Identifying Soybean Resistance to Cercospora Leaf Blight

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Cercospora Leaf Blight (CLB) and Purple Seed Stain of Soybean

- Caused by *Cercospora* cf. *flagellaris*, *C.* cf. *sigesbeckiae* and *C. kikuchii*

Wikipedia.org

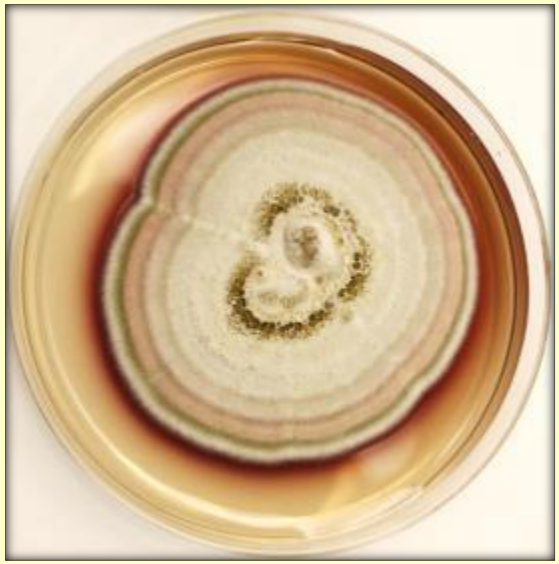
- CLB is one of the most important soybean diseases in Louisiana and other states of the mid-south

CLB Symptoms

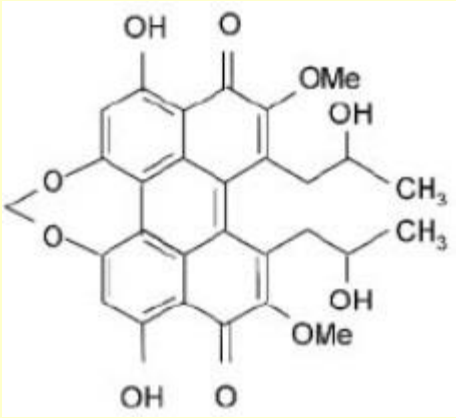
Extreme Symptoms

Photo by: Ray Schneider, 2012

Cercosporin



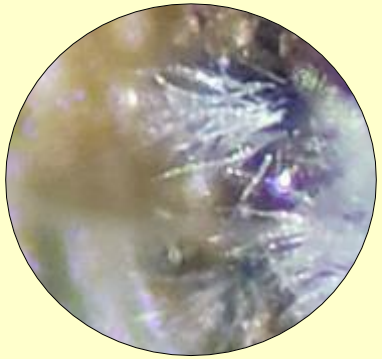
- Host non-specific toxin
- Absorbs light energy and produces reactive oxygen species (ROS)
- ROS damage cell membranes of the host cells
- Virulence factor



Taylor et al., 2006

Resistance Assay

- Currently no screening methods available outside of traditional field testing
  - Unreliable disease pressure
  - Variation across locations
  - Laborious and space-intensive



- Inoculum (spores) not easily produced

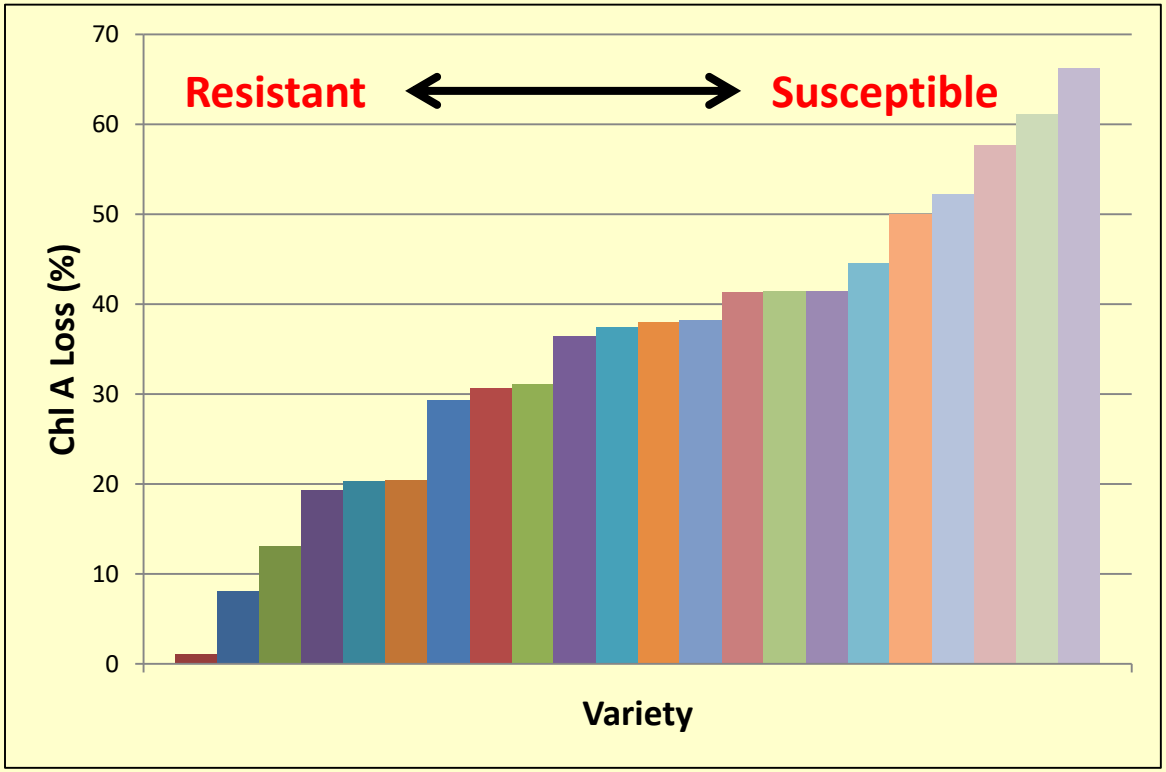
- Need fast and repeatable screening methods to expedite breeding practices

