

**2007 GCSA
Chapter Cooperative Research Program**

Project title: Management and Biology of Brown Ring Patch on Annual Bluegrass Greens

Total Funding Request: \$62,000 over 2 years from the GCSA Environmental Institute for Golf, California State GCSA and 6 Regional California GCSA Chapters

Abstract

Brown Ring Patch can cause severe damage to high value turfgrass plantings throughout the western, midwestern and northeastern U.S. The causal agent, *Waitea circinata* var *circinata* is a new, invasive pathogen of turfgrass in the U.S. Control of the pathogen has been erratic, and fungicide resistant isolates of the pathogen have been found in California populations. Little is known about its biology and there are no established guidelines for the cultural or biological management of this pathogen. This study will examine its population structure using amplified fragment length polymorphism (AFLP) analysis, the genetic basis of fungicide resistance, the most effective fungicide programs for control of the disease and determine the effects of nitrogen fertility and applications of fungicides and plant growth regulators on the development of the disease under field conditions. Understanding the population structure of this pathogen may help determine the origin and possible mode of spread for the pathogen. Determining the mechanisms of fungicide resistance and results from field trials evaluating the effect of fungicides, plant growth regulators and fertility on disease development will help establish effective management guidelines.

Project Team

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Project Rationale

Waitea circinata var *circinata* was recently discovered as a new, invasive pathogen of turfgrass in the U.S. affecting high value golf course putting greens (de la Cerda et al. 2007, Chen et al. 2007). Previously only found as a turfgrass pathogen in Japan causing “Brown Ring Patch” (Toda et al. 2005), it was only first detected by the Turf Disease Diagnostic Lab at UC Riverside from golf courses in Washington and California beginning in 2003. Since then, it has been detected at over 50 locations in California, Oregon, Washington and Nevada and most recently in Illinois, Ohio, Pennsylvania, New York, New Jersey, Connecticut, Maine, Rhode Island and Massachusetts (Figure 1).



Figure 1. Distribution of Brown Ring Patch in the U.S. on annual bluegrass (●) and rough bluegrass (○).

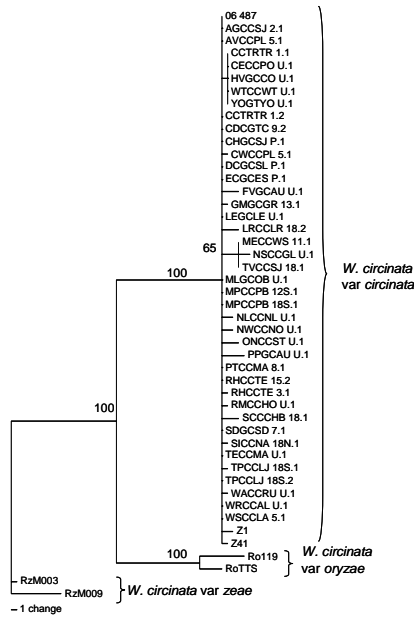


Figure 1. ITS-sequence similarity between *Waitea circinata* (*Rhizoctonia*) pathogens.

method of spread (Milgroom 1996; Milgroom and Peever 2003). Recent work in the Douhan and Wong labs has shown that the pathogen is widespread in multiple states. Analysis of the ITS-region of approximately 50 isolates has indicated that there is appreciable genetic variation (~ 3%) in this region. This amount of variation is consistent with a pathogen that has been present, yet un-noticed as a disease agent, for a long period of time. Thus, we are proposing to use AFLP markers to further explore this hypothesis since we have only looked at a single locus and AFLP markers span across the entire genome and are more appropriate markers for this kind of study. This type of analysis will also let us examine genotypic diversity across California and other states to determine if there has been any possible spread across wide geographic distances.

