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

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Introduction: Kikuyugrass

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

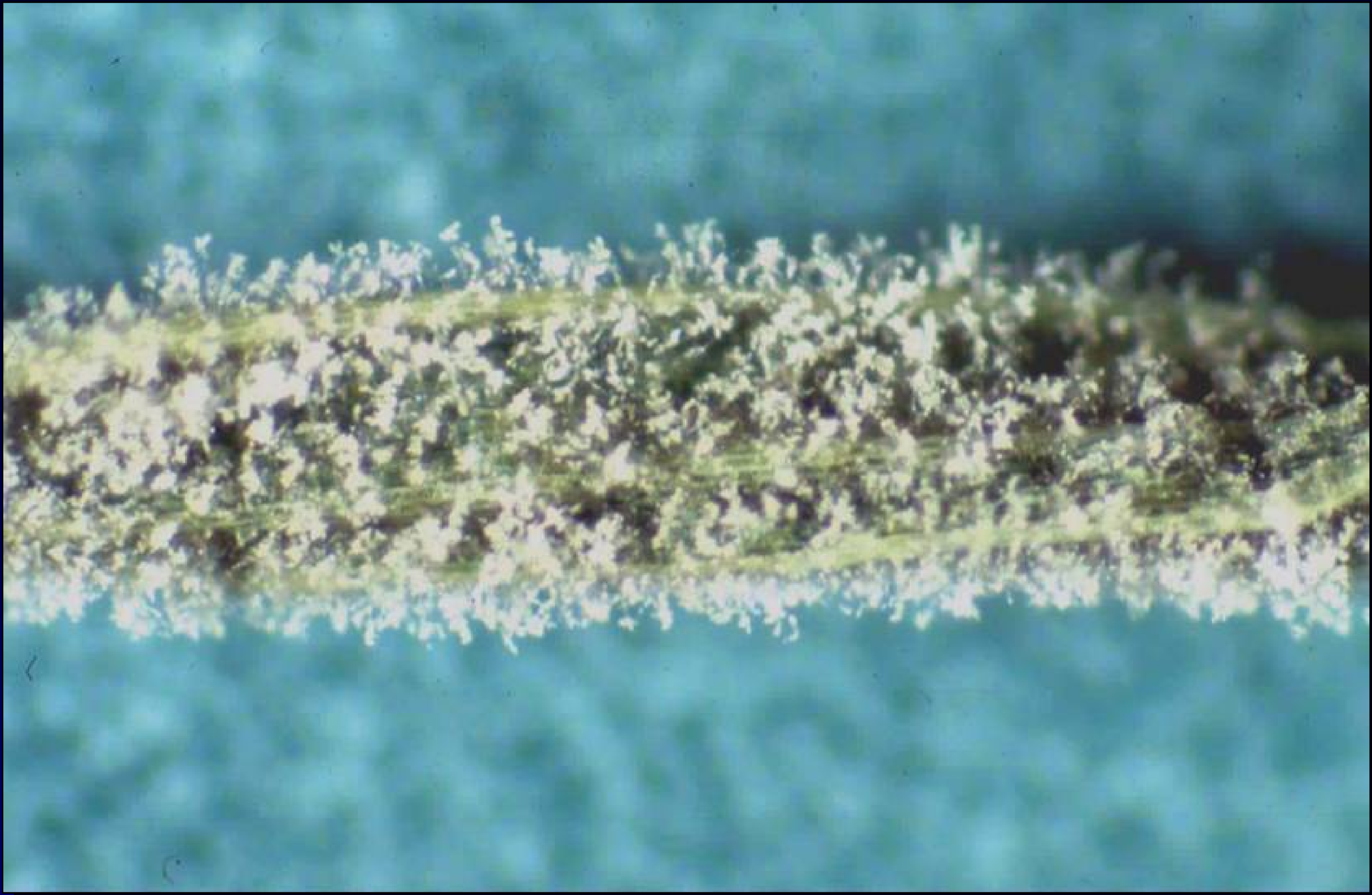
Introduction: Kikuyugrass

- 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

