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TITLE: Investigation of Turf Disease Decline for Potential Development of Biological Control Methods

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Investigation of Disease Decline for Development of Biocontrol of Turf Diseases

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SUMMARY OF REPORT FOR 1992 PROJECT YEAR

In response to environmental concerns and increasing restrictions on the use of chemical pesticides alternative disease control methods must be developed to reduce our reliance on these materials. Biocontrol of plant disease through the use of "beneficial" microorganisms that are antagonists of disease-causing microorganisms is one such alternative. Since March 1991 we have been investigating sites where disease has declined naturally as sources of potential biocontrol agents and ecological information that will be used to develop and implement biocontrol methods for turf diseases. We hypothesize that disease decline may be due to increased activity of indigenous microorganisms antagonistic to the pathogen. Of 147 microbial isolates (from a UCR bermudagrass plot showing decline of spring dead spot caused by *Leptosphaeria korrae*) tested so far, 41 bacteria and 19 fungi inhibited growth of *Sclerotium rolfsii* (cause of southern blight) by antibiosis; 6 fungi parasitized *S. rolfsii*. Growth of *Rhizoctonia solani* (cause of brown patch) was inhibited by 25 bacteria and 26 fungi. Tests with *L. korrae* are not yet completed. In greenhouse experiments, 2 bacterial isolates, JT78 and JT80, were most effective at reducing disease caused by *S. rolfsii* and *R. solani* in perennial rye. We observed no detrimental effect on plants by these biocontrol agents, even when applied at high concentrations. We have begun field testing potential biocontrol agents at two bermuda grass plots naturally infected with *L. korrae*; results are pending. Identification of additional disease decline sites and collections from these sites is continuing.

INTRODUCTION

Public concerns regarding the environmental and human health impact of chemical pesticide usage has led to increasing restrictions on the use of these materials. This situation demands a shift in emphasis from chemical control to alternative disease control methods. Biological control of plant pathogens is one promising alternative and its application to the control of turfgrass diseases deserves serious attention.

One approach to biological control of plant disease is the use of "beneficial" microorganisms that are antagonists of disease-causing microorganisms. Microbial antagonists include organisms that limit the growth, reduce the population, or interfere with the disease-causing ability of a pathogen. Sites where disease has occurred but has subsequently declined or disappeared (particularly under conditions otherwise favorable to the pathogen) may indicate the activity of natural antagonists of the pathogen. Such "disease-decline" sites are a likely source of potential biological control agents from among the natural microbial antagonist population.

While conducting field research on turf diseases over the past several years, Dr. Howard Ohr (Cooperator on this project) has consistently observed a decline of diseases caused by *Leptosphaeria korrae* (spring dead spot) and *Sclerotium rolfsii* (southern blight). This disease decline has been characterized by an initially severe disease that, without fungicide application, is reduced over several years until the pathogen can no longer be isolated. The nature of this disease decline is presently unknown. Although this decline apparently occurs over several years, we believe that a state of disease suppression might be effected in a short time by deliberate application of agents responsible for the disease decline.

The phenomenon of disease decline has previously been observed in other systems, most notably in wheat take-all decline. The decline in take-all disease in the presence of a susceptible host plant and the pathogen, *Gaumannomyces graminis*, is gradual and damage due to the disease must be sustained for a number of years before disease is reduced to acceptable levels. Although several agents may be involved in take-all decline, fluorescent pseudomonad bacteria isolated from take-all decline soil can reduce disease when applied to plants grown in soil not previously suppressive to the pathogen, without the need to wait several years for disease suppressiveness to develop naturally.

LONG-TERM RESEARCH OBJECTIVES

1. Determine nature of decline of turf diseases caused by *Leptosphaeria korrae* and *Sclerotium rolfsii.*

2. Identify potential biocontrol agents from among microorganisms isolated from disease decline areas and their effectiveness against other important turf pathogens.

3. Understand the ecology of potential biocontrol agents, including interactions with other microorganisms and response to environmental conditions.

4. Develop effective biocontrol strategies for turf diseases.

PROCEDURES AND RESULTS

Overall Approach. This project to develop biocontrol methods for turfgrass diseases has been funded and in progress since March 1991 (approximately one and a half years). The approach has been to investigate sites where disease has declined naturally to recover potential biocontrol agents from the microflora at these sites. As discussed in the Introduction, disease decline at these sites may be due to increased activity of microorganisms antagonistic to the pathogen. In addition, ecological information obtained from our investigations will be valuable in developing and implementing biocontrol methods. This final report for the 1992 project year will focus on progress made since November 1991.

