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

INVESTIGATORS:

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SUMMARY

Increasing restrictions on the use of chemical pesticides demands a shift in emphasis from chemical control to alternative disease control methods. One alternative is the biological control of plant disease through the use of "beneficial" microorganisms that are antagonists of disease-causing microorganisms. This is the first year of a project to investigate sites where disease has declined naturally as potential sources of information and biological materials for the development of biological control methods. Increased activity of antagonistic microorganisms may be associated with disease decline expressed at a site over several seasons or within the green, recovered central areas that often appear within brown, symptomatic patches of turf as the disease spreads.

Study was begun with a bermudagrass field plot previously inoculated with Leptosphaeria korrae (cause of spring dead spot) at the University of California at Riverside Experiment Station. Disease had spread sufficiently so that green, symptomless patches were obvious in the center of brown, diseased areas, hence, a comparison of microbial profiles from each of these areas could be performed. Thus far, 135 different bacteria and fungi have been isolated from this UCR field plot. These microorganisms are being tested for the ability to reduce growth of several turfgrass pathogens (Leptosphaeria korrae, Sclerotium rolfsii, and Rhizoctonia solani) in culture. Experiments are also underway to test the most promising microorganisms for their ability to reduce disease in the greenhouse.

Identification of disease decline sites in California, studies to determine the disease-suppressiveness of turf samples from these sites, and a comparison of virulence of pathogens from these sites are planned for the second half of this project year.

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 biological control 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 biological control strategies for turf diseases.

PROCEDURES AND RESULTS

Overall approach. The approach of this project is to investigate sites where disease has declined naturally as potential sources of information and biological materials for the development of biological control methods. Disease decline expressed as a general disease reduction and perhaps eventual disappearance at a particular site over several seasons might involve increasing activity of microorganisms antagonistic to the pathogen. Another phenomenon that may be associated with increased activity of antagonistic microorganisms is the green, recovered central area that appears within a brown, symptomatic patch of turf as the disease spreads.

Experimental site. For the past two years, Dr. Casale's laboratory has been investigating natural suppression of Phytophthora root rot of avocado as a basis for developing biological control methods for this disease. Although the general approach of the turfgrass project is similar to that of the avocado research, specific techniques to be used with turfgrass were new to the laboratory and, obviously, quite different than the avocado system. In light of this, and from our experience with the Phytophthora root rot/avocado system, we decided the most appropriate plan would be to begin the turfgrass project by focusing on one field plot at the UCR Experiment Station to work out methods of studying disease decline and isolating potential biological control agents. These techniques could then be used in studying other sites. The plot selected 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 spread sufficiently that green, symptomless patches were obvious in the center of brown, diseased areas. The bulk of the research performed so far has been done on this particular field plot.

Our immediate interest was the green patches within diseased areas at this site. The strategy was to isolate and compare the profile of microorganisms present in (1) those areas where disease was obvious and (2) the green central areas in which the grass had recovered. If the project continues, we will continue monitoring this site over a longer term in anticipation of a complete decline of the disease as has been observed previously.

Isolation of microorganisms. Microorganisms were isolated from soil collected from the root zone of bermudagrass at sites within brown, symptomatic areas and green, recovered areas. Each soil sample was shaken in sterile water. Serial dilutions of each soil suspension were spread onto various growth media. Microorganisms were also isolated from roots and crowns by placing small pieces of these plant tissues onto growth media. 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. The inoculated media were incubated at room temperature and monitored for growth of microbial colonies.

Each isolation plate typically produced a mixed culture of many different bacteria and fungi. Individual colonies were transferred separately to fresh growth media. Pure cultures of fungi and bacteria isolated in the above manner were tentatively identified and appropriately stored for future use. Based on colony color and morphology, growth on specific media, and microscopic examination, the rather labor-intensive procedure described above has resulted in 135 different microorganisms (88 bacteria and 50 fungi) isolated from the single UCR field plot so far.

Testing microorganisms for antagonism of pathogens in culture. The microorganisms isolated from the UCR field plot are currently being tested for the ability to reduce growth of several turfgrass pathogens (Leptosphaeria korrae, Sclerotium rolfsii, and Rhizoctonia solani) in culture. In these tests, one of the pathogens and one test microorganism are placed on opposite sides of agar growth medium contained in a petri dish. As the colonies grow together the interaction is observed for inhibition of growth or direct "attack" (e.g., parasitism) of the pathogen by the test organism. Results of these tests are pending.

The results of these laboratory tests must be interpreted with caution. 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 these points in mind, however, the antagonism-in-culture tests will serve to prioritize microorganisms for further testing of their biocontrol potential.

Greenhouse tests. Experiments are underway to test the most promising microorganisms for their ability to reduce disease in the greenhouse. Greenhouse flats (approximately 18 inches square) of turf are each inoculated in one corner with one of the pathogens. When disease has been established, a test organism will be applied by spraying each flat with bacterial cultures, fungal spore suspensions or fragmented fungal mycelium, as is appropriate for the particular organism being tested. The rate of disease spread will be compared with that of pathogen-inoculated flats that have not been treated with a test organisms.

EXPERIMENTS TO BE PERFORMED

Because disease is cyclic in nature and its expression is dependent on the season, pathology in the field cannot be done year-round. Beginning this project in March put us at the end of the period during which spring dead spot could be observed. With the onset of cool weather again, we will have a better opportunity to observe and work on disease in the field. Therefore, much of the research during this first project year will be performed after submission of this report. In addition to continuing the work described above, the project will be expanded during this full disease season to include the following.

Identification of disease decline 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 are continuing to solicit golf course managers and farm advisors for the location of sites that suggest the occurrence of disease decline. Turf samples (with associated soil removed intact) will be collected from areas that have tentatively been identified as exhibiting the disease decline phenomenon described above. This turf will be transferred to greenhouse flats and inoculated with the appropriate pathogen to test for suppression of disease compared to turf from non-disease decline areas. Microorganisms will be isolated from disease-suppressive samples and evaluated as described above. When available, soil analyses and records of pesticide application will be included in our evaluations.

Relative virulence of pathogens from "disease-decline" sites. 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. A reduction in the virulence of pathogens in other plant disease systems has been linked to infection of the pathogen with a virus or other similar agent. The virulence of pathogens isolated from disease decline sites will be compared with that of pathogens from areas that show no indication of disease decline. If this line of research is promising, future experiments could be performed to determine whether reduced virulence can be transferred (i.e., it is infectious) to highly virulent pathogens by culturing them together with low virulent isolates from disease decline areas.

Principal Investigator William L. Casale Date 11/1/91
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