- 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)**

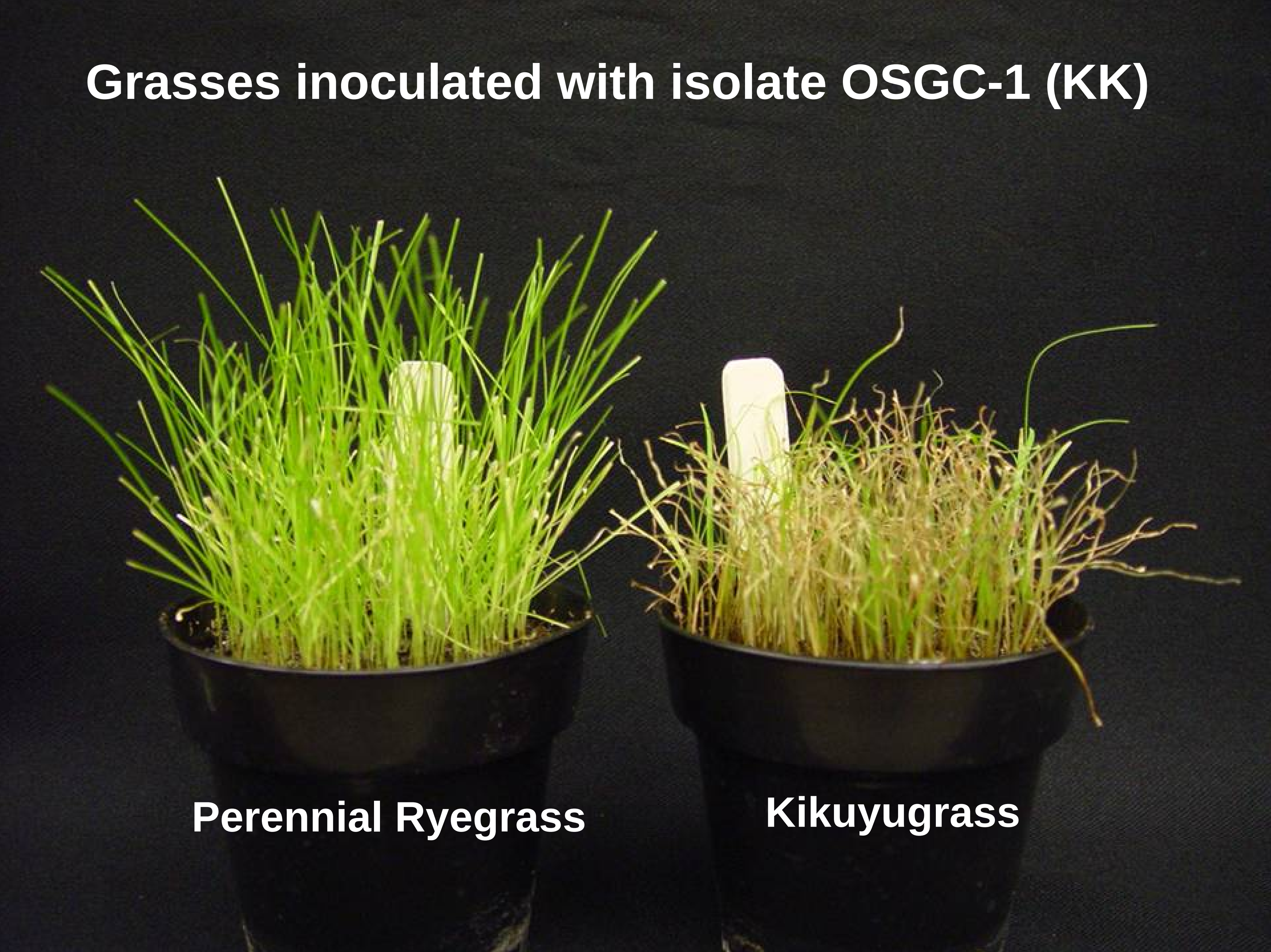

Gray Leaf Spot – Lexington KY 1999


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

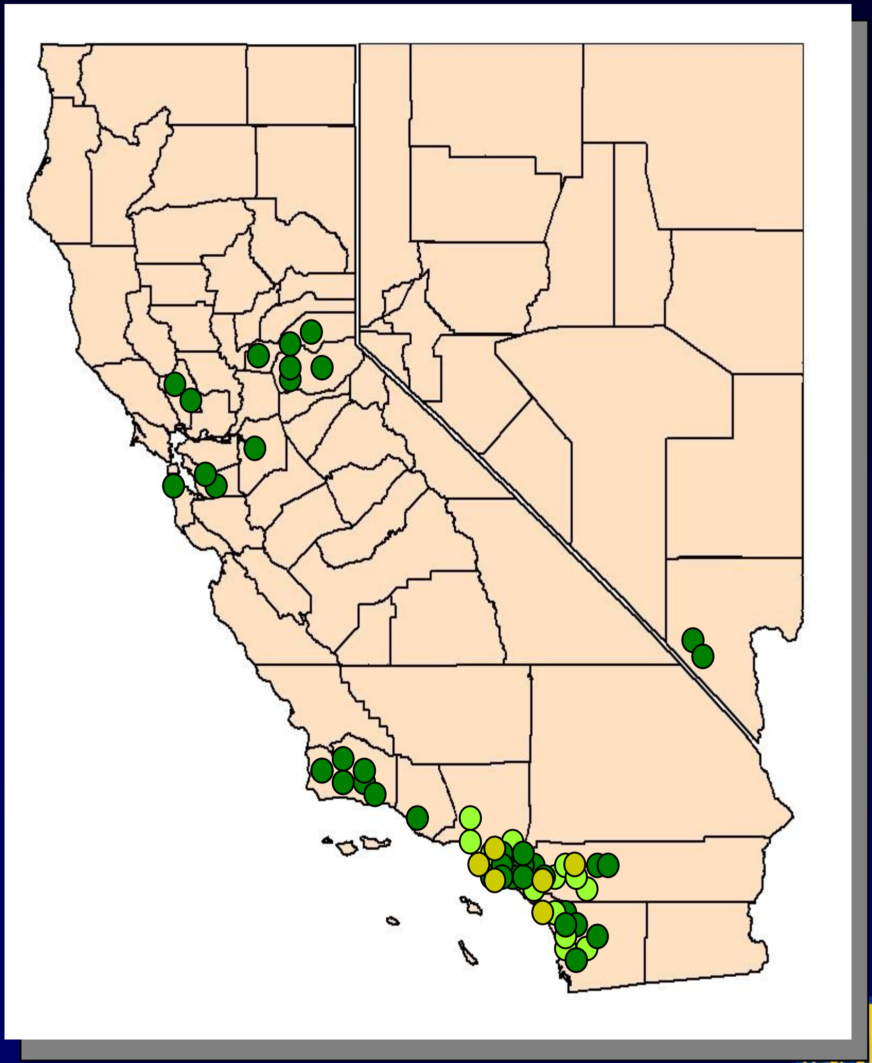

Grasses inoculated with isolate OSGC-1 (KK)
Perennial Ryegrass
Kikuyugrass

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?

