

**An Update on Gray Leaf Spot Research in California 2005**  
**Frank P. Wong, Ph D.**  
**Department of Plant Pathology**  
**University of California, Riverside, CA.**

**Introduction**

In 2003, the California turf industry was affected by the emergence of Gray Leaf Spot (*Pyricularia grisea*) as a new disease of perennial ryegrass and kikuyugrass. 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 (Wong 2005).

In response to the outbreaks of Gray Leaf Spot (GLS), a research proposal was submitted to the local, state and national GCSAA for funding. The cooperative research group is comprised of a mix of different academic, industry and private groups, including the labs of Dr. Frank Wong at the University of California at Riverside, and Dr. Wakar Uddin at the Pennsylvania State University, Drs. Wendy Gelernter and Larry Stowell of PACE Consulting, and Mike McCullough, Director of Turfgrass services for the Northern California Golf Association. Thirty-eight thousand five hundred dollars in total was contributed by the California and national organization to fund the project for 2004 - 2005.

This is an update on the research that has been performed so far on GLS and the status of some of the projects funded by GCSAA.

**Incidence of GLS Infections in California**

- Thirty locations had positive diagnoses (made by either UCR or PACE) for GLS in 2003
  - 21 perennial ryegrass
  - 9 kikuyugrass
  - 1 location had both
- Eleven samples were diagnosed with GLS in 2004, all were from perennial ryegrass.
- As of July 28, 2005, GLS has been diagnosed from 5 locations in California
  - Perennial Ryegrass
    - Menlo Park (July 6)
    - Oceanside (July 11)
  - Kikuyugrass
  - Costa Mesa (July 1)
    - La Jolla (July 1)
    - Los Angeles (July 1)

In both 2003 and 2004, epidemics started in the middle to late July and was associated with high temperatures and humidity, and lasted until October in some inland valleys.



**Figure 1. Location of GLS outbreaks in 2003 (left) and 2004 (right). Positive diagnoses from perennial ryegrass are in dark green, while those from kikuyugrass are in light green.**

### **GLS Specificity towards Kikuyugrass and Perennial Ryegrass**

In greenhouse assays, isolates recovered from kikuyuygrass in 2003 were found to be infective against kikuyuygrass but not perennial ryegrass. Isolates recovered from perennial ryegrass were only infective on perennial ryegrass and not kikuyugrass. It appears that kikuyugrass isolates represent a new race of the fungus that only affects kikuyuygrass, although it is highly likely that the biology and epidemiology of the new race is very similar to that of the perennial ryegrass races.

### **GLS Sensitivity to QoI-fungicides**

QoI-fungicides like Heritage, Compass and Insignia are some of the most highly effective products available for GLS control, but reports of resistance to these fungicides had been reported in the Midwest and east coast since 2000.

Seventy four isolates of GLS from 9 California locations collected in 2003 and 2004 were tested in the laboratory against azoxystrobin, the active ingredient in Heritage fungicide.

The majority of the isolates collected were highly sensitive to azoxystrobin, with the exception of some isolates from Menlo Park which showed some resistance to azoxystrobin. **(Table 1).**

**Table 1. Characterization of Isolate Sensitivity to QoI Fungicides**

Course Location	Isolates	Host <sup>a</sup>	Avg. ED <sub>50</sub> (ppm) <sup>b</sup>	Status <sup>c</sup>
Anaheim	10	PR	0.022	sensitive
Carlsbad	10	PR	0.023	sensitive
Oceanside	10	KK	0.019	sensitive
Fallbrook	10	PR	0.048	sensitive
Newport Coast	10	PR	0.027	sensitive
Irvine	5	PR	0.024	sensitive
Menlo Park	1	PR	3.429	partial resistance
	1	PR	3.544	partial resistance
	1	PR	4.200	partial resistance
	1	PR	4.500	partial resistance
	1	PR	0.029	sensitive
	1	PR	0.017	sensitive
	1	PR	0.021	sensitive
	1	PR	0.025	sensitive
	1	PR	0.019	sensitive
	1	PR	0.021	sensitive
Rancho Bernardo	10	PR	0.021	sensitive
Sensitive Average			0.027	
Resistant Average			3.918	

<sup>a</sup> PR = perennial ryegrass, KK = kikuyugrass

<sup>b</sup> sensitivity as measured by concentration at parts per million (mg/L) of fungicide needed to inhibit growth by 50%

<sup>c</sup> isolates classified as partially resistant have the F129L mutation, see below for explanation.

Those isolates recovered from Menlo Park showing reduced sensitivity to azoxystrobin were approximately 140-times less sensitive.

The Menlo Park isolates showed evidence of a F129L target site mutation, which confers partial resistance to the QoI fungicides. The G143A mutation has not yet been found in California, but has been found in resistant populations in other parts of the U.S. (Table 2, Kim et. al 2003). At the Menlo Park location, Heritage had been applied three to four times a year on fairways starting approximately in 2000. Although the number of applications required for the development of practical resistance varies, the discovery of resistant isolates in California underlines the importance of fungicide rotation and using multi-site, contact fungicides in a GLS control program.

Generally, the QoIs are fully cross-resistant to each other, thus Heritage resistant isolates are also resistant to Compass and Insignia and vice versa.

