

# Evaluation of Soybean Germplasm Collections for Disease Reaction and Prospects for Developing Disease-Resistant Varieties

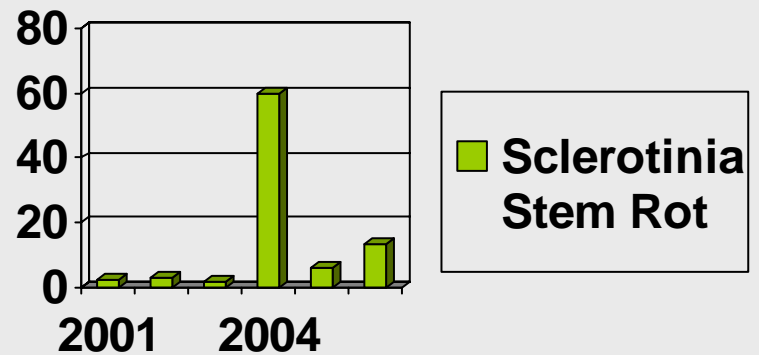
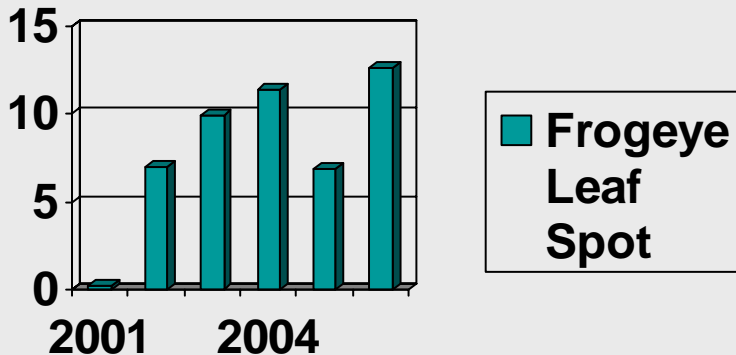
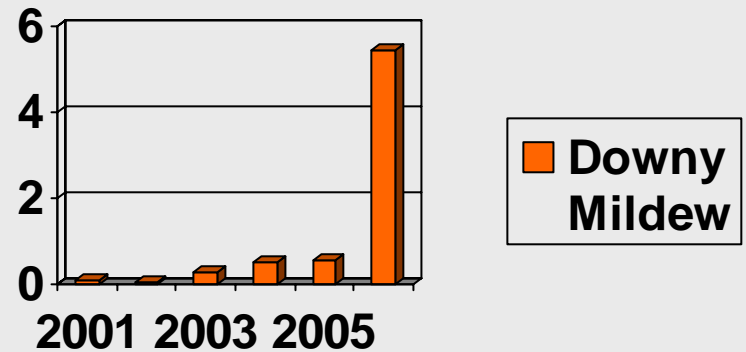
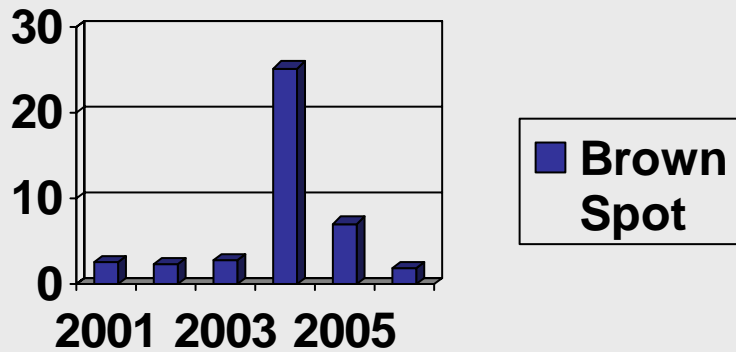


David R. Walker  
USDA-ARS

Soybean/Maize Germplasm, Pathology and Genetics  
Research Unit  
Urbana, IL

# Soybean yield losses to diseases, 2001-2006

(millions of bushels)



Estimates by the Univ. of Missouri-Columbia, Delta Res. Center  
(<http://aes.missouri.edu/delta/research/soyloss.stm>)