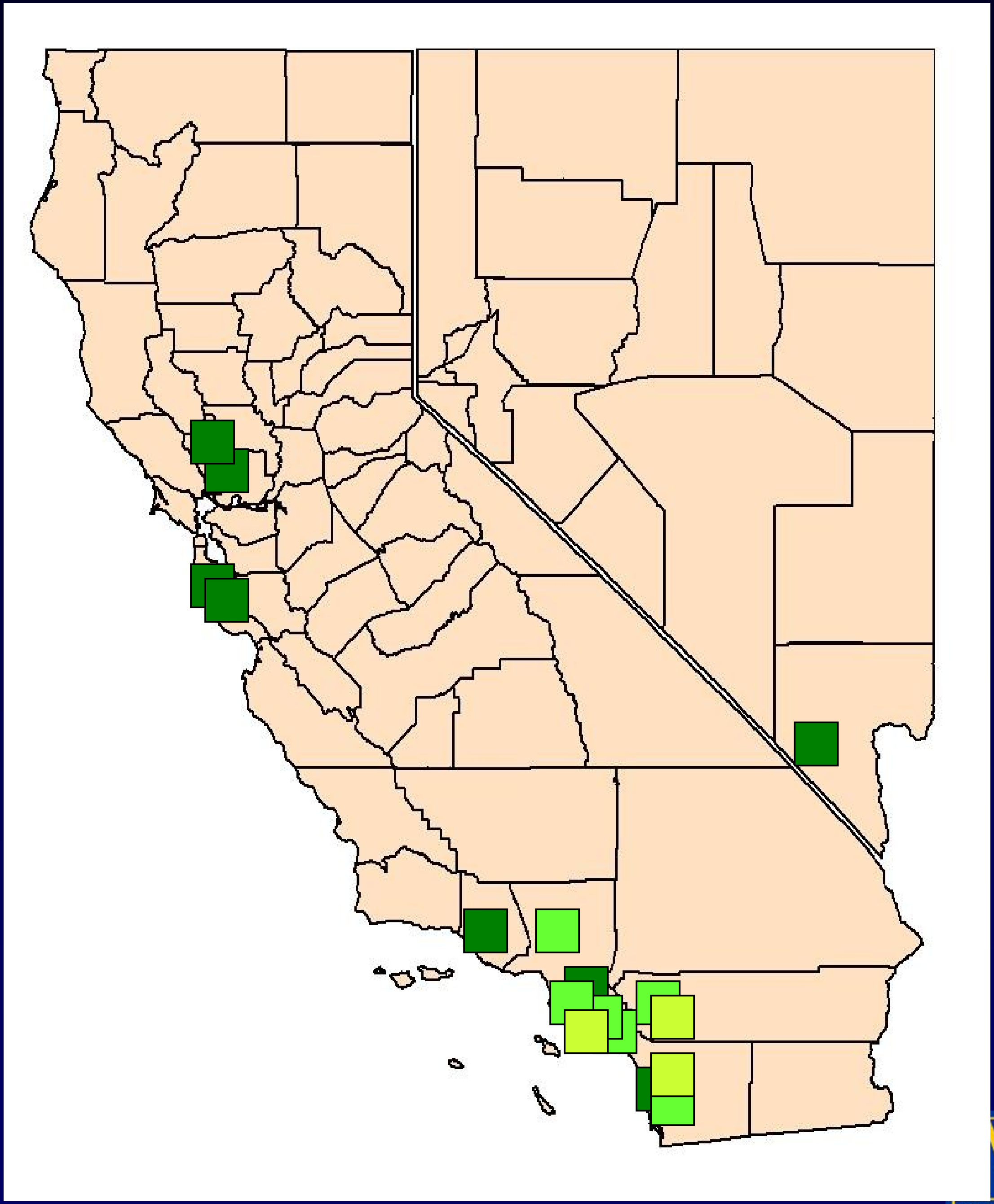
- Determine the genetic structure of *P. grisea* populations from the western U.S.
- Compare *P. grisea* isolates from populations in the western and eastern U.S.

