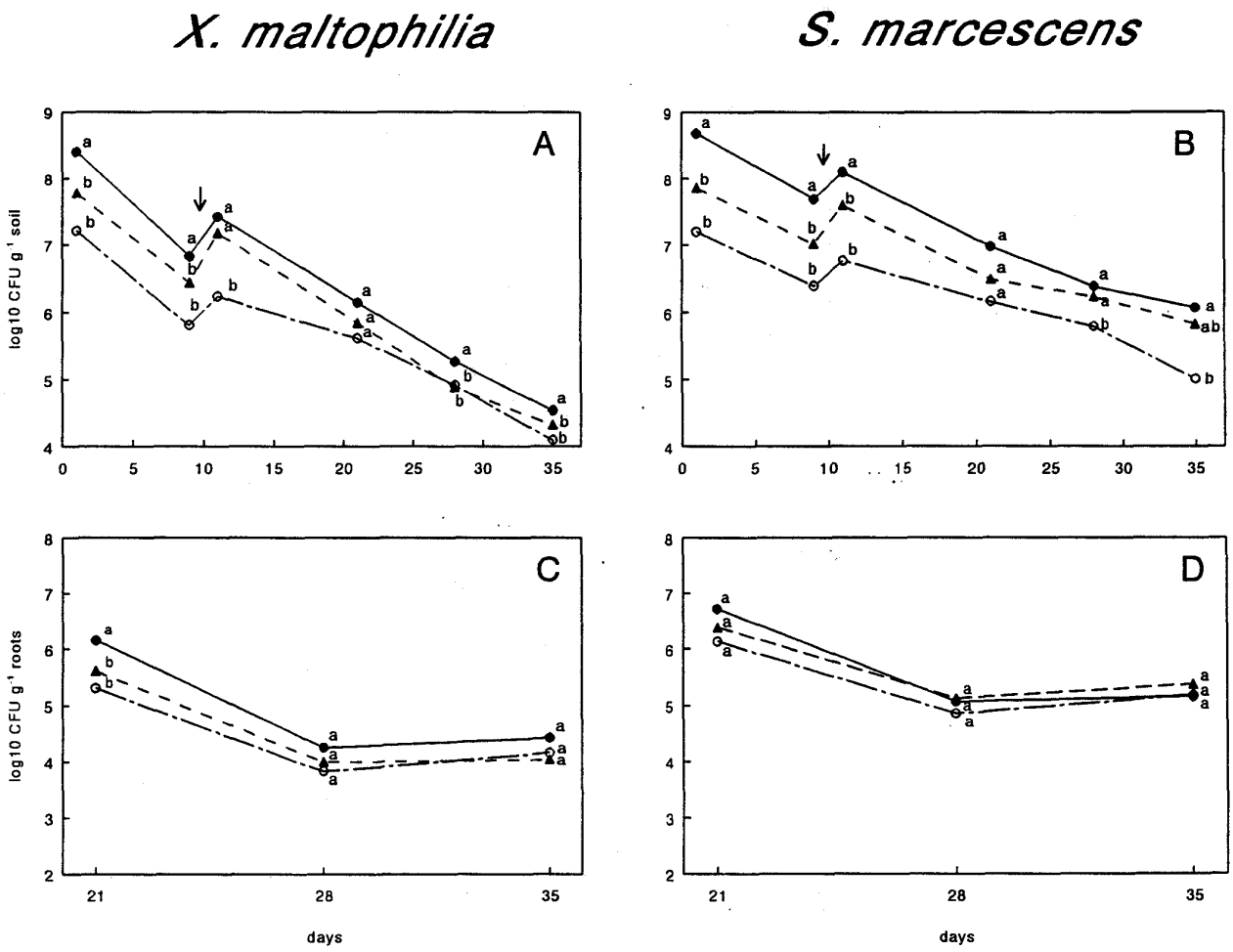

Figure 7. Populations of bacteria inoculated at three different concentrations in nonrhizosphere soil (A,B) and the rhizosphere of turfgrass (C,D). Soil populations of *Xanthomonas maltophilia* (A) and *Serratia marcescens* (B) were monitored over a 35 days. Arrows depict time of second application of bacteria. Populations of *X. maltophilia* 34S1 (C) and *S. marcescens* 9M5 (D) in the rhizosphere at 21, 28 and 35 days after planting. (●), conc. = 10^{10} cfu ml^{-1}; (▲), conc. = 10^{9} cfu ml^{-1}; (○), conc. = 10^{8} cfu ml^{-1}. Values followed by common letters within each observation represent no significant difference at P = 0.05.

Table 1. Summer patch disease suppression by bacterial isolates recovered from enrichment procedures[1].