# Resistance to soybean rust (SBR) may or may not involve:

- Reddish-brown (RB) instead of tan lesions
- Necrosis of tissue in vicinity of lesions (HR)
- Comparatively low densities of lesions
- Slow development of disease on infected tissue
- Slow movement of disease up through canopy
- Limited sporulation from uredinia
- Reduction in viability of urediniospores released from lesions

# Challenges to evaluating resistance

- Environmental influence on epidemiology and disease development
- Stage of plant development at time of infection and time of evaluation
- 13 Maturity Groups (MGs) of soybean
- Poor germination and/or low vigor of some PIs
- Injury and lesions caused by other pests and pathogens
- Potential geographic and temporal variation among *P. pachyrhizi* isolates

# Looks can be deceiving – Example 1

## Bossier City, LA, September 2007

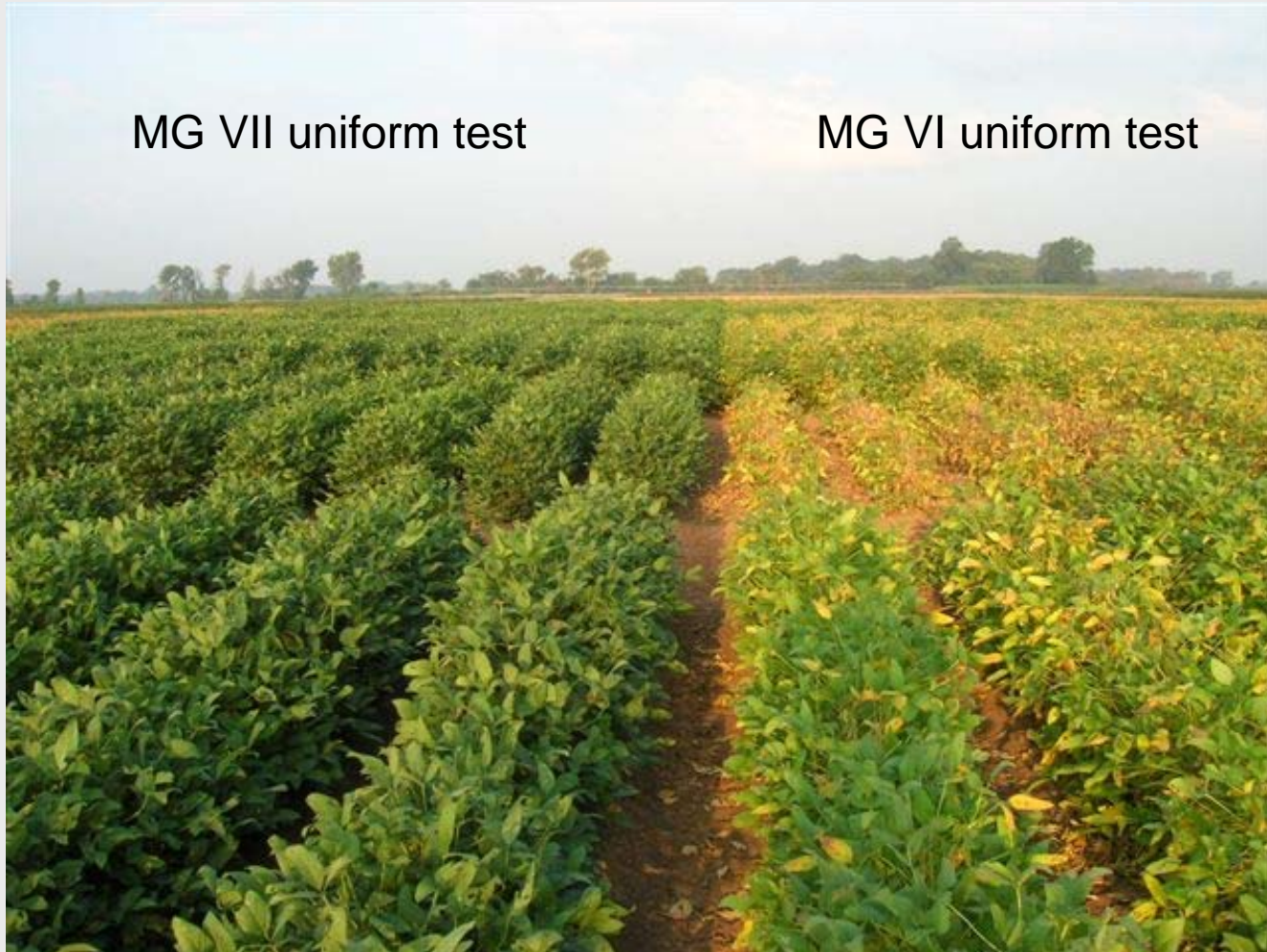
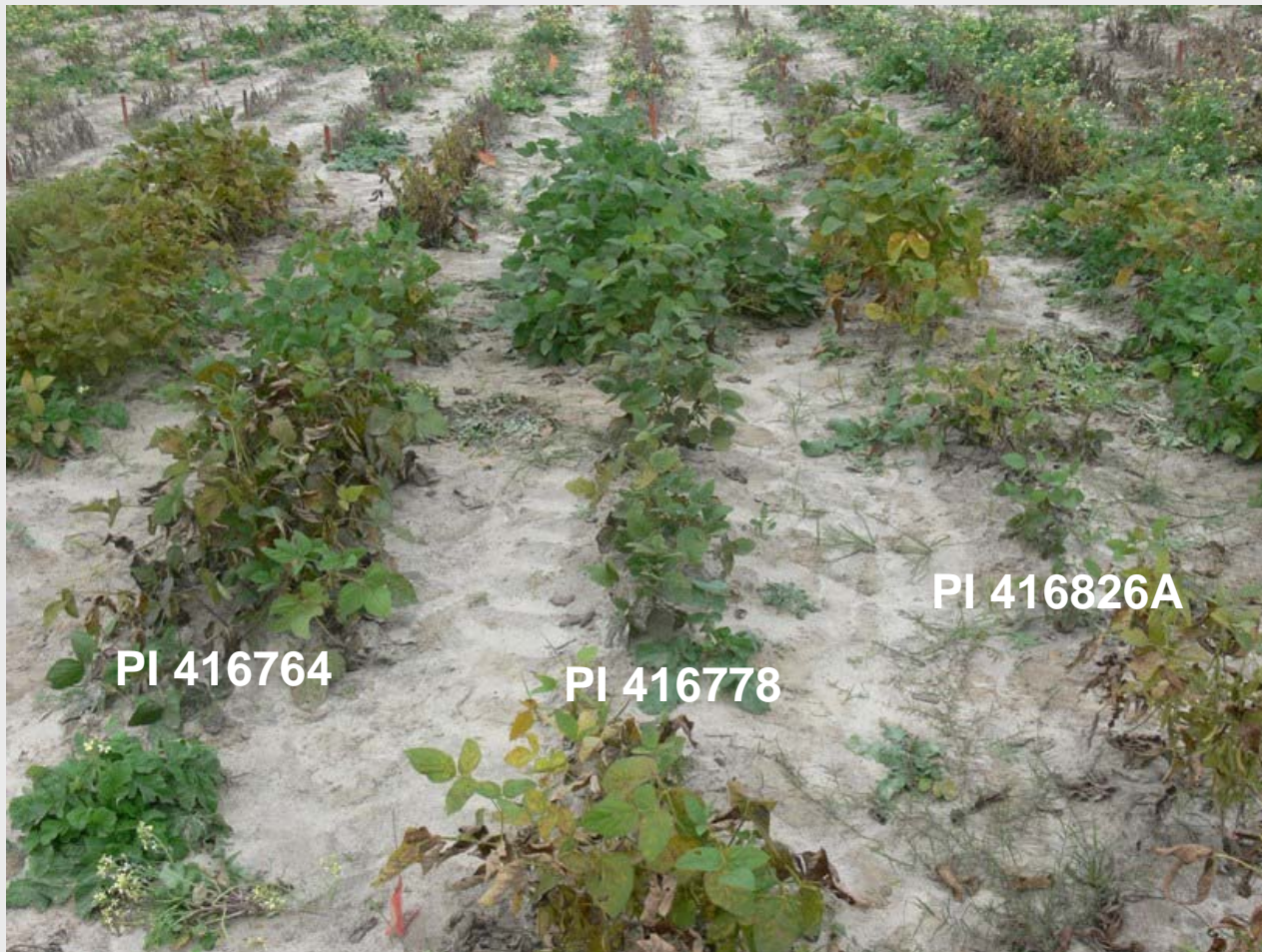