Isolation of Potential Biocontrol Agents. The initial site (UCR 10-I) selected for study contains bermudagrass that had previously been inoculated with *Leptosphaeria korrae* (cause of spring dead spot) by Dr. Ohr. By the spring of 1991, disease had developed such that green, symptomless patches were obvious in the center of brown, diseased areas. Microorganisms were isolated by standard methods from soil collected from the root zone of bermudagrass on either side of the margin between brown, symptomatic areas and green, recovered areas. Several different non-selective and semi-selective growth media were used for isolations to allow for the recovery of a wide range of bacteria and fungi. This rather labor intensive process resulted in the isolation of 147 different microorganisms (97 bacteria and 50 fungi) from this site.

We isolated from the UCR 10-I plot at what has since proved to be the appropriate time, since by the spring of 1992 we could no longer detect the disease. If our hypothesis is correct, the antagonistic microorganisms should have been at their highest populations during the time we collected, that is, just prior to the disappearance of disease symptoms. While testing potential biocontrol agents recovered from UCR 10-I, we are also continuing to collect from other disease decline sites (see below).

Antagonism of Pathogens in Culture. The UCR 10-I isolates were tested for the ability to reduce growth of several turfgrass pathogens (*Rhizoctonia solani*, *Sclerotium rolfsii* and *Leptosphaeria korrae*) in culture. One of the pathogens and one test microorganism were placed on opposite sides of agar growth medium contained in a petri dish. As the colonies grew together the interaction was observed for inhibition of growth or direct "attack" (e.g., parasitism) of the pathogen by the test organism. The results of these experiments with *R. solani* and *S. rolfsii* are shown in Table 1 (antagonistic bacteria) and Table 2 (antagonistic fungi). Tests with *L. korrae* are not completed.

Table 1. Antagonism of *Rhizoctonia solani* and *Sclerotium rolfsii* in culture by bacterial isolates from field plot UCR 10-I.

	Total	*Rhizoctonia solani*	*Sclerotium rolfsii*
No. bacteria tested	97	97	97
No. bacteria expressing:			
Antibiosis	46		
Antibiosis on CMA only		4	26
Antibiosis on PDA only		5	3
Antibiosis on CMA & PDA		16	12
No. fluorescent bacteria	35		
Fluorescent bacteria that inhibit growth on iron-deficient medium		35	15

Table 2. Antagonism of *Rhizoctonia solani* and *Sclerotium rolfsii* in culture by fungal isolates from field plot UCR 10-I.

	Total	*Rhizoctonia solani*	*Sclerotium rolfsii*
No. fungi tested	50	50	50
No. fungi expressing:			
Antibiosis	29		
Antibiosis on CMA only		7	12
Antibiosis on PDA only		5	0
Antibiosis on CMA & PDA		14	7
No. fungi expressing:			
Parasitism	15		
Parasitism on CMA only		3	1
Parasitism on PDA only		3	4
Parasitism on CMA & PDA		9	1

Microorganisms that exhibit antagonism of a pathogen in culture will not necessarily be effective biological control agents. On the other hand, potentially effective biological control agents may not demonstrate the antagonism of a pathogen under the particular cultural conditions used for the laboratory experiments. With this in mind, however, the antagonism-in-culture tests will serve to prioritize microorganisms for further testing of their biocontrol potential.

Effect of Antagonists in the Absence of Pathogens. Four bacteria were selected for the first biocontrol experiments. Bacterial antagonists were applied to plants in the absence of a pathogen to assess any detrimental effect they might have on the plants and to determine the maximum concentrations of bacteria that could be applied for biocontrol experiments. Perennial rye seed was planted into nonsterile Maddox soil:sand (1:1) in 4-inch styrofoam cups. Plants were grown for 10 days and trimmed to 3.18 cm prior to inoculation. Bacteria were grown on YDC agar medium for 48 h at 24 C, harvested in sterile distilled water and adjusted to concentrations of 1×10^6 to 1×10^9 cfu/ml. Bacterial suspensions (50 ml) were poured into each of 3 replicate cups containing perennial rye.