Isolate	SP[2] rating	Isolate	SP rating
Exp. 1		**Exp. 2**	
N1-7	4.2	N2-4	3.2
N1-9	3.3	N2-6	3.1
N1-13	3.5	N2-7	3.2
N1-15	3.3	Uninoc.	4.9
Uninoc.	4.9	IC	1.0
IC	1.3		
Exp. 3		**Exp. 4**	
N3-5	3.3	N4-3	4.0
N3-10	3.3	N4-5	4.6
N3-13	3.8	N4-7	4.7
N3-16	3.7	N4-11	4.4
N3-19	3.4	N4-15	4.1
Uninoc.	5.0	Uninoc.	5.0
IC	1.25	IC	1.9
Exp. 5		**Exp. 6**	
N5-13	4.1	N6-18	4.4
Uninoc.	4.4	Uninoc.	5.0
IC	1.7	IC	2.6
Exp. 7		**Exp. 8**	
N11-1	3.2	N12-2	3.0
N11-6	3.3	N12-4	3.6
N11-8	3.5	N12-9	3.3
N11-9	3.2	N12-10	3.3
N11-13	3.2	N12-16	3.5
N11-15	3.7	N12-17	3.4
N11-16	3.3	N12-20	3.2
N11-17	3.3	Uninoc.	5.0
N11-18	3.5	IC	1.8
N11-19	3.1		
Uninoc.	4.8		
IC	1.4		

[1]Experiments represent values of disease suppression by inoculation of bacteria on Kentucky bluegrass var. Baron in growthchamber assays in a single experiment. All isolates were tested in a minimum of two experiments, resulting in suppression of 50% or greater compared to control plants. Controls for each experiment are given, where Uninoc. = healthy control plants and IC = disease control plants.

[2]SP rating: summer patch disease rating based on a nine point logarithmic scale where .5 = 100% foliar area is diseased, 3 = 50% diseased and 5 = 0% diseased.

Table 2. Characteristic traits of bacterial isolates with suppressiveness against summer patch symptom development.

Isolate	Glucanase	Chitinase	Protease	Lipase	AFA	RCA (cfu/gm root)	Description
N1-7	-	+	+	-	-	$5.2x10^4$	*Bacillus* sp.
N1-9	-	+	+	+	-	$3.8x10^6$	Gm -
N1-13	-	+	+	-	-	$3.8x10^4$	Gm +
N1-15	-	-	+	-	-	$2.4x10^6$	Gm -
N2-4	-	+	+	+	+	$3.2x10^7$	*Serratia* sp.
N2-6	-	+	+	+	+	$4.8x10^6$	*Serratia marcescens*
N2-7	+/-	+	+	+	+	$2.5x10^7$	*Serratia* sp.
N3-5	-	+	-	-	+	ND*	Gm stain ND
N3-10	-	+	+	-	+	$2.8x10^5$	Gm +
N3-13	-	+	-	-	-	ND	Gm stain ND
N3-16	+/-	-	+	-	+	$5.5x10^6$	Gm -
N3-19	-	-	-	-	-	$9.6x10^5$	Gm +
N4-3	-	+	+	-	+	$9.0x10^4$	Gm +
N4-5	-	+	+	+	+	$1.4x10^7$	*Serratia marcescens*
N4-7	+	+	+	+	+	$1.3x10^6$	Gm -
N4-11	-	+	+	+	+	$5.0x10^7$	Gm -
N4-15	+	+	+	+	-	$6.4x10^5$	Gm -
N5-13	-	-	+	-	-	$4.9x10^5$	Fluorescent pseudomonad
N6-18	-	-	+	-	-	ND	Gm -
N11-1	-	-	+	-	-	$1.3x10^7$	Fluorescent pseudomonad
N11-6	+/-	-	+	-	-	$5.8x10^6$	Fluorescent pseudomonad
N11-8	-	+	+	+	+	$2.4x10^6$	Gm -
N11-9	-	-	+	-	-	$5.7x10^6$	Fluorescent pseudomonad
N11-13	+/-	+	+	-	+	$2.4x10^6$	Fluorescent pseudomonad
N11-15	-	+	+	-	+	ND	*Bacillus* sp.
N11-16	-	+	+	-	+	$6.6x10^5$	Fluorescent pseudomonad
N11-17	-	-	+	-	+	$9.5x10^6$	Fluorescent pseudomonad
N11-18	+	-	+	+	+	$5.8x10^7$	Fluorescent pseudomonad
N11-19	+/-	-	+	+	+	$8.3x10^6$	Fluorescent pseudomonad
N12-2	+/-	-	ND	-	+	$8.9x10^6$	Fluorescent pseudomonad
N12-4	+	-	ND	+	+	ND	Fluorescent pseudomonad
N12-9	-	-	ND	+	+	ND	Gm -
N12-10	-	+	ND	-	+	ND	Gm -
N12-16	-	+	ND	-	+	$1.3x10^4$	Gm -
N12-17	-	+	ND	-	-	$1.4x10^4$	*Bacillus* sp.
N12-20	+	-	ND	-	-	ND	Gm +

*ND = not determined