Photo courtesy of Blair Buckley, LSU

# Looks can be deceiving - Example 2

## Three accessions with resistance to SBR

Quincy, FL 30 October 2007



# Evaluations of USDA soybean germplasm collection at locations in the U.S., 2004-2007

<b>Year(s)</b>	<b>Location(s) &amp; institution(s)</b>	<b>Assay site</b>	<b>Number of PIs</b>	<b>Mat. Groups</b>
<b>2004-05</b>	<b>Ft. Detrick – First round (USDA-ARS)</b>	<b>GH</b>	<b>16,595</b>	<b>000-X</b>
	<b>Ft. Detrick – Second round</b>	<b>GH</b>	<b>3,215</b>	<b>000-X</b>
	<b>PIs w/ putative resistance</b>	<b>-</b>	<b>805</b>	<b>000-X</b>
<b>Late 2005</b>	<b>Attapulgus, GA (UGA; w/ suppl lighting)</b>	<b>Field</b>	<b>776</b>	<b>000-X</b>
<b>Early 2006</b>	<b>Griffin, GA (UGA)</b>	<b>GH</b>	<b>328</b>	<b>000-X</b>
<b>Late 2006</b>	<b>w/ suppl lights: Attapulgus, GA (UGA) Quincy, FL (ARS)</b>	<b>Field</b>	<b>426 181</b>	<b>000-X III-VIII</b>
	<b>No suppl lights: Alexandria, Baton Rouge &amp; Bossier City, LA; Fairhope, AL</b>	<b>Field</b>	<b>104-270</b>	<b>000-X; III-VIII</b>
<b>Late 2007</b>	<b>w/ suppl lights: Alexandria &amp; Baton Rouge, LA; Attapulgus, GA; Quincy, FL; Blackville, SC (Clemson)</b>	<b>Field</b>	<b>403</b>	<b>000-X</b>
	<b>No suppl lights: Bossier City, LA (LSU) &amp; Fairhope, AL (Auburn)</b>	<b>Field</b>	<b>293</b>	<b>IV-X</b>

# 2007 USDA germplasm tests



# Collaborators in 2007 evaluations

<b>Emerson Shipe &amp; John Mueller</b>	<b>Clemson U</b>	<b>Blackville, SC</b>
<b>Roger Boerma &amp; Dan Phillips</b>	<b>U of Georgia</b>	<b>Attapulugus, GA</b>
<b>David Walker &amp; Glen Hartman; David Wright &amp; Jim Marois</b>	<b>USDA-ARS or U of Florida</b>	<b>Quincy, FL</b>
<b>David Weaver</b>	<b>Auburn U</b>	<b>Fairhope, AL</b>
<b>Ray Schneider</b>	<b>LSU</b>	<b>Baton Rouge, LA</b>
<b>Steve Moore</b>	<b>LSU</b>	<b>Alexandria, LA</b>
<b>Blair Buckley</b>	<b>LSU</b>	<b>Bossier City, LA</b>
<b>Randy Nelson</b>	<b>USDA-ARS</b>	<b>Urbana, IL</b>

# Evaluation strategy

- Try to synchronize plant maturity (growth stage) with late summer/early fall rust epidemic in the Southeast
- Plant late (between late July and early September)
- Extend photoperiod using portable lighting units
- Divide germplasm accessions into tests containing PIs with similar MGs
- Devised by Roger Boerma and Dan Phillips (University of Georgia) in 2005

# Locations with supplementary lighting in 2007

- GA, SC, FL and 2 locations in LA
- 403 entries in MGs 000 to X divided into 4 tests:
  - 1. MGs 000 to I
  - 2. MGs II to IV
  - 3. MGs V to VII
  - 4. MGs VIII to X
- 26 N.A. cultivar checks (from all MGs except X)
- Two replications
- Late planting dates (mid-July to early September)
- Supplementary lighting provided for 1 month after planting to extend photoperiod