**Table 2. Typical Characteristics of F129L and G143A Mutations in GLS**

Target Site Mutation	Typical Reduction in Sensitivity	Control with high labeled rate of QoI fungicide	Documented in Isolates from

F129L	100 to 200 X “partial resistance”	low to moderate	CA, IL, KY, MD
G143A	1000 to 2000 X “complete resistance”	none	IL, KY

### Genetic Related of California Populations of GLS to those from the Eastern U.S.

Over 60 isolates of GLS from California have been sent to Wakar Uddin at Penn State University for genetic analysis to determine the identity and origin of the California populations. Genetic analysis of isolates using restriction fragment length polymorphisms has been used previously to elucidate the relatedness of isolates in prior research performed there (Figure 2, Viji et al 2003)

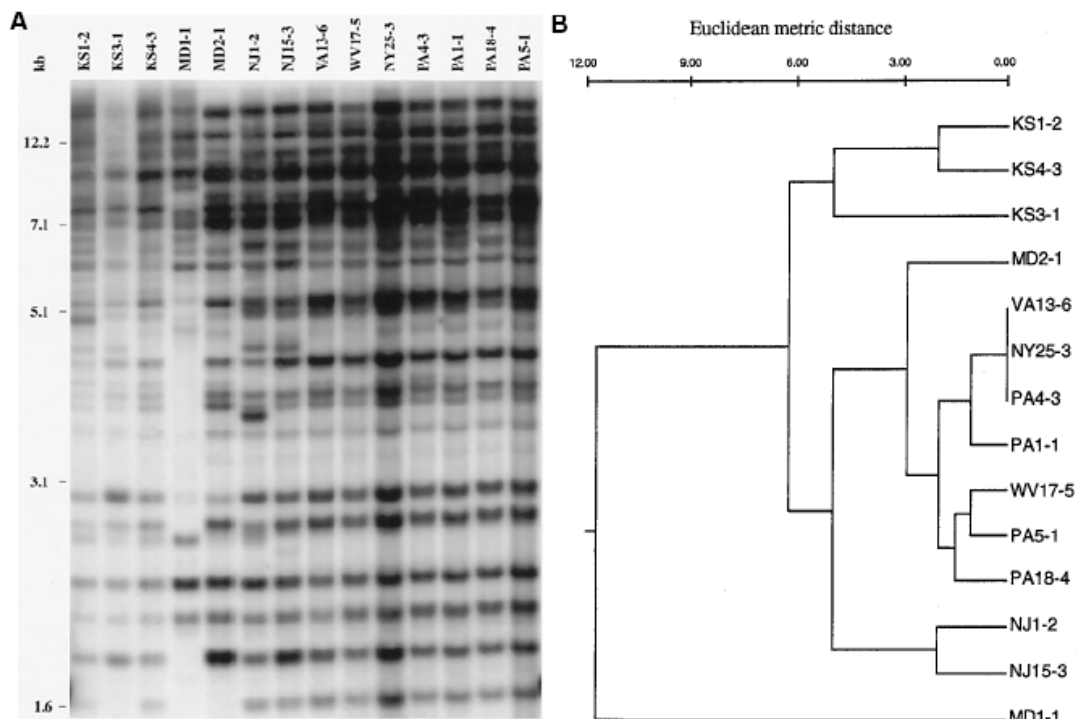


Fig. 3. Comparison of genetic similarity among *Pyricularia grisea* isolates from perennial ryegrass using Pot2 fingerprinting probe. A, Fingerprints of representative isolates from Kansas (KS), Maryland (MD), New Jersey (NJ), Virginia (VA), West Virginia (WV), New York (NY), and Pennsylvania (PA). B, Phenogram derived by UPGMA (unweighted pair group method, arithmetic mean) based on Pot2 restriction fragment length polymorphism data.

### Figure 2. DNA Fingerprinting of GLS Isolates to Determine Genetic Relatedness (excerpted from Viji et al. 2003)

#### Conclusions and Outlook

GLS appears to have established itself in California with outbreak happening in 2003, 2004 and 2005 since its initial discovery on turfgrass in California in 1999. Frequent and preventive fungicide applications are recommended for its control, starting approximately in the first week of July and lasting until September or October. Already we have seen the development of QoI resistance in one location, although performance at other locations appears to be okay.

Determining the origin of the pathogen remains a top priority and the cooperative research with Penn State University should help answer this question. More isolates from different locations are needed though and please send samples to our lab if GLS is suspect on your course.

### **Literature Cited**

Kim, Y.; Dixon, E. W.; Vincelli, P.; and Farman, M. L. 2003. Field resistance to strobilurin (QoI) fungicides in *Pyricularia grisea* caused by mutations in the mitochondrial cytochrome b gene. *Phytopathology* 93:891-900.

Mueller, K. E. 1972. Gray leaf spot of St. Augustinegrass. *California Turfgrass Culture*. 22:19-20.

Uddin, W.; Viji, G.; and Stowell, L. 2002. First report of gray leaf spot on perennial ryegrass turf in California. *Plant Disease* 86:75.

Viji, G.; Wu, B.; Kang, S.; Uddin, W.; Huff, D. R. 2001. *Pyricularia grisea* causing gray leaf spot of perennial ryegrass turf: Population structure and host specificity. *Plant Disease* 85:817-826.

Wong, F. P. , Gelernter, W., and Stowell, L. 2005. First Report of *Pyricularia grisea* Causing Gray Leaf Spot on Kikuyugrass (*Pennisetum clandestinum*) in the United States. *Plant Dis.* 89:433.