Practically, fungicidal control of this pathogen has been problematic. Often, repeated fungicide applications are required to halt the disease. Recent work by the Wong Lab has already identified that this pathogen is completely resistant to benzimidazole fungicides and potentially QoI-resistant isolates have already been identified from California populations (Rios et al. 2006). Laboratory trials have shown that fungicide timing is crucial for the control of the pathogen. In these tests, preventive applications of fungicides provided near complete control while



Figure 3. Symptoms of *Waitea circinata* var. *circinata* on annual bluegrass (*Poa annua*).



Figure 4. Long term damage caused by *Waitea circinata* var. *circinata* on annual bluegrass.

curative applications provided only 20 to 78% control (Wong and Kaminski 2007). The role of nitrogen in the management of this disease is unknown. Nitrogen is known to increase the severity of *R. solani* (Brown Patch) (Cubeta and Vilgalys 1997), but its effects are not well documented for other *Rhizoctonia* diseases (Couch 1995). Anecdotally, many of the locations with chronic Brown Ring Patch have been using low nitrogen fertility programs to increase ball roll and greens speed. Lack of recovery due to inadequate fertility seems to be a plausible reason for the increased severity of the disease at these locations. Also, the effect of plant growth regulators, such as Primo (trinexapac-ethyl) is unknown for Brown Ring Patch. Trinexapac-ethyl is commonly used on putting greens to reduce plant size in order to have increased ball roll on the greens surface (King et al. 1997) and its use has been observed in slowing recovery from Brown Ring Patch.

The overall objectives of this proposed multi-year study is to understand the biology and population structure of *W. circinata* var *circinata* and what management practices are most effective in controlling the disease. Based upon the results of the study, we would hope to implement sustainable best management strategies for golf course superintendents.

Benefits and Expected Results for Golf Course Superintendents

From a practical perspective, the information obtained by this study will provide tangible guidelines for effective turfgrass management practices. For a basic biology standpoint, the proposed research will allow for a better understanding of this new pathogen in the U.S. As an invasive pathogen, understanding the population structure in the U.S. will help us understand the pathogen's spread, perhaps helping to serve as a model system for the dissemination of invasive pests on golf courses.

This research is timely in nature and will provide much needed information to golf course superintendents. Due to the recent discovery of this pathogen in other parts of the U.S., this research project also provides us with the opportunity to be the first to fully understand the biology and management of this pathogen at a national level.

The tangible products that will be produced by this project include publications in *Golf Course Management*, University extension bulletins, management guidelines and information for educational presentations made at GCSA seminars. Information generated by this study will also provide suitable material for publication in scientific journals such as *Plant Disease* or *Crop Science*.

Project Objectives:

- 1) Determine the population structure of *W. circinata* var *circinata* isolates using amplified fragment length polymorphism analysis (AFLP)
- 2) Characterize the molecular basis of benzimidazole and QoI-resistance in *W. circinata* var *circinata*
- 3) Determine effective fungicide programs, based on material selection and application timing.
- 4) Examine the effects of nitrogen fertility and a plant growth regulator (trinexapac-ethyl) on disease development on annual bluegrass putting greens

Project Action Plan and Methodology:

Winter 2008

- Determine the population structure of *W. circinata* var *circinata* isolates using amplified fragment length polymorphism analysis (AFLP)
 - Over 60 isolates from California, the Midwest and Northeast have been collected and identified as *W. circinata* var *circinata*. Total genomic DNA will be extracted from isolates and digested with EcoRI and MseI restriction enzymes and specific adapter will be ligated onto the ends of the restriction fragments. Selective PCR amplification reactions will be performed with primers specific to the EcoRI and MseI adaptors that include two selective nucleotides at the 3' ends. A small population will be used to determine the most effective primer pairs that generate adequate polymorphism for AFLP analysis.
 - Population genetic structure analysis may help to elucidate the origin and spread of the pathogen. If this pathogen is truly new to California, we would predict very little genotypic diversity and spread via clonal reproduction. However, if we find substantial genotypic diversity and no population subdivision, then this may indicate that this pathogen has been present for a long time and that other factors, such as environmental changes or altered turf management practices, have resulted in the 'appearance' of this disease. Preliminary analysis is in favor of the latter prediction. Therefore, our proposed research on the effects of management practices and disease development would be highly relevant at understanding this 'new' disease. Characterizing the isolates would also allow us to use different individuals in the fungicide resistance allele characterization and in inoculation studies for disease development in the field and in the greenhouse.

