Gray Leaf Spot of Kikuyugrass: An Invasive Pest of an Invasive Pest

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California Invasive Plant Council Symposium
September 20-21, 2007
San Diego, CA
Introduction: Kikuyugrass

- *Pennisetum clandestinum*
- C-4/warm season grass
- Optimal growth is 16-32°C (60-90°F)
- Invasive weed in urban landscapes
- Classified as a federal noxious weed

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Introduction: Kikuyugrass

- Native to Central / East Africa
- Introduced as an erosion control grass in California in 1918
- Established throughout:
  - central coast
  - southern coast & inland valleys
  - Hawaii/Mexico

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- Very invasive in urban settings
- Stoloniferous growth
- Good shade, cold and heat tolerance
- Common in landscapes, parks, sports turf plantings
Grey Leaf Spot (Pyricularia grisea) on Perennial Ryegrass (Lolium perenne)
Vincelli, Uddin and Viji, *Plant Disease*
Rice Blast (Pyricularia grisea)
Gray Leaf Spot on St. Augustinegrass
Gray Leaf Spot on Perennial Ryegrass
Newport Coast, CA 2003
GLS on Kikuyugrass (*Pennisetum clandestinum*)
GLS on kikuyugrass, Huntington Beach 2006
Grasses inoculated with isolate OSGC-1 (KK)
Current Management Issues for Gray Leaf Spot

- Due to the damage potential on sports turf – the disease is heavily managed from July to October
  - It has not yet been a major issue on non-sports turf plantings
- Reduced nitrogen in summer months
- Water use management
- Regular fungicide applications
  - $150 to 600 per acre
  - 30 – 80 acres
  - 4 to 6 applications ($18,000 - $288,000)
- QoI-fungicide resistance has already developed at several locations within 2 years of use
Geographic Distribution of Gray Leaf Spot in the West

- Gray Leaf Spot has been diagnosed from > 75 locations in California and Nevada since 2003
- Perennial ryegrass
- Kikuyugrass
- St. Augustine
Questions & Objectives

• Where did the kikuyugrass populations of *Pyricularia grisea* originate?
• How closely are kikuyugrass populations related to populations from other hosts?

• Determine the genetic structure of *P. grisea* populations from the western U.S.
• Compare *P. grisea* isolates from populations in the western and eastern U.S.
Genetic Characterization of Pyricularia grisea Populations from Turfgrass

- Restriction Fragment Length Polymorphism (RFLP) analysis
  ▪ (Viji et al 2001, Farman 2001)
- Amplified Fragment Length Polymorphism (AFLP) analysis
  ▪ (Tredway 2005)
- Mating type idiomorph distribution
  ▪ (Tredway 2003)
**P. grisea Populations**

- Populations from 17 locations collected in 2006
  - 8 perennial ryegrass
  - 6 kikuyugrass
  - 3 St. Augustine
# P. grisea Populations

**Single Spore Isolate Origins**

<table>
<thead>
<tr>
<th>Collection Date</th>
<th>Population</th>
<th>City</th>
<th>Host</th>
<th>Isolates</th>
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<tr>
<td>7/7/2006</td>
<td>TIPIOS</td>
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<tr>
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Mating Type Idiomorph Distribution

- Mat 1-1 and Mat 1-2 specific primers were used to amplify mating type idiomorphs from extracted genomic DNA (Tredway 2003)
  - Mat 1-1 (552 bp)
    - L1 5-ATGAGAGCCTCATCAACGGCAACG-3
    - L2 5-ACAGGATGTAGGCATTGCAGGAC-3
  - Mat 1-2 (390 bp)
    - T1 5-ACAAGGCAACCATCTTGGACCCTG-3
    - T2 5-CCAAAACACCGAGTGCCATCAAGC-3
- Products visualized by agarose gel electrophoresis and ethidium bromide staining
GLS Mating Types

Mat 1-1

Mat 1-2

Kikuyugrass

Perennial Rye
### Mating Type Distribution

<table>
<thead>
<tr>
<th>Host</th>
<th>Mat 1-1</th>
<th>Mat 1-2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ryegrass (Lolium perenne)</td>
<td>0</td>
<td>149</td>
<td>149</td>
</tr>
<tr>
<td>St. Augustinegrass (Stenotaphrum secundatum)</td>
<td>24</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Kikuyugrass (Pennisetum clandestinum)</td>
<td>239</td>
<td>22</td>
<td>261</td>
</tr>
<tr>
<td>Rice (Oryzae sativa)</td>
<td>180</td>
<td>0</td>
<td>180</td>
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## Mating Type Distribution: Kikuyugrass Populations

