2004 GCSAA Research Funding Program: National and Chapter

Title of Project: Biology, Diversity and Epidemiology of Gray Leaf Spot (*Pyricularia grisea*) Populations Affecting Perennial Ryegrass and Kikuyugrass in California

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Total Amount of funding requested from GCSAA and Regional Chapters:
$10,000 National
$6,000 per supporting regional California GCSA Chapter
Total: $52,000

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INTRODUCTION

Grey Leaf Spot (GLS), caused by the fungus *Pyricularia grisea*, is a major disease of turfgrass that appeared in epidemic proportions in 2003 in California on both perennial ryegrass and kikuyugrass. The occurrence of this disease on a new host (kikuyugrass), on a new life stage of turf (mature perennial ryegrass plants) and under arid conditions that would not have been predicted by current disease models presents a unique opportunity to learn more about its host range, biology, environmental requirements and control. The information obtained from this study both is needed for developing sound GLS management in California and can be put into immediate use by superintendents across the country in terms of improved forecasting of disease occurrence, timing preventive management strategies, selecting the optimal control measures and identifying fungicide resistant disease populations. Additionally, superintendents in the southwest and Hawaii who manage either kikuyugrass or overseeded perennial ryegrass will gain additional benefits through better characterization of GLS under their unique combination of environmental and turf type conditions.

The disease has been known to occur in the U.S. on St. Augustinegrass since 1957 (Malca 1957) and on annual ryegrass since the 1970s (Bain 1972, Carver 1972). It was only identified as causing disease on perennial ryegrass fairways since 1991 (Landschoot 1992) and since then has caused severe losses on perennial ryegrass fairways in the mid-Atlantic states and has been observed in the mid-Western and New England states. Although known to be present in California on St. Augustinegrass since the 1970s (Mueller 1972), the disease had not been reported on perennial ryegrass until 2001 (Uddin 2002) and only reported on kikuyugrass in 2003.

In 2003, the disease was diagnosed on perennial ryegrass from a number of locations across the state, from Sacramento to San Diego and as far inland as the Sierra foothills and Riverside County, encompassing an area approximately 500 miles long by 70 miles wide within the state. Infections on kikuyugrass were limited to the southern part of the state. The disease caused severe turf loss in some locations before superintendents realized that GLS was present. Turf loss due to GLS began in August and has continued to be active in some areas through October.

The disease requires several hours of leaf wetness or high humidity and temperatures above 68ºF for infection. The optimal temperature range of the pathogen is 79 to 84ºF (Uddin 2003b), and the pathogen can cause damage on turf in as little as three days under these conditions. Although the summer precipitation in California that triggered some of the outbreaks is rare, irrigation coupled with periods of high humidity or coastal fog, appear adequate for disease development. The period of high to moderate temperatures favorable for disease development can historically last from July to November in some locations, especially in southern California. Additionally, there is little information available regarding the risk of disease development on perennial ryegrass overseeded in the fall, or the biology of the pathogen on kikuyugrass.

Due to the recent arrival of the disease on perennial ryegrass and kikuyugrass, there is little scientific data from California available to assist superintendents manage the disease. Due to the climatic differences between California and areas which have traditionally been affected by the disease (i.e. the mid-Atlantic states), the pattern of GLS development may not match what is known about the epidemiology of the disease in other locations. Although superintendents in many locations in the U.S. routinely apply fungicides to fairways, those in California rarely do. The added cost of spraying fairways will greatly increase the management budgets of superintendents, and in some cases exceed the financial capability of some courses. More information on the biology and epidemiology GLS in California is needed to help develop sound management strategies. The information gathered by the proposed research will also assist superintendents in other parts of the U.S. by helping them understand how the disease develops and needs to be managed under different climactic and geographic conditions.

OBJECTIVES AND PROPOSED PROJECTS

1. Determination of the origin and diversity of GLS affecting perennial ryegrass and kikuyugrass in California. Since the disease was only recently detected on these turf types in California, it is important to understand where the pathogen originated from and the similarity of California populations to others in the U.S.

Additionally, the similarity between isolates from perennial ryegrass, kikuyugrass and St. Augstinegrass in California as well as other weedy hosts such as crabgrass must be determined to know if infections on one turf type pose a threat to neighboring turf types. Finally, the information generated will help determine if the pathogen was imported into the state, or if new strains spontaneously arose within California to cause the recent epidemics.

2. Host-specificity of GLS strains to perennial ryegrass and kikuyugrass. The ability of strains of GLS to cross infect different turf types has been demonstrated for some strains. There is no information available for the cross-infectivity of GLS strains from perennial rye to kikuyugrass or vice-versa. Additionally, there is no evidence that St. Augustinegrass strains infect perennial ryegrass, but it is unknown at this time if they attack kikuyugrass. Understanding the host range of the strains present in California will help superintendents better assess the risk of GLS outbreaks on their specific turf types.

3. Temperature and leaf wetness requirements for infection of kikuyugrass. The environmental conditions required for infection of kikuyugrass are unknown at this time. Understanding these requirements would help superintendents predict the outbreak of epidemics and allow for the best timing of fungicide applications for control.

4. Temperature and leaf wetness requirements for infection of perennial ryegrass seedlings. Although known that newly planted perennial ryegrass is much more susceptible to GLS, there is little data available with regard to the susceptibility of ryegrass seedlings to the disease. Many of the courses in California overseed warm season turf fairways in the fall during periods that can be conducive for the development of the disease, thus the susceptibility of these seedlings must be investigated.