Genetic Characterizarion 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
-



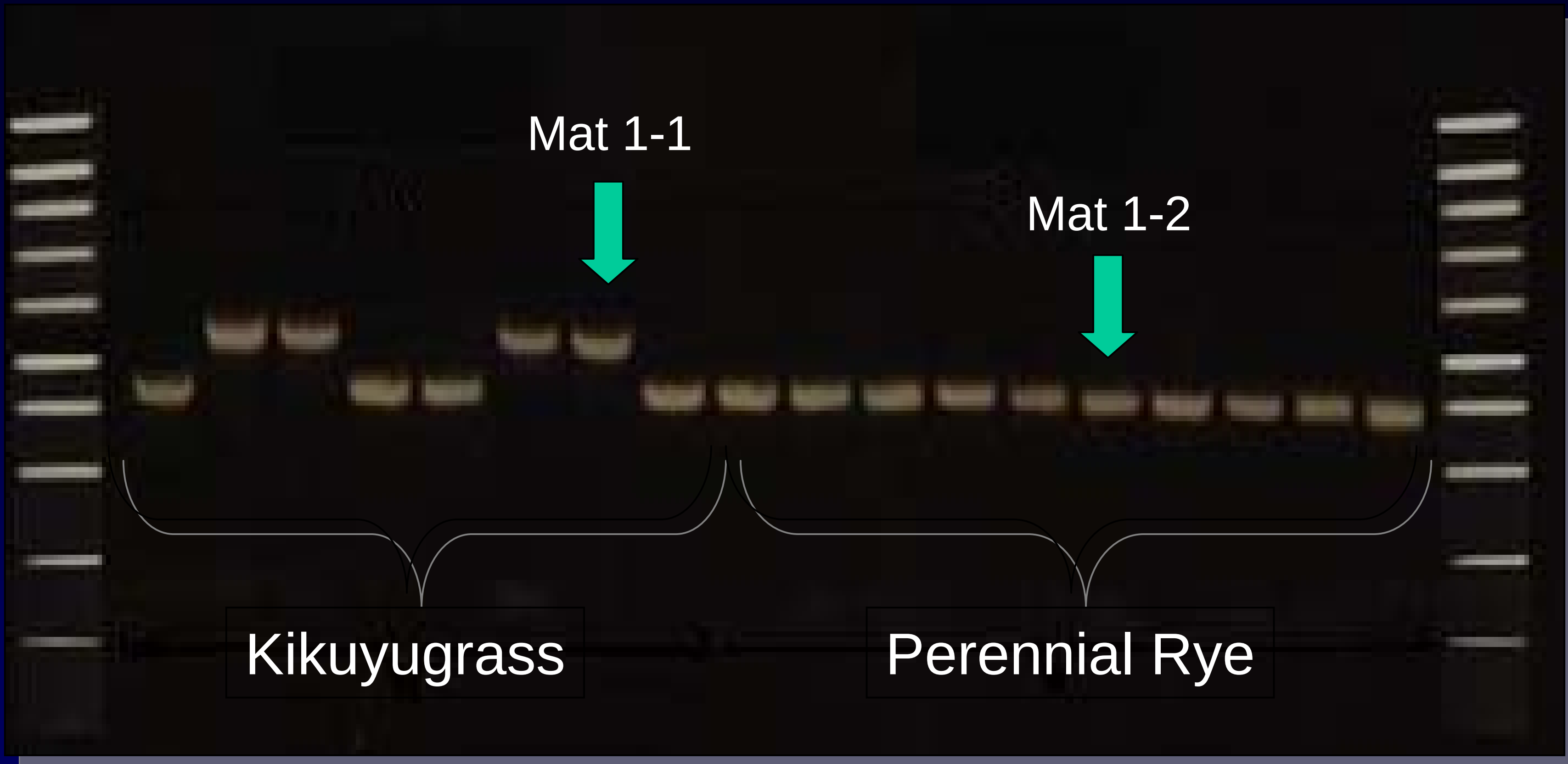
P. grisea *Populations*
Single Spore Isolate Origins

Collection Date	Population	City	Host	Isolates
7/7/2006	TIPIOS	Oceanside	perennial ryegrass	36
8/17/2006	MPCCMP	Moorpark	perennial ryegrass	10
9/6/2006	NVCCNV	Napa	perennial ryegrass	36
9/22/2006	ALCCSJ	San Jose	perennial ryegrass	21
10/6/2006	TOSTNA	Napa	perennial ryegrass	8
10/11/2006	SSGCLV	Las Vegas	perennial ryegrass	5
10/11/2006	VCOMSB	Santa Barbara	perennial ryegrass	5
10/13/2006	SHCCMP	Menlo Park	perennial ryegrass	10
10/14/2006	HAGCLH	La Habra Heights	perennial ryegrass	20
7/6/2006	NBCCNB	Newport Beach	kikuyugrass	2
8/17/2006	SCCCHB	Huntington Beach	kikuyugrass	71
8/31/2006	HCCCLA	Los Angeles	kikuyugrass	87
9/19/2006	BPGCSD	San Diego	kikuyugrass	89
9/21/2006	CCCCRI	Riverside	kikuyugrass	22
10/14/2006	HAGCLH	La Habra Heights	kikuyugrass	2
8/28/2006	SDSALS	San Diego	St. Augustinegrass	3
9/5/2006	UCI	Irvine	St. Augustinegrass	6
9/29/2006	HDMVND	Moreno Valley	St. Augustinegrass	20

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-ACAGGATGTAGGCATTCGCAGGAC-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