No browning, yellowing or wilting was observed. Although there were differences in the mean growth (increased height) of perennial rye among treatments, none were judged statistically different than the control (= no bacteria added) (Table 3). There is a suggestion that isolate JT78 at the higher concentrations may actually have increased the growth of perennial rye, although the increase was not judged statistically significant in this experiment. We are performing additional experiments to determine if this apparent growth enhancement by JT78 is real.

Table 3. Growth of perennial rye in response to bacterial isolates from field plot UCR 10-I.

Bacterium	Log concentration	Increased height (cm) after 10 days
JT78	9	3.17 a[x]
JT78	8	3.17 a
Control	-	2.11 ab
JT22	9	1.90 ab
JT80	8	1.90 ab
JT35	7	1.69 ab
JT78	6	1.69 ab
JT22	8	1.48 ab
JT35	6	1.48 ab
JT78	7	1.48 ab
JT80	6	1.27 ab
JT80	7	1.27 ab
JT80	9	1.05 ab
JT22	6	0.84 b
JT22	7	0.84 b
JT35	9	0.84 b
JT35	8	0.63 b

[x] Means within a column with a common letter are not significantly different according to Tukey's test (α=0.1).

A similar experiment was conducted using a peat moss:sand (1:1) planting mix (UC PP IV). Differences in treatment means were even smaller (and not statistically significant) than those in the previously described soil:sand experiment. We believe this is at least in part due to the suppression of microbial activity in the peat moss:sand planting mix. A soil:sand mix will be used for all subsequent experiments since this more realistically represents the field situation.

Biocontrol of Disease in the Greenhouse. The biocontrol ability of selected UCR 10-I isolates is being tested in the greenhouse. Perennial rye was seeded into soil:sand mix in 6-inch pots. After 2 months, the pots treated with an aqueous suspension of one of the test biocontrol agents. The plants were inoculated with sclerotia of *S. sclerotium* or cornmeal:sand cultures of *R. solani*. Disease in biocontrol treatments was compared to treatments with the pathogen alone (no test biocontrol agent). Initial results indicate that 2 bacteria, JT78 and JT 80, were most effective at reducing disease caused by *S. rolfsii* and *R. solani* in perennial rye.

Field Trials with Potential Biocontrol Agents. We have set up our first field plots to test potential biocontrol agents. Two bermuda grass plots in Fresno, CA, naturally infected with *L. korrae* (spring dead spot), were used for the initial field trials, begun on 9/15/92. Treatments consisted of a bacterial biocontrol agent (JT80), a fungal biocontrol agent (KA159), a combination of JT80 and KA159, and three chemical fungicides (Rubigan, Lynx, and Bayleton). From these experiments, our first attempts at applying biocontrol agents to turfgrass in the field, what we learn about applying biocontrol agents in the field and how well they survive will be as important as any actual disease control that we may observe. We included only one bacterium, one fungus and the combination of the two in order to gain experience with each type of biocontrol agent without setting up too large an experiment until we have the experience in application of biocontrol to turfgrass that these trials will provide.

Identification of Additional Disease Decline and Field Test Sites. To determine the extent of the disease decline phenomenon and to provide a broad and varied range of source material for further research, we have been solicit golf course managers and farm advisors for the location of sites that suggest the occurrence of disease decline. Janet Hartin, a UC Cooperative Extension Farm Advisor, is collaborating with us during a one year sabbatical leave (Ms. Hartin will also be working with Dr. Vic Gibeault and Dr. Marylynn Yates during her sabbatical). Ms. Hartin's input to the project is identifying disease and disease decline sites in southern California (especially the Palm Springs area), sampling from these sites, and locating potential cooperators for experimental field plots.

I have presented the objectives and results of this project at a recent Turfgrass Research Conference and Field Day at UCR (9/15/92) attended by several hundred golf course managers, ornamental specialists and industry representatives. There was considerable interest in the project. I made a number of contacts with golf course and park managers that expressed a desire to collaborate on and provide sites for field testing potential biocontrol agents.

Relative Virulence of Pathogens from "Disease-Decline" Sites. Were are continuing to collect isolates of pathogens from active disease sites and as well as from areas suspected as being in disease decline. Although a major focus of this research project is on microbial antagonists, some occurrences of disease decline could be due to reduced virulence of the pathogen. When more pathogen isolates have been collected, we will compare their virulence. If reduced virulence is associated with disease decline, additional research will investigate the potential of this phenomenon to biocontrol. A reduction in the virulence of pathogens in other plant disease systems has been linked to infection of a pathogen with a virus or other similar agent.

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