5. Determination of the risk of resistance development for GLS to QoI, benzimidazole and sterol biosynthesis inhibitor fungicides. Of the fungicides available for GLS control, three classes have single site mode of action and are susceptible to resistance development. Already, QoI (azoxystrobin [Heritage], trifloxystrobin [Compass], and pyraclostrobin [Insignia]) resistance has been detected in nine states in the eastern and Midwestern U.S.. (Uddin 2003, Kim 2003). Three courses in California have already reported that azoxystrobin was ineffective in stopping GLS epidemics that developed this year. Resistance to all three fungicides has not yet been examined in California. The presence of resistance would greatly hinder the ability of superintendents to control the disease and must be addressed.

METHODOLOGY

1. Isolates of GLS will be collected from both perennial ryegrass and kikuyugrass on courses from courses located in different geographic areas of California. GLS was diagnosed by Drs. Stowell and Wong from 22 locations ranging from the Sierra foothills and San Francisco Bay area in northern California to the southern California coast and inland valleys. Ten to twenty isolates would be collected from each location. Molecular methods will be employed to determine the lineage and diversity of California populations (Viji 2001) and genetic information will be compared with that of strains from other geographic locations (such as the mid-West, mid-Atlantic and New England states) known graminaceous and weed hosts.

2. A subset of California isolates collected from (1) will be tested for their pathogenicity on perennial ryegrass, kikuyugrass and St. Augustinegrass in glasshouse assays. Previous work (Uddin 2003b) indicates that perennial ryegrass strains do cross infect fescue, but there is no data available on their ability to infect kikuyugrass or vice versa. Also, it is not known if St. Augustinegrass strains are able to infect kikuyugrass or not.

3. The temperature and leaf wetness requirements for kikuyugrass infection will be investigated by testing the ability of the fungus to cause disease at four temperatures (68, 75, 82 and 90ºF) and 12 leaf wetness durations (3 to 36 hrs) in laboratory assays using the methodologies described by Uddin *et. al.* (Uddin 2003a). Kikuyugrass will be grown in the glasshouse and infected with a spore suspension of kikuyugrass-specific strains, incubated at the different temperature and leaf wetness regimes and the effect of both temperature and leaf wetness on disease development will be determined. Information will be used to develop a disease forecasting model specific for the development of GLS epidemics on kikuyugrass.

4. Similar to (3), newly germinated, one-, two- and four-week old perennial ryegrass seedlings will be assayed for their susceptibility to GLS infection under four temperature and 12 leaf wetness regimes. Due to the increased susceptibility of seedlings to the disease, temperature and leaf wetness optima may be broader than those defined for mature turf. The information gathered will be used to identify critical periods for GLS infection on newly overseeded perennial ryegrass.

5. Isolates collected in (1) will be tested in the laboratory for their sensitivity to fungicides representative of the three classes of site-specific fungicides used for their control: azoxystrobin (Heritage, a QoI), thiophanate-methyl (Clearys 3336, a benzimidazole) and propiconazole (Banner, a sterol biosynthesis inhibitor). Included in the tests will be isolates from courses where no control from Heritage applications was reported.

EXPECTED OUTCOMES AND BENEFITS

The information obtained from this study can be put into immediate use by superintendents across the country in terms of improved forecasting of disease occurrence, timing preventive management strategies, selecting the optimal control measures and identifying fungicide resistant disease populations. Superintendents in the southwest and Hawaii who manage either kikuyugrass or overseeded perennial ryegrass will gain additional benefits through better characterization of GLS under their unique combination of environmental and turf type conditions. Additionally, the information generated by the proposed research will complement on-going field studies and fungicide efficacy testing at University of California turf research plots and field experiments to be conducted in collaboration with California superintendents. These experiments will be conducted using funding sources outside of those requested from GCSAA and state and regional GCSA chapters.

The proposed research specifically addresses two issues important to California turfgrass culture with regard to disease development on kikuyugrass and overseeded perennial ryegrass. Additionally, understanding the origin and diversity of the pathogen in California could shed light on how the disease has emerged over the last decade in the U.S. as a major pathogen of perennial ryegrass. Finally, the determination of fungicide resistance risk addresses a problem that affects all areas managing GLS which would greatly impact the ability of superintendents to successfully manage the disease.

LITERATURE CITED

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BUDGET REQUEST

	% of Time on Project	**Requested 2004/2005**
Personnel		
Post-doctoral Research Associate (Penn. State University)	50	16,000
Lab Assistant II (UC Riverside)	50	12,000
Employee Benefits (approx. 30%)		8,400
Supplies and Expenses		
DNA extraction, Gel Electrophoresis, Southern Hybridization (Penn State Univ.)		5,000
Glasshouse experiments - pots, plants, etc. (UC Riverside)		3,000
Resistance monitoring - media, Petri plates (UC Riverside)		3,600
Travel		
Trips/Purpose/Costs		2,000
TOTAL REQUEST		**$52,000**

Budget Justification:

Personnel. One 50% FTE post-doctoral researcher to perform the molecular analyses (DNA extraction, gel electrophoresis, Southern blots), located at Penn. State University in the lab of Dr. Uddin. One 50% FTE Lab Assistant II to collect isolates, perform host-range assays, and temperature/leaf wetness assays.

Supplies and Expenses. Usual laboratory disposables such as plates, pipette tips, gel supplies, gloves etc. Reagents and kits for performing DNA extractions, gel electrophoresis and Southern blots. Costs for host plant assays. Supplies for plant maintenance such as soil, pots, trays, growth lamps. Publication costs.

Travel. Transportation and mileage costs to collect samples; travel to and from Pennsylvania and California for meetings and data analysis.

Budget Support Summary:

The proposed research will be submitted to the GCSAA Nation Research Program for funding to the California State GCSA and to regional California GCSA Chapters (San Diego, Hi-Lo, Southern California, Central Valley, Sierra Nevada and Northern California) for the Chapter Cooperative Research Programs.