Spring/Summer 2008

- Conduct fungicidal control studies. Fungicides from the QoI (azoxystrobin, pyraclostrobin) and DMI (propiconazole, tebuconazole, myclobutanil, triadimefon) classes, flutolanil, polyoxin-D, and fludioxonil will be used in field studies to determine the effect of fungicide application timing at sites where the disease has been problematic. Fungicide applications will be initiated at different intervals based on soil temperatures, a possible environmental predictor for outbreaks. The trials will be replicated in two locations in southern California (Riverside, San Diego) and one location in northern California (San Jose).

- Examine the effects of nitrogen fertility and a plant growth regulator (trinexapac-ethyl) on disease development on annual bluegrass putting greens
 - Experimental treatments to be implemented include nitrogen in CaNO₃ and urea forms applied at the equivalent rates of 0, 6.1, 12 and 24 kg nitrogen /ha. The effect of the plant growth regulator (PGR) trinexapac-ethyl will also be examined at the rates of 0, 24, 48 and 96 g/ha. Treatments will be applied in combination in a split-block design. Independently funded fungicide efficacy trials will be performed in conjunction with the fertility/PGR trials. Disease will be evaluated approximately two times per month from each site.
- Assemble and identify additional *W. circinata* var *circinata* isolates from locations in California and other U.S. States through the diagnostic lab to use in additional AFLP studies.

Fall 2008/Winter 2009

- Complete the AFLP characterization of *W. circinata* var *circinata* isolates and analyze the population structure.
- Characterize the molecular basis of benzimidazole and QoI-resistance in *W. circinata* var *circinata*
 - Beta tubulin genes from isolates appearing sensitive and resistant to benzimidazole fungicides will be sequenced and characterized. Mutations in fungal beta tubulin are commonly associated with benzimidazole resistance in fungi. (Davidse 1986)
 - Cytochrome b genes from isolates appearing sensitive and resistant to QoI fungicides will be sequenced and characterized. Mutations in cytochrome b are commonly associated with QoI resistance in fungi. (di Rago et al. 1989)

Spring/Summer 2009

- Repeat field trials examining the effect of fungicides, fertility and plant growth regulators.

Fall 2009

- Summarize and publish project results, finish additional experiments

Project Results Dissemination Plan

Key results from this project will be disseminated through several routes including:

- Local and national GCSA meeting presentations
- Publication of results in *Golf Course Management*
- Publication in scientific journals such as *Plant Disease*, *Phytopathology* and *Crop Science*

Budget Description

The requested funds will be used to support the research efforts of one PhD level student (C. Chen) in Dr. Wong's lab by providing funds for her salary, experiment supplies and travel to research locations. Since the limit for the Chapter Cooperative Research Program is \$10,000/year and, State and Regional California Chapters have historically limited research budgets, we are only requesting \$62,000 from these funding agencies. According to the proposal guidelines, the total contributions from the California State and Regional chapters will match or exceed the \$10,000 spent by the national GCSA towards funding this project. However, individual chapters are free to contribute more than the \$3,000 requested. Due to the importance of the problem, costs not covered by state and national GCSA-funding will be covered by other resources from Wong's program.

Budget Summary

<i>Expenditure Category</i>	<i>Expected Costs</i>	<i>Justification</i>
Staff Salaries	\$41,634	2 years support for a PhD student stipend and fees
Staff Benefits	\$25,727	
Supplies & Expenses	\$15,000	Consumable lab and field supplies, PCR and AFLP supplies, Petri plates, media, etc.
Travel	\$5,000	Travel to field sites and research meetings
Equipment	---	none requested
GRAND TOTAL	\$ 87,361	
REQUESTED FROM GCSA	\$62,000	

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