<table>
<thead>
<tr>
<th>Host</th>
<th>Mat 1-1</th>
<th>Mat 1-2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBCCNB</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>SCCCHB</td>
<td>55</td>
<td>16 (23%)</td>
<td>71</td>
</tr>
<tr>
<td>HCCCLA</td>
<td>87</td>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>BPGCSD</td>
<td>83</td>
<td>6 (7%)</td>
<td>89</td>
</tr>
<tr>
<td>CCCCRI</td>
<td>22</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>HAGCLH</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Mating type assays

- Perithecia have been produced in vitro between Mat 1-1 and Mat 1-2 kikuyu grass isolates
- Viability of ascospores is being examined
- Unique for U.S. populations of *Pyricularia grisea*
AFLP Analysis

- 100-200 ng of genomic DNA digested with *EcoRI* and *Msel*
- Double-stranded *EcoRI* and *Msel* adaptors ligated to digested DNA
- One sets of selective primers used
  - *EcoRI*-AA
  - *Msel*-CA (Tredway 2005)
- 37 polymorphic locations scored for each isolate
AFLP Analysis
Eastern U.S. Populations

• Penn State (W. Uddin)
  ▪ Perennial ryegrass – 19 isolates
  ▪ Kansas, Maryland, New Jersey, Virginia, West Virginia, New York, Pennsylvania

• North Carolina State Univ. (L. Tredway)
  ▪ Tall fescue (*Festuca arundinacea*) – 10
  ▪ Weeping lovegrass (*Eragrostis curvula*) – 2
  ▪ St. Augustinegrass - 3
Perennial Ryegrass vs Kikuyugrass
Cluster analysis of genetic distances using unweighted pair-group method (UPGMA); bootstrap values based on 1000 reps.
Summary of Results

- 434 isolates of *P. grisea* were analyzed
- Mat 1-1 and Mat 1-2 mating type idiomorphs are present in west coast populations of *Pyricularia grisea*
  - Perennial ryegrass isolates are all Mat 1-2 (149)
  - St. Augustine isolates are all Mat 1-1 (24)
  - Both are present in kikuyugrass isolates (261)
    - Mat 1-1 (92%)
    - Mat 1-2 (8%)
      - BPGCSD (7%)
      - SCCCHB (23%)
Summary of Results

• AFLP data supports separation of isolates from kikuyugrass, perennial ryegrass and St. Augustinegrass into distinct clades by host
  ▪ The kikuyugrass clade appears to have two groups
  ▪ Mat 1-1 and Mat 1-2 appear present in both kikuyugrass groups
Conclusions

- AFLP and mating type distribution data indicate *P. grisea* populations from St. Augustine and perennial ryegrass from the West are similar to those from the East.

- The kikuyugrass population appears unique:
  - AFLP analysis separates these from other clades.
  - Host specificity for infection.
  - The presence of both mating types and higher diversity suggests the possibility of sexual recombination/reproduction in these populations.
  - Alternately – the diversity could be a result of host diversity.
    - Only a few kikuyugrass genotypes have been reported using isozyme analysis (Wilen et al. 1995).
Conclusions

• Management in sports turf continues to be a problem
• The kikuyugrass populations of *P. grisea* represent a “new” lineage of the pathogen that has not been seen in the U.S. before
  ▪ Possible sexual recombination = increased diversity
  ▪ Spread to other hosts (weeds & crops)
• This pathosystem is being examined as a potential model for pathogen evolution/invasion in urban ecosystems
Acknowledgements

• GCSAA Institute for Environmental Research
• California State GCSAA
  ▪ San Diego, Northern California, Southern California, Sierra Nevada, Hi-Lo Desert GCSAs
• University of California Invasive Pests Program
• Larry Stowell & Wendy Gelernter, PACE Consulting
• Lane Tredway, North Carolina State University
• Wakar Uddin, Penn State University
• The APS Turf Working Group