Leaf Disk Resistance Assay

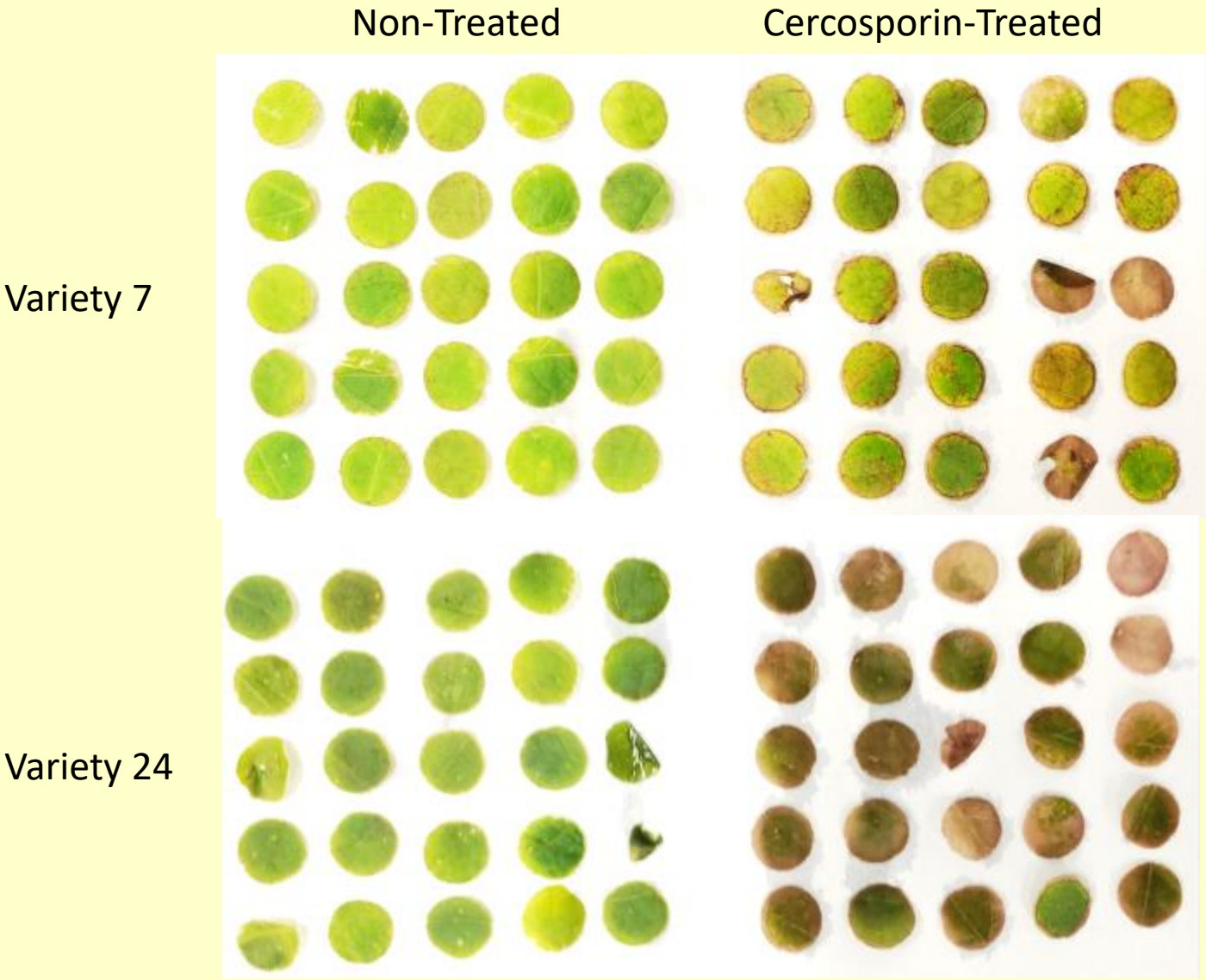
- Greenhouse-grown plants
- Leaflets from upper 3 nodes collected
- Washed, cut with hole-punch, placed in dishes
- Split into untreated and cercosporin-treated
- Placed under 14/10 lights
- Photographed and measured
  - Chlorophyll A via spectrophotometer