Attapulgus, GA 19 Sept 07

# Germplasm test plots with lighting units

Baton Rouge, LA

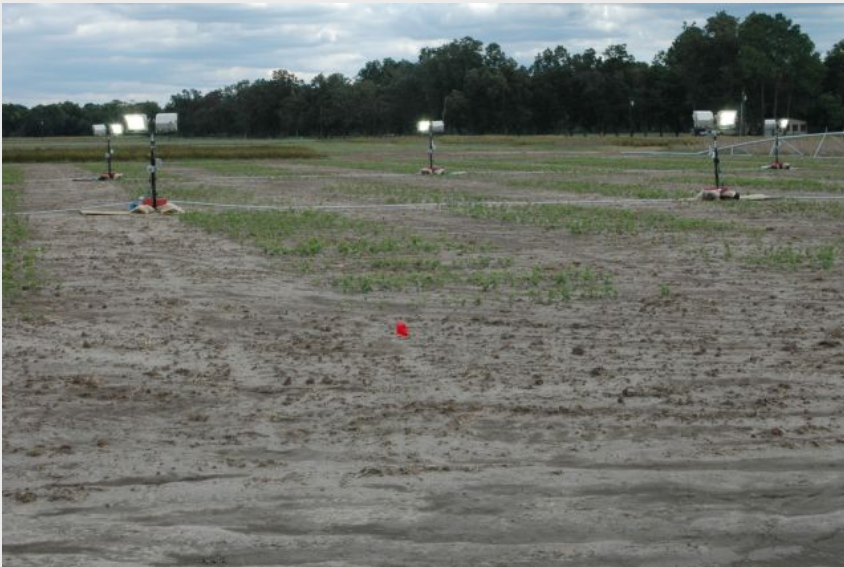


Photo courtesy of Ray Schneider

Quincy, FL



# Locations with no supplementary lighting

- Fairhope, AL and Bossier City, LA
- 293 entries in MGs IV to X
- Two replications
- Late planting dates



# 2007 evaluations in Quincy, FL

- Two replications x 446 plots planted 16-17 August
- Four tests, with earlier MGs planted closer to two light towers than later MGs
- Lights run from dusk until 10 pm for one month
- Plots inoculated once with spore suspension and grown in field adjacent to heavily infected plants



# Rating of germplasm accessions at Attapulcus, GA 2005-2006

- Plots rated on a scale of 1 to 5 (lower numbers indicate less disease)
- Involved visual evaluation of disease at the whole-plant level in the field
- 2005 ratings based on composite assessment of:
  - Disease incidence (frequency of plants with symptoms)
  - Disease severity (percent of area with lesions)
  - Disease development up through canopy
- 2006 ratings based on incidence and severity on leaf samples inspected in the field

# Evaluation of germplasm in Quincy 2007

- Complicated by heavy whitefly infestation
- Further complicated when soil pathogen complex struck plots in late September
- Soil pathogens included *Fusarium*, *Phytophthora*, *Pythium* and *Rhizoctonia*



# Switch to lesion counts and sporulation observations

- Five mature leaflets collected from each plot row and taken to lab
- Lesions counted in a 1.0 cm<sup>2</sup> area using a microscope
- Mean infection frequency calculated from the four leaves with the highest lesion densities
- Presence of sporulating or non-sporulating lesions on each leaflet was noted
- Sporulation rating (1-5) given based on number of sampled leaves with sporulating uredinia

# Five-point sporulation rating scale used at Quincy in 2007

- 1 = No sporulating lesions observed
- 2 = Sporulating lesions on a single leaflet
- 3 = Sporulating lesions on 2 leaflets or from 3-4 when average no of lesions per leaflet was  $<5.0$
- 4 = Sporulation on 3-4 leaflets
- 5 = Sporulation on all 5 leaflets