Mating Type Distribution

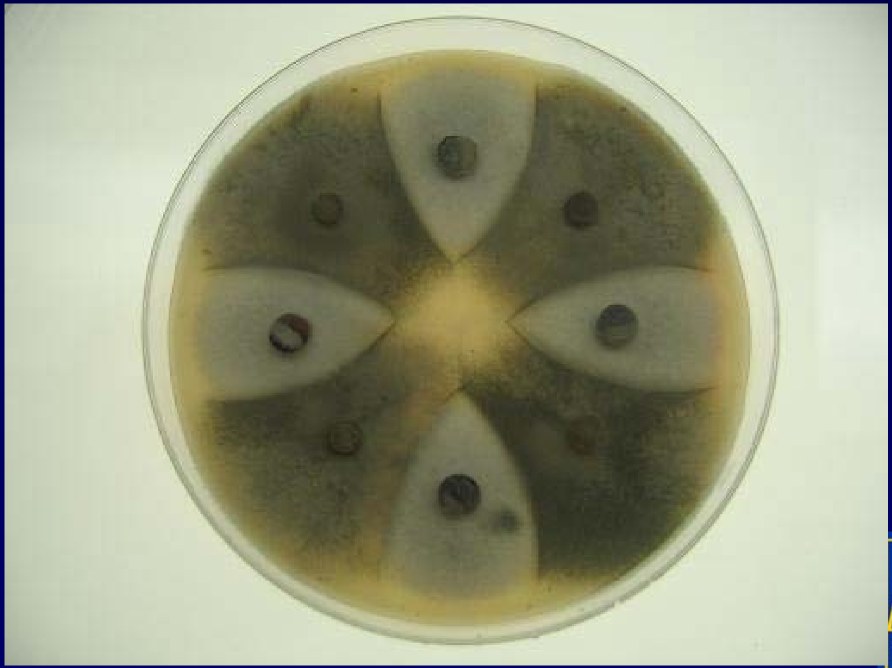
Host	Mat 1-1	Mat 1-2	Total
Ryegrass (*Lolium perenne*)	0	149	149
St. Augustinegrass (*Stenotaphrum secundatum*)	24	0	24
Kikuyugrass (*Pennisetum clandestinum*)	239	22	261
Rice (*Oryzae sativa*)	180	0	180

Mating Type Distribution: Kikyuygrass Populations

Host	Mat 1-1	Mat 1-2	Total
NBCCNB	2	0	2
SCCCHB	55	16 (23%)	71
HCCCLA	87	0	87
BPGCSD	83	6 (7%)	89
CCCCRI	22	0	22
HAGCLH	2	0	2

Mating type assays

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



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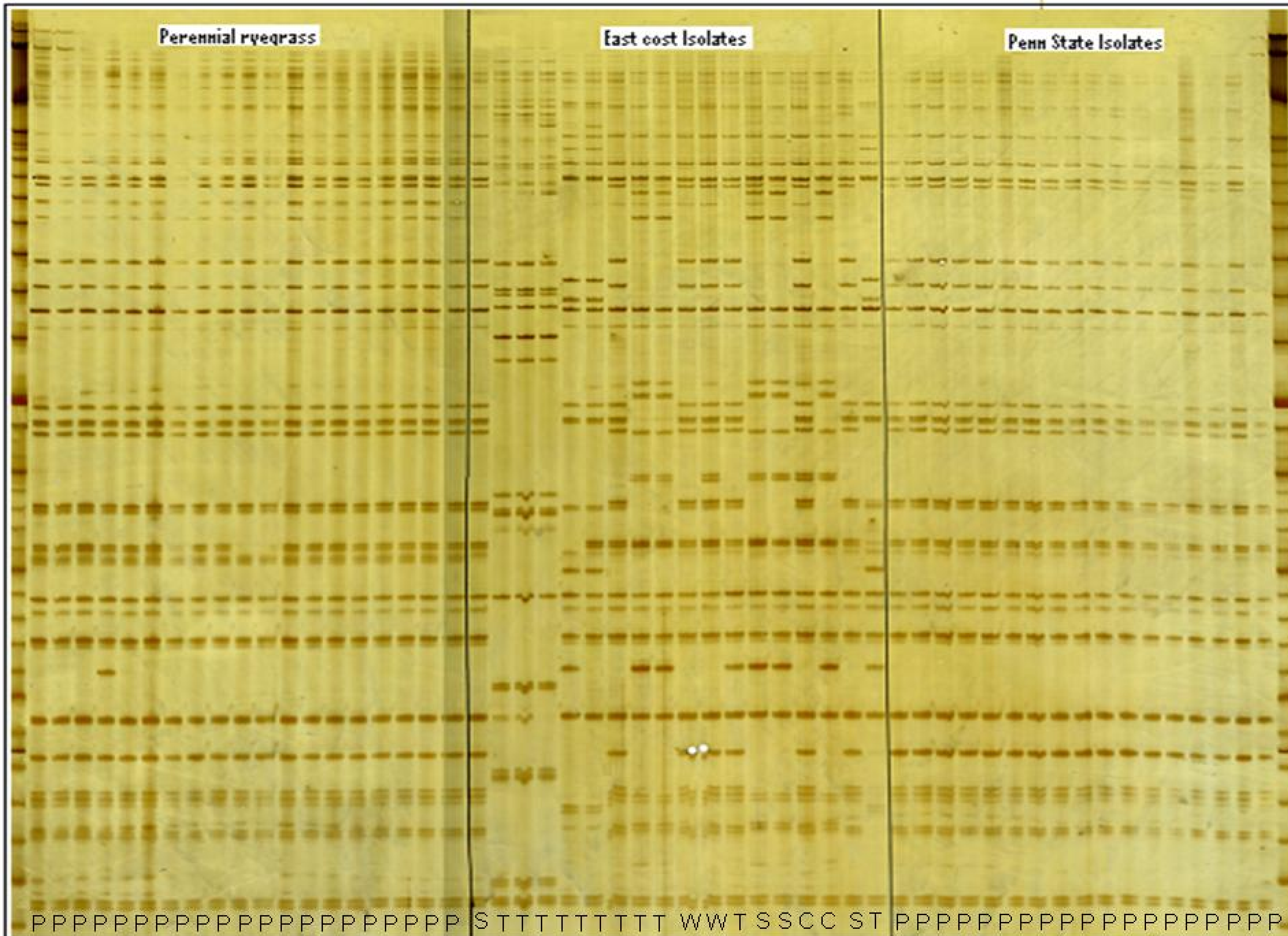
AFLP Analysis

- 100-200 ng of genomic DNA digested with *EcoR*I and *Mse*I
- Double-stranded *EcoR*I and *Mse*I adaptors ligated to digested DNA
- One sets of selective primers used
  - *EcoR*I-AA
  - *Mse*I-CA (Tredway 2005)
- 37 polymorphic locations scored for each isolate

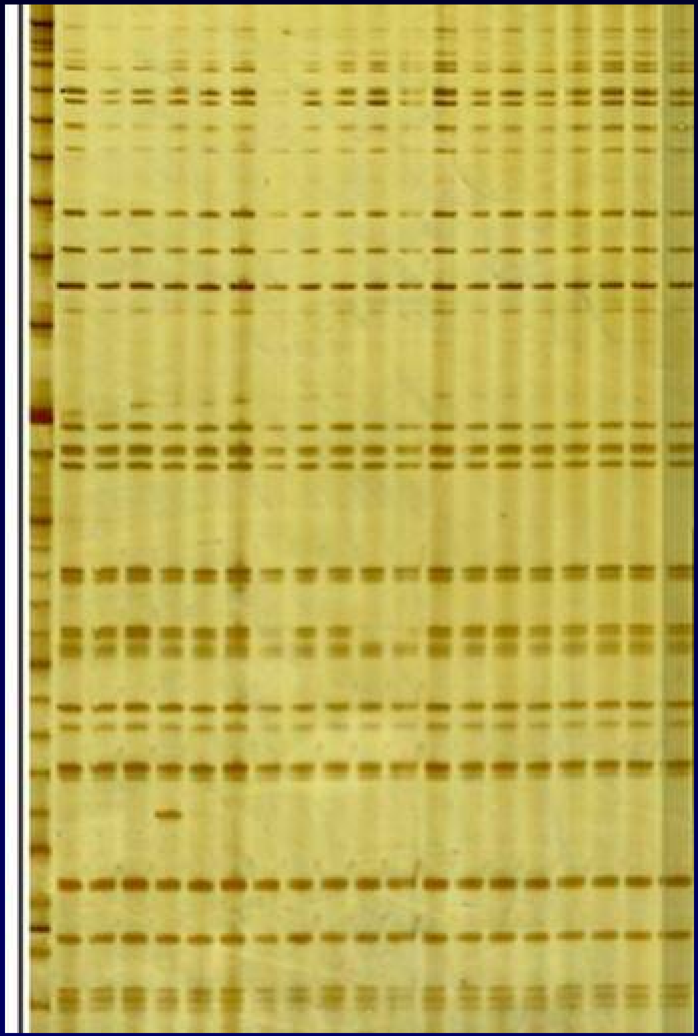
AFLP Analysis Eastern U.S. Populations

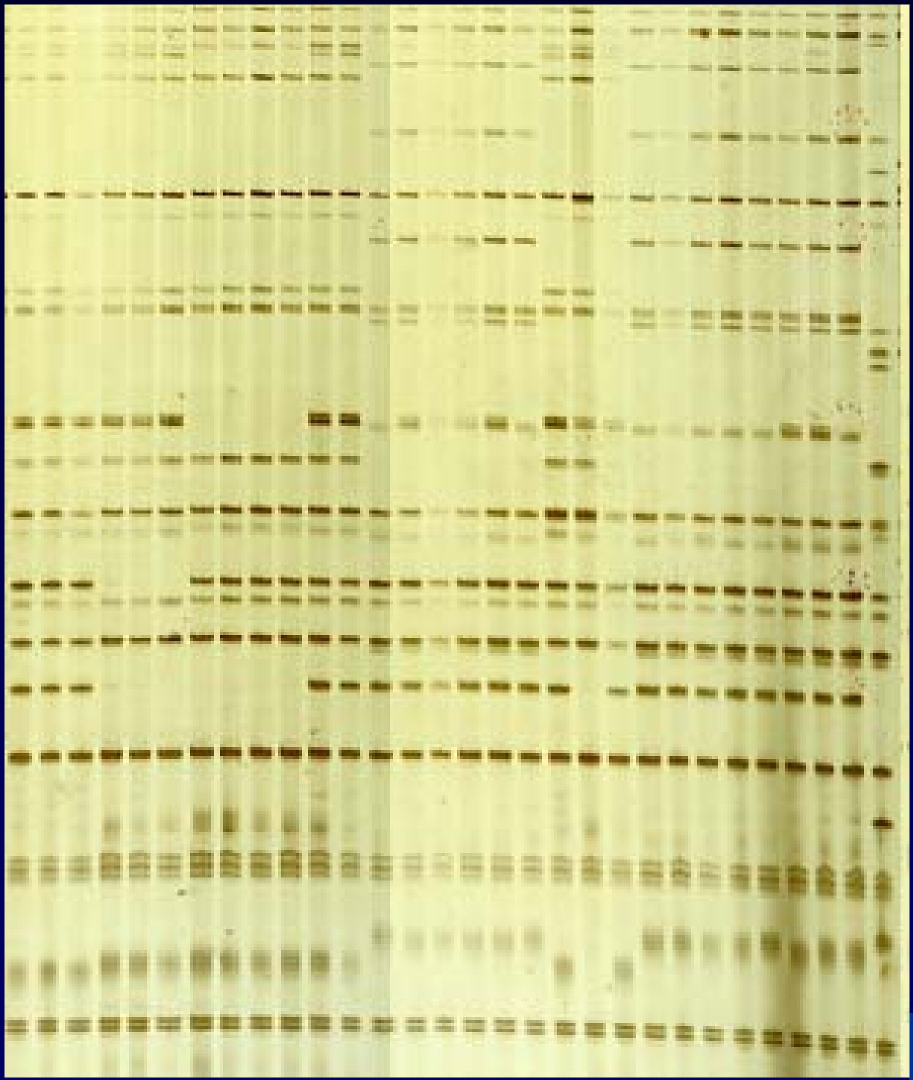
- Penn State (W. Uddin)
  - Perennial ryegrass – 19 isolates
  - Kansas, Maryland, New Jersey, Virginia, West Virginia, New York, Pennsylvania
- North Carolina State Univ. (L. Tredway)
  - Tall fescue (*Festuca arundinacea*) – 10
  - Weeping lovegrass (*Eragrostis curvula*) – 2
  - St. Augustinegrass - 3



Perennial ryegrass
East cost Isolates
Penn State Isolates
P P P P P P P P P P P P P P P P P P P P P S T T T T T T T T T WWT S S C C ST P P P P P P P P P P P P P P P P P P P

Perennial Ryegrass vs Kikuyugrass





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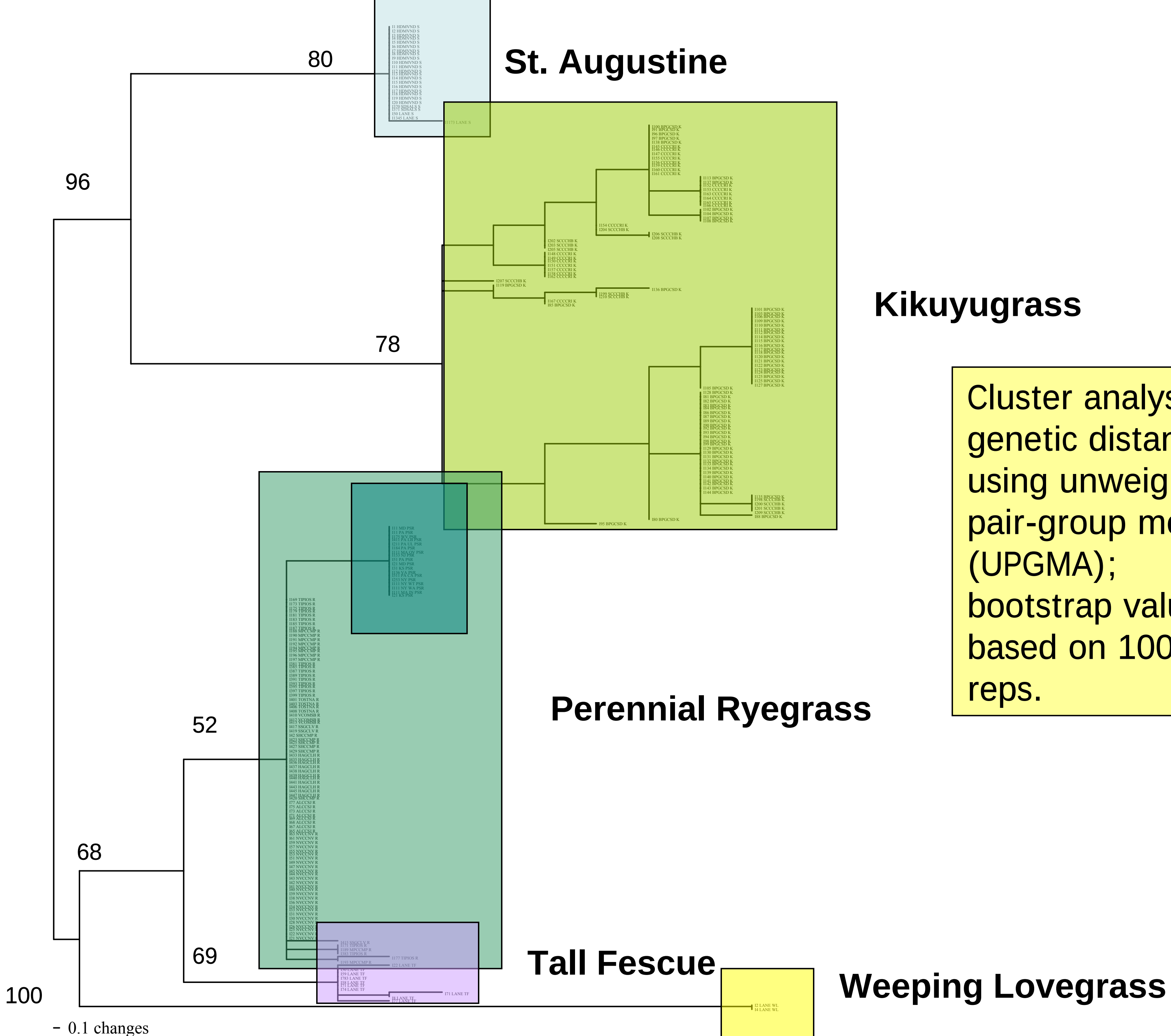
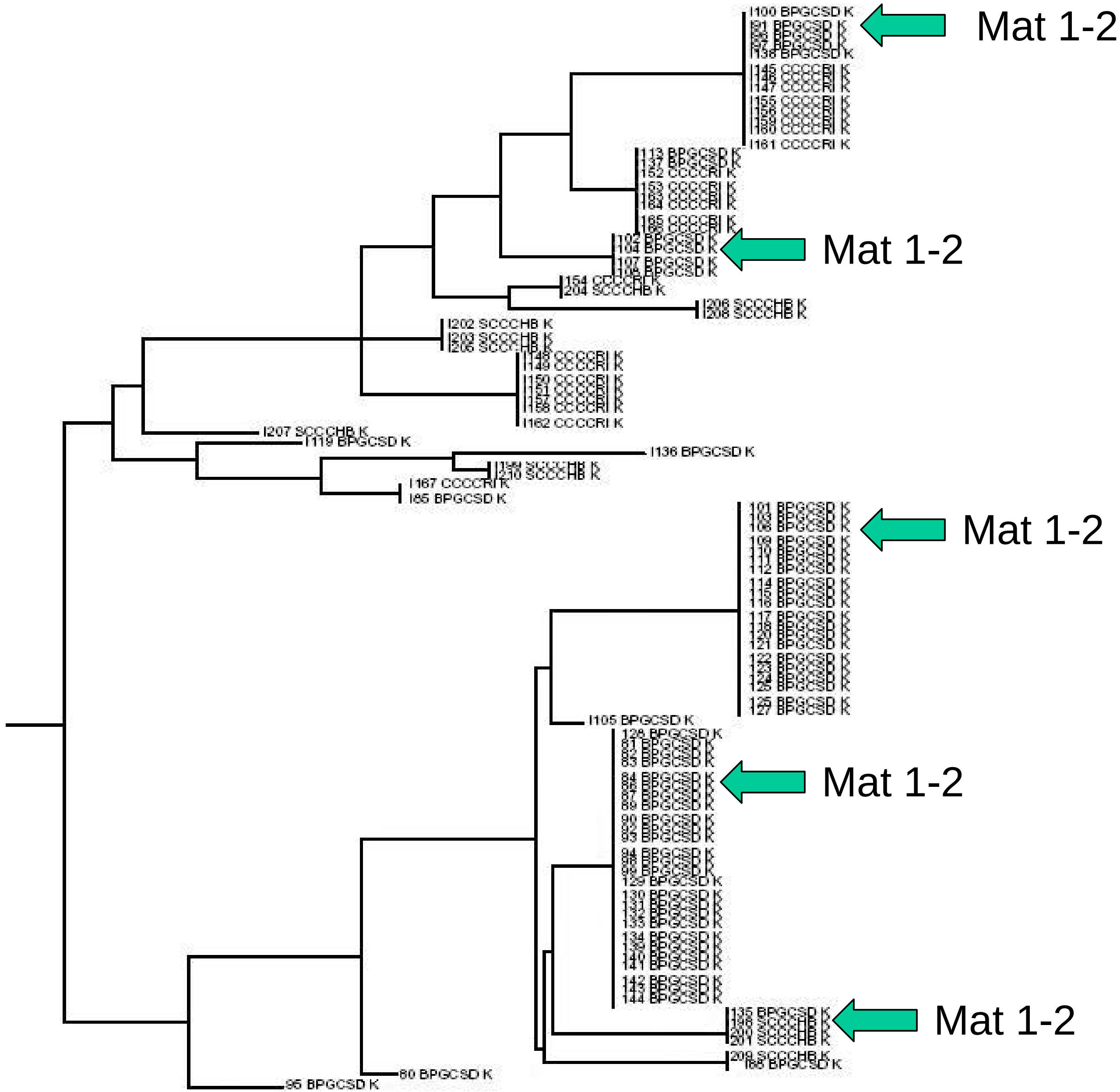


Cluster analysis of genetic distances using unweighted pair-group method (UPGMA); bootstrap values based on 1000 reps.


I100 BPGCSD K
Mat 1-2
I138 BPGCSD K
I145 CCCCRI K
I147 CCCCRI K
I155 CCCCRI K
I161 CCCCRI K
I113 BPGCSD K
I152 CCCCRI K
I153 CCCCRI K
I165 CCCCRI K
Mat 1-2
I107 BPGCSD K
I154 CCCCRI K
I204 SCCCHB K
I202 SCCCHB K
I203 SCCCHB K
I148 CCCCRI K
I150 CCCCRI K
I162 CCCCRI K
I207 SCCCHB K
I119 BPGCSD K
I136 BPGCSD K
I167 CCCCRI K
I65 BPGCSD K
Mat 1-2
I105 BPGCSD K
Mat 1-2
Mat 1-2
80 BPGCSD K
95 BPGCSD K

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

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