Leaf Disk Resistance Assay

- Assay utilizing cercosporin (toxin) applied to leaf disks
- 3-6 days, minimal material cost



Leaf Disk Resistance Assay



Leaf Disk Resistance Assay

Resistant

Tolerant

Susceptible

Variety	%Chl Loss
8	1.05
2	8.07
14	13.09
9	19.31
17	20.29
C	20.46
A	29.25
1	30.60
B	31.05
21	36.40
7	37.48
18	37.95
6	38.27
22	41.33
3	41.39
16	41.45
19	44.50
11	49.99
13	52.21
26	57.66
4	61.12
24	66.26

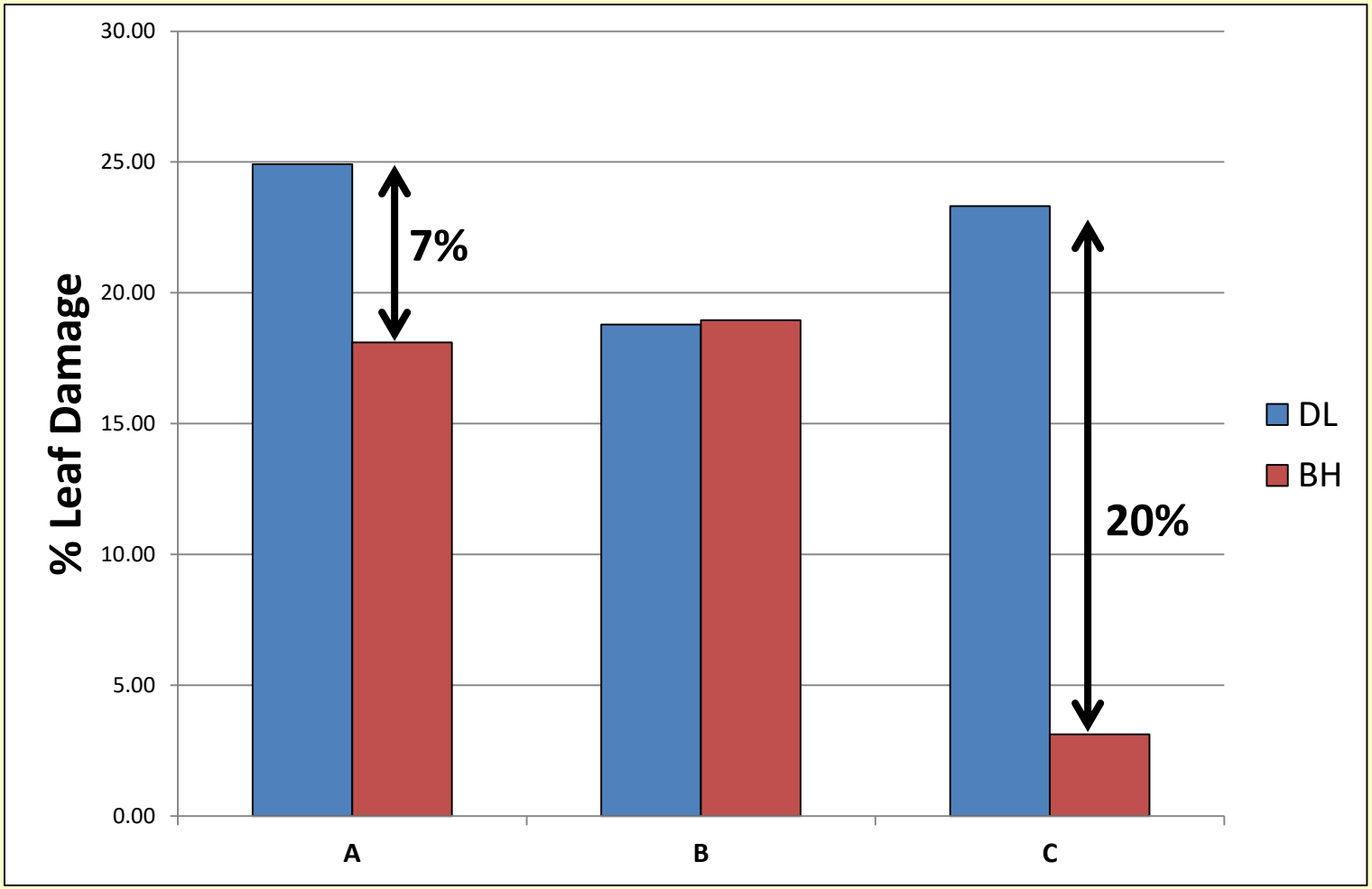
Variety	%Chl Loss
17	0.00
9	0.00
18	7.74
6	8.59
7	24.84
21	27.76
3	28.45
16	29.19
22	31.77
A	32.11
C	36.39
B	39.32
24	39.64
2	43.83
4	44.78
1	52.00
8	53.79
26	55.95
14	56.59
19	57.83
11	59.15
13	72.06

60% Same Category

32% Category Shift

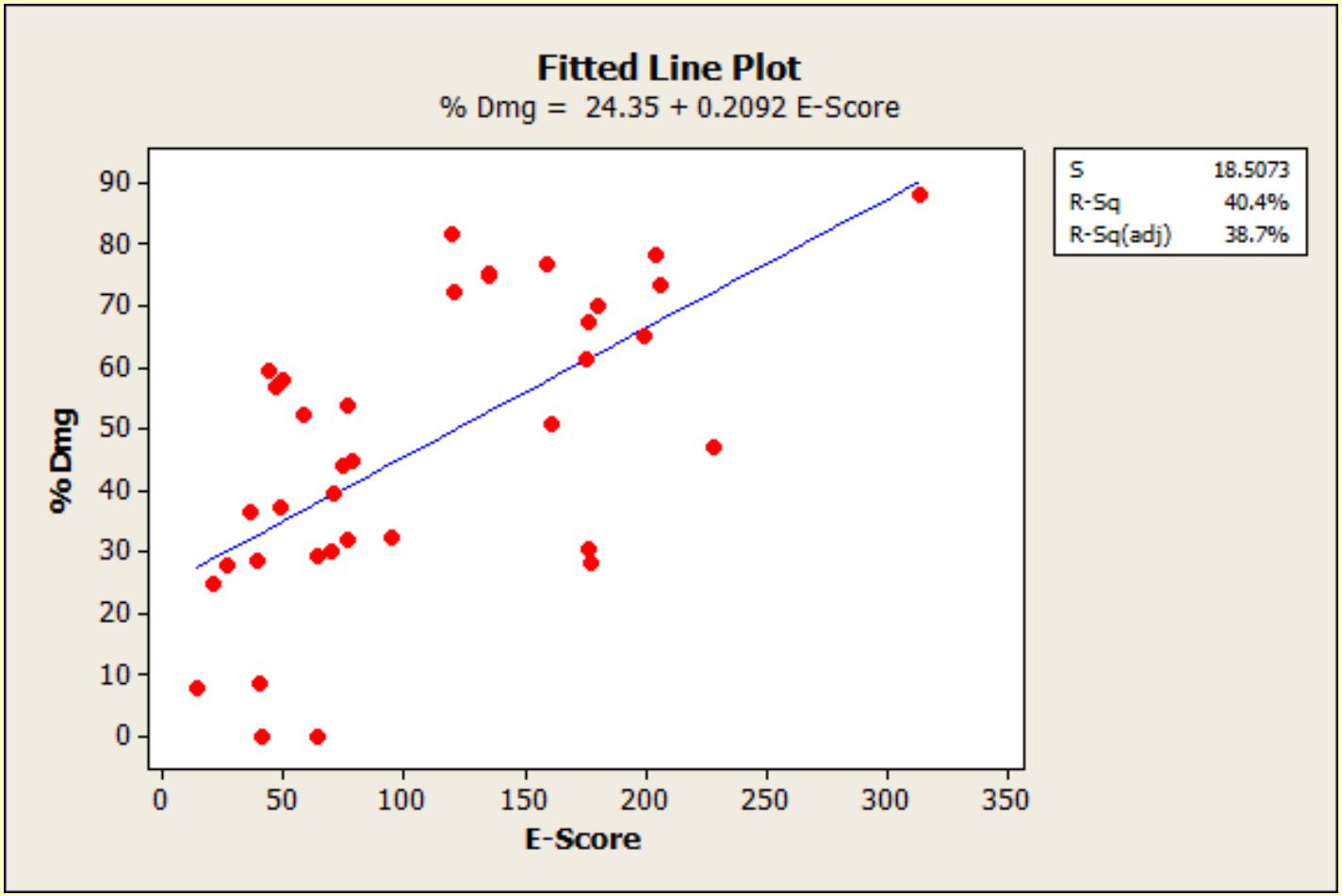
8% Unexplained

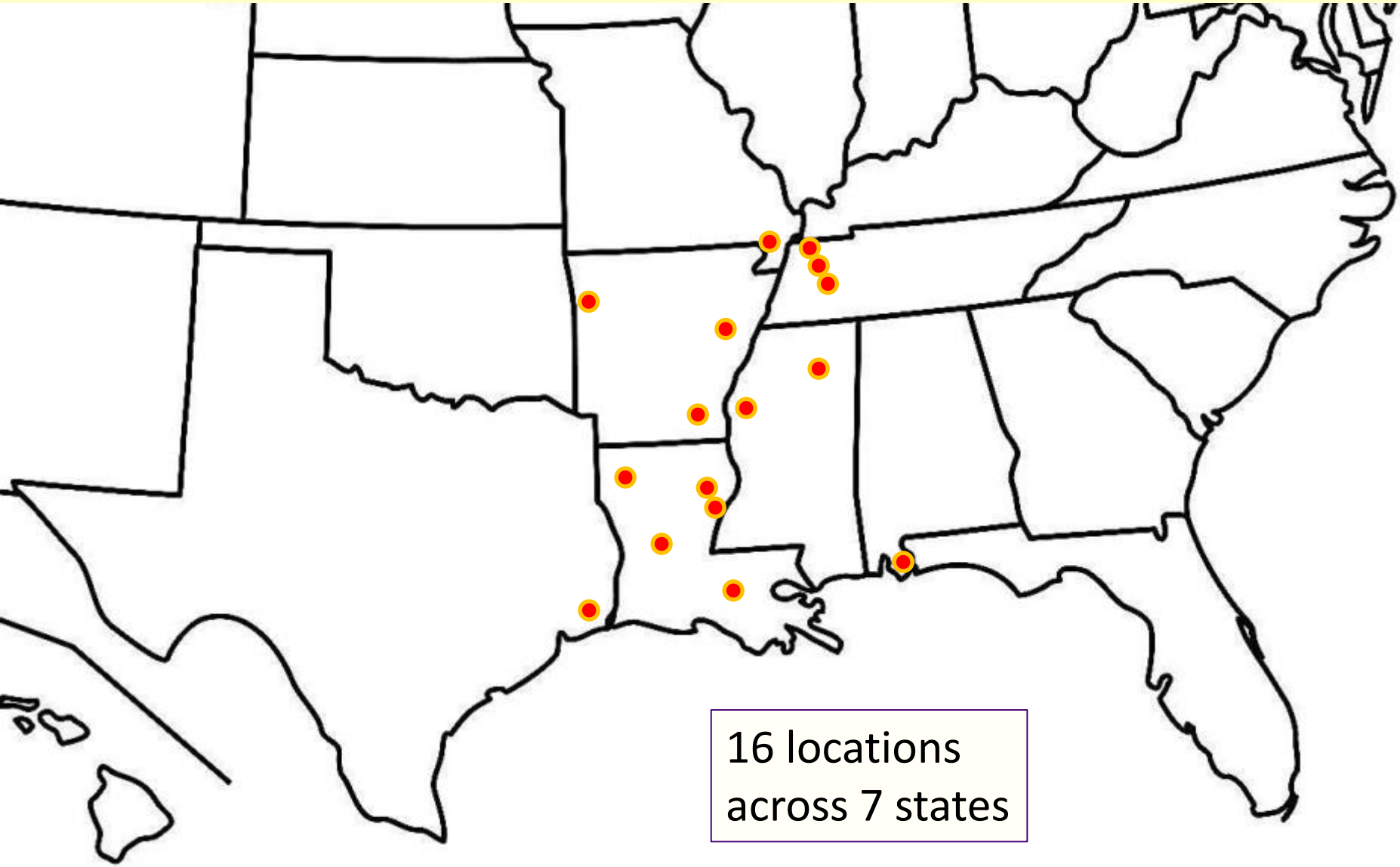
Soil Type and Resistance



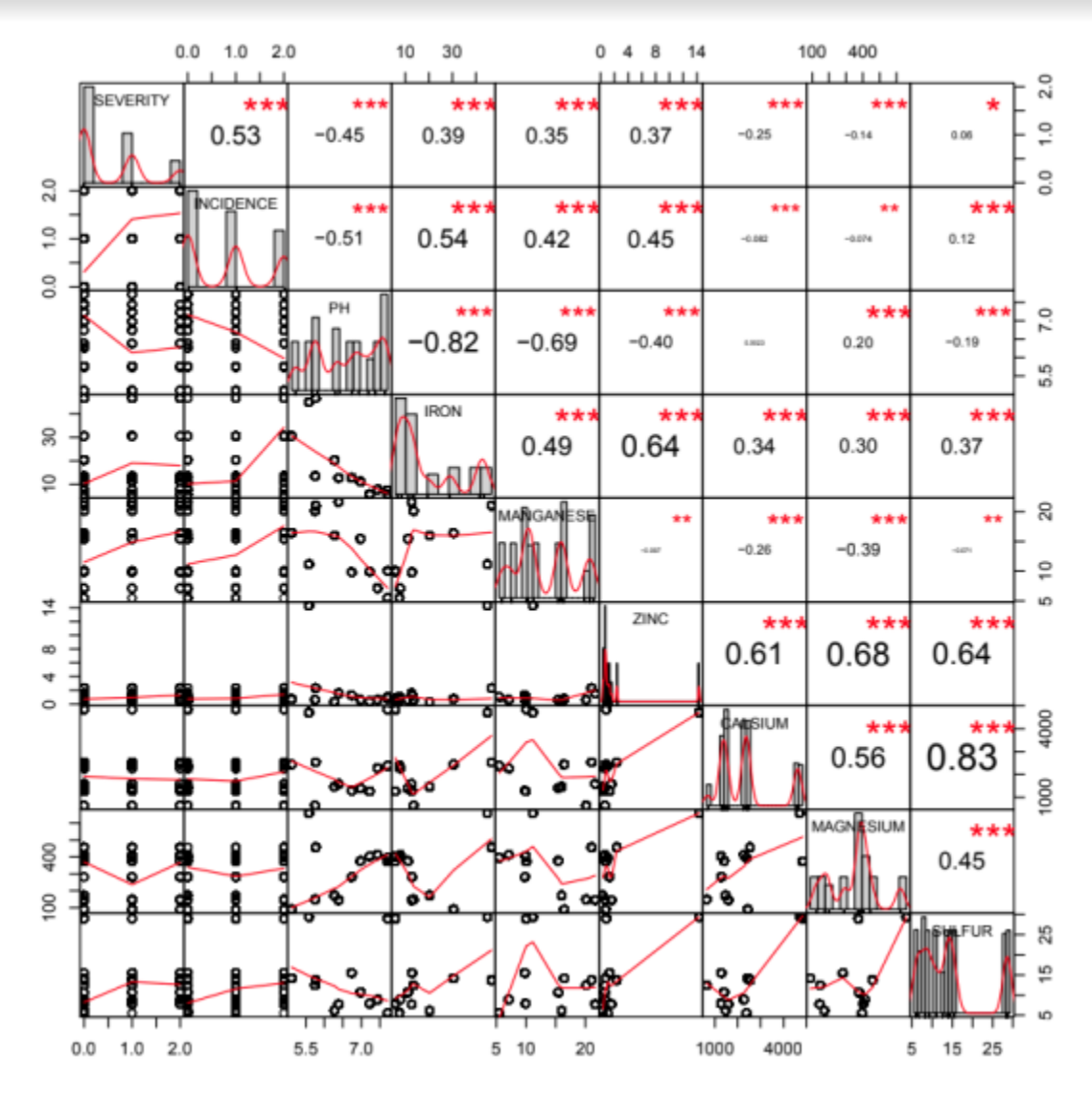
Nutrition and Resistance

Pearson correlation = 0.635 (**Moderately Strong**)
P-Value = 0.000 (**Significant**)




16 locations
across 7 states

No Soil Nutrient Correlations



Not accurate within field

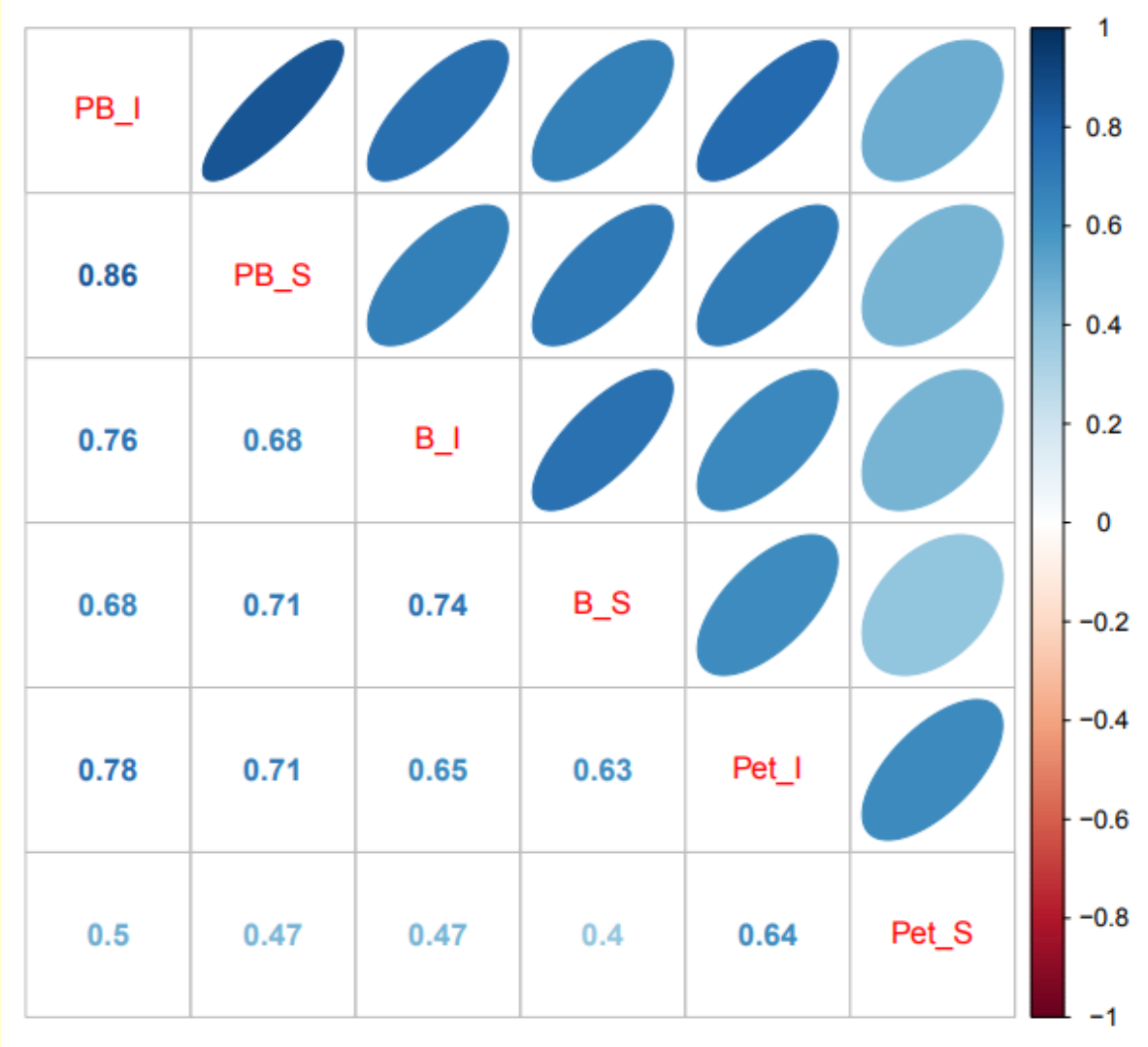
Does not account for leaf content

Does not account for varietal differences

Disease Ratings

- Numerous systems previously used (Petiole, Purpling/Bronzing, Blighting, CLB 0-9, CLB%)
- Simplified based upon previous data (2016/17)
- Ease of use, less error
- Uniformity across locations, easier statistical analyses
- More trustworthy data

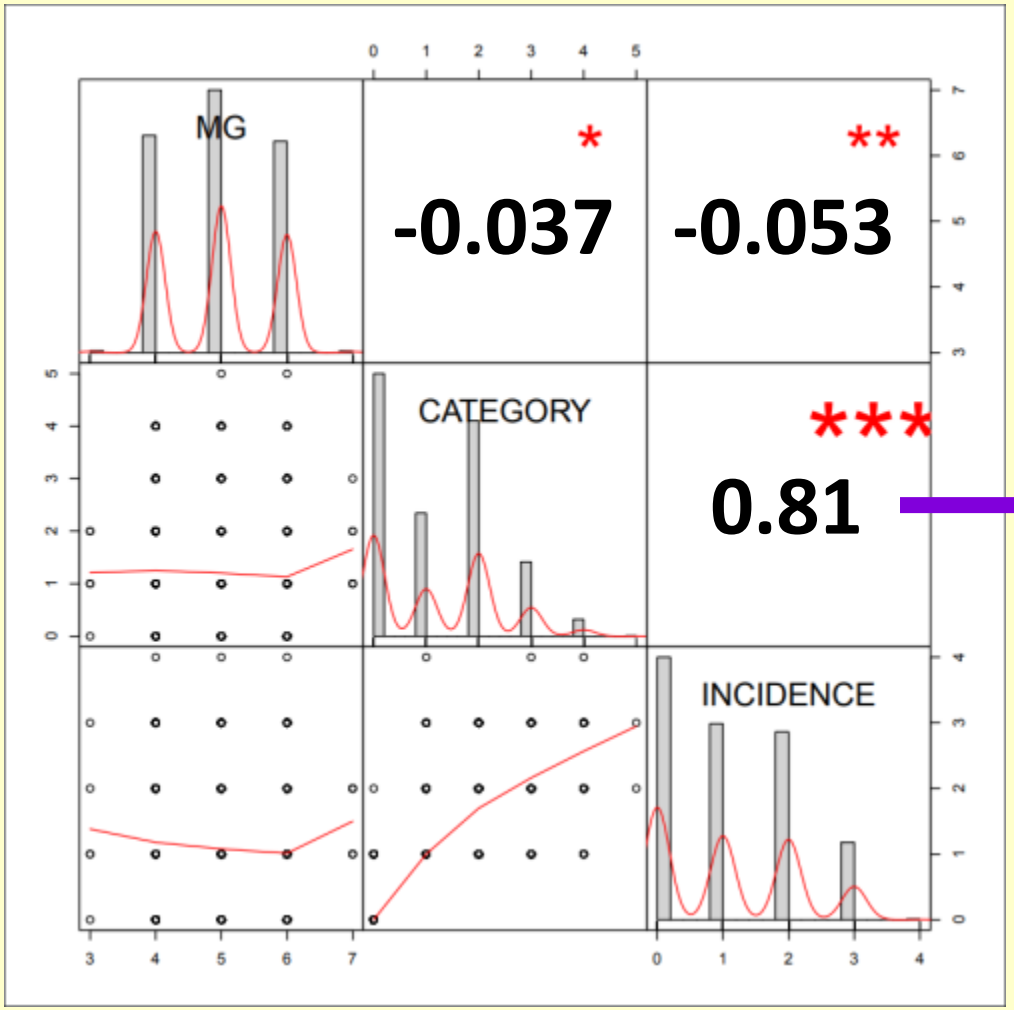
Symptoms are Correlated



CLB Rating Criteria

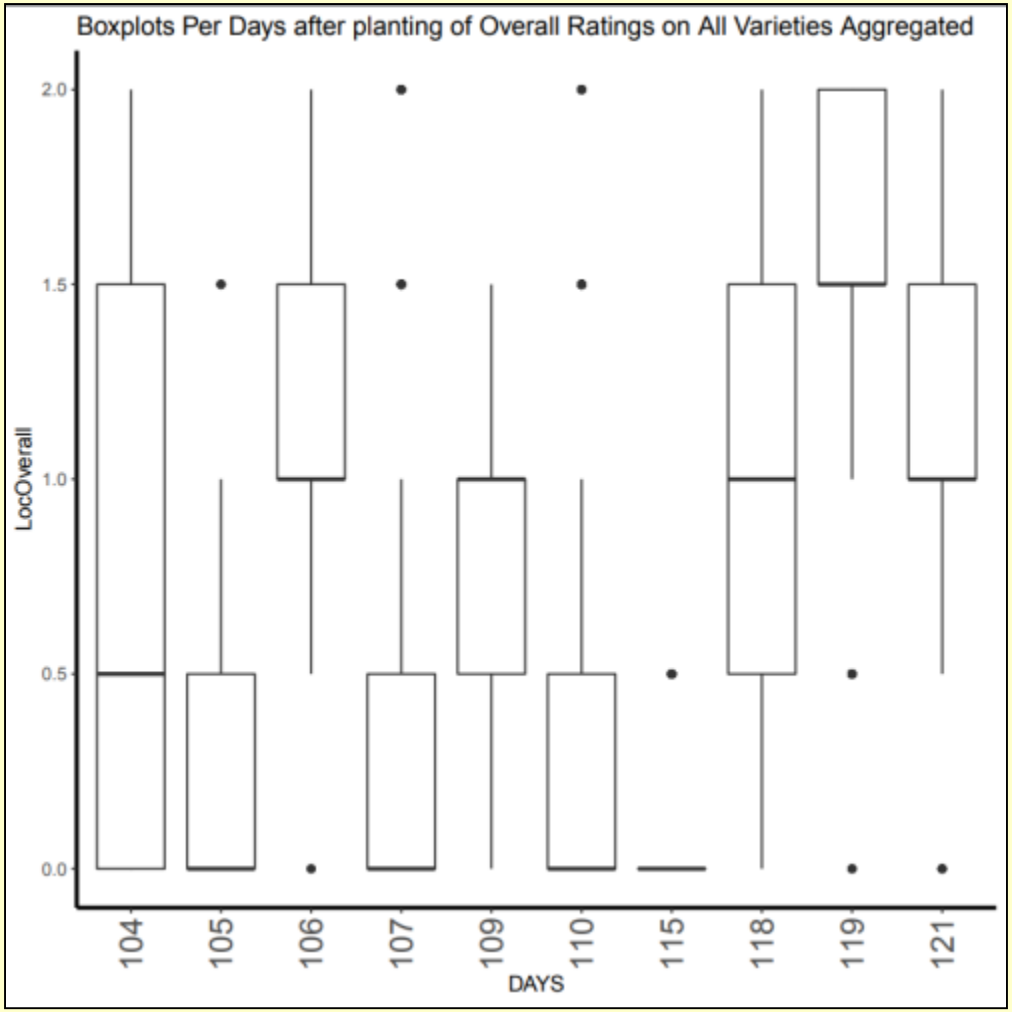
- Categorical Rating Scale
  - Severity 0-6
  - Incidence 1-4

Maturity Group not Significant



Severity and Incidence
Highly Correlated

Days After Planting



Good ratings through all of R5-6

Slightly higher in later ratings

Importance of similar rating times across locations

Breeding Line Resistance

- 8 Locations across 4 states
- Each location is an observational replicate (n=1)

- From 2016/17, 2/520 resistant
  - **PI 548548**
  - **PI 592756**
- From 2018, 3/460 resistant
  - **PI 534646**
  - **PI 536636**
  - **PI 576857**

Commercial Cultivar Resistance

- 13 Locations rated for CLB across 7 states over 3 years
- 3-4 replicates per location

- Ratings were normalized with weight given to severity

- 20% Resistant, 60% Tolerant, 20% Susceptible

Commercial Cultivar CLB Resistance

- **S16-14458**
- **S14-9017R**
- **DeltaGrow 4967LL**
- **R13-13997**
- **S15-3772RY**
- **S14-9051R**
- **S16-11222**
- **S11-16653**
- **S13-10592C**
- **S14-15138R**
- **S11-20242**
- **Progeny4930LL**
  - Very resistant in AR, tolerant elsewhere

Commercial Cultivar Tolerance