# Examples of germplasm accessions with apparent resistance

Entry	MG	Lesions per cm <sup>2</sup>	Sporulation rating (1-5)
PI 470227B	III	6.5	3.0
L85-2378 ( <i>Rpp1</i> )	III	0.1	1.0
'Williams 82'	III	>45.9	5.0
PI 476905A	V	4.6	1.0
PI 567059	V	1.2	1.0
PI 605773	V	0.8	1.0
PI 605891A	V	0.5	1.0
'5601T'	V	>34.5	4.5

## Some of the germplasm accessions with apparent resistance

Entry	MG	Lesions per cm <sup>2</sup>	Sporulation rating (1-5)
PI 417503	VI	0.0	1.0
PI 605891B	VI	22.1	1.5
'Dillon'	VI	>49.5	5.0
PI 417132	VII	14.2	1.0
'FT-2'	VII	16.8	1.5
'Benning'	VII	>41.8	5.0
PI 416826A	VIII	1.0	1.0
PI 567025A	VIII	10.8	1.5
'Prichard	VIII	>50.0	5.0

# Comparison of ratings from multiple locations in 2006-2007 (MG VI)

<b>Entry</b>	<b>Quincy 07 (Les/cm<sup>2</sup>)</b>	<b>Quincy 07 Sporul (1-5)</b>	<b>Quincy 06 Rating (1-5)</b>	<b>Attapulgus 06 Rating (1-5)</b>	<b>Alexandria 06 Rating (0-8)</b>
<b>PI 417503</b>	<b>0.0</b>	<b>1.0</b>	<b>3.5</b>	<b>2.7</b>	<b>0.5</b>
<b>PI 506965</b>	<b>&gt;45.1</b>	<b>5.0</b>	<b>4.0</b>	<b>3.3</b>	<b>5.0</b>
<b>PI 507009</b>	<b>8.8</b>	<b>1.0</b>	<b>3.0</b>	<b>4.0</b>	<b>4.0</b>
<b>PI 567190</b>	<b>4.1</b>	<b>1.0</b>	<b>3.0</b>	<b>1.8</b>	<b>3.5</b>
<b>PI 605891B</b>	<b>&gt;22.1</b>	<b>1.5</b>	<b>2.8</b>	<b>4.3</b>	<b>2.5</b>
<b>PI 615437</b>	<b>18.5</b>	<b>1.0</b>	<b>3.0</b>	<b>3.7</b>	<b>2.0</b>
<b>PI 635999</b>	<b>11.0</b>	<b>1.5</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>'Dillon'</b>	<b>&gt;49.5</b>	<b>5.0</b>	<b>5.0</b>	<b>3.0</b>	<b>8.0</b>
<b>'Musen'</b>	<b>&gt;50.0</b>	<b>5.0</b>	<b>5.0</b>	<b>4.7</b>	<b>-</b>

# Comparison of ratings from multiple locations in 2006-2007 (MG VIII)

<b>Entry</b>	<b>Quincy 07 (Les/cm<sup>2</sup>)</b>	<b>Quincy 07 Sporul (1-5)</b>	<b>Quincy 06 Rating (1-5)</b>	<b>Attapulgus 06 Rating (1-5)</b>	<b>Alexandria 06 Rating (0-8)</b>
<b>PI 416826A</b>	<b>1.0</b>	<b>1.0</b>	<b>3.0</b>	<b>2.7</b>	<b>2.5</b>
<b>PI 416873B</b>	<b>4.4</b>	<b>1.0</b>	<b>3.5</b>	<b>2.0</b>	<b>-</b>
<b>PI 462312</b>	<b>14.1</b>	<b>1.5</b>	<b>-</b>	<b>-</b>	<b>-</b>
<b>PI 506947</b>	<b>16.4</b>	<b>1.0</b>	<b>2.7</b>	<b>2.0</b>	<b>1.0</b>
<b>PI 567031B</b>	<b>4.6</b>	<b>1.0</b>	<b>2.8</b>	<b>2.3</b>	<b>0.0</b>
<b>PI 567046A</b>	<b>16.5</b>	<b>1.5</b>	<b>2.8</b>	<b>1.7</b>	<b>1.0</b>
<b>PI 567085B</b>	<b>&gt;34.2</b>	<b>4.0</b>	<b>4.3</b>	<b>2.3</b>	<b>6.2</b>
<b>'Maxcy'</b>	<b>&gt;43.8</b>	<b>5.0</b>	<b>5.0</b>	<b>2.7</b>	<b>-</b>
<b>'Prichard'</b>	<b>&gt;50.0</b>	<b>5.0</b>	<b>5.0</b>	<b>-</b>	<b>-</b>

# Example MG VIII entries – Quincy 2007

PI	Quincy 07 (Les/cm <sup>2</sup> )	Quincy 07 Sporul (1-5)	Griffin GH 06 LD / LT / Sporul	Comments
PI 200487	10.4	1.5	9.3 / RB / N	
PI 200488	7.4	1.0	- / - / -	Suscept to soil pathogens
PI 203398	13.0	1.0	8.0 / RB / N	
PI 230971	>30.3	4.5	18.7 / RB / N	R gene at <i>Rpp2</i> locus



# Reactions of lines with known SBR resistance genes

Entry	Gene	MG	Quincy 07 (Les/cm <sup>2</sup> )	Quincy 07 Sporul (1-5)	Quincy 06 Rate (1-5)	Attap 06 Rate (1-5)
LG85-2378	<i>Rpp1</i>	III	0.1	1.0	3.6	2.3
'Williams 82'	-	III	>45.9	5.0	4.9	5.0
PI 417503	?	VI	0.0	1.0	3.5	2.7
'Dillon'	-	VI	>49.5	5.0	5.0	3.0
PI 230970	<i>Rpp2</i>	VII	>25.6	3.0	3.7	2.0
PI 506764	<i>Rpp(?)</i> Hyuuga	VII	>26.0	2.0	-	3.3
'FT-2'	(LG C2)	VII	16.8	1.5	2.5	2.3
'Benning'	-	VII	>41.8	5.0	5.0	4.7
PI 462312	<i>Rpp3</i>	VIII	14.1	1.5	-	-
'Prichard'	-	VIII	>50.0	5.0	5.0	-

# Rust resistant and susceptible MG VIII accessions – 31 Oct 2007



# Lessons learned in 2007

- Inspection with a microscope may be necessary to distinguish rust lesions from other disease symptoms and injuries
- Lesion type and number may be indications of resistance, but are not necessarily correlated with sporulation levels
- Rate or sample on more than one date if possible
- Microscopic examination may provide clues about different mechanisms of resistance

# Breeding and mapping strategies

- Cross elite parents x resistant germplasm
- Advance populations using modified single seed descent or develop recombinant inbred line (RIL) populations
- $F_2/F_{2:3}$  populations may be adequate for mapping lesion type genes (RB vs. TAN)
- Dual-purpose RIL populations for breeding and for mapping resistance genes and QTLs
- Marker-assisted selection (MAS) for single resistance genes and to combine genes

# Considerations in choosing PI sources of rust resistance genes

- Resistance to other diseases and pests
- Morphology and growth habit
- Apparent yield potential (e.g., seed size, pod set)
- Maturity Groups of resistant parent and potential elite parents to be used in crosses

# Value of resistance gene mapping

- Mapping and tagging resistance genes with DNA markers will allow:
  - Early generation selection
  - Selection in absence of pathogen
  - Easier transfer of recessive and incompletely dominant genes (especially during backcrossing)
  - Pyramiding of resistance genes
- DNA markers can also be used to:
  - accelerate recover of recurrent parent genome in backcrosses
  - investigate relatedness of resistant accessions

# Acknowledgements

- USDA-ARS
- United Soybean Board
- Auburn University
- Clemson University
- University of Florida – North Florida Research and Education Facility
- University of Georgia
- Louisiana State University

# Acknowledgements

David Wright	NFREC	Quincy, FL
Jim Marois	NFREC	Quincy, FL
Tristan Mueller	NFREC	Quincy, FL
Maynard Douglas	NFREC	Quincy, FL
Kelley O'Brien	NFREC	Quincy, FL
Brian Kidd	NFREC	Quincy, FL
Nick Dufault	Penn State	Working in Quincy
Katey Pauls	USDA-ARS	Urbana & Quincy
Todd Bedford	USDA-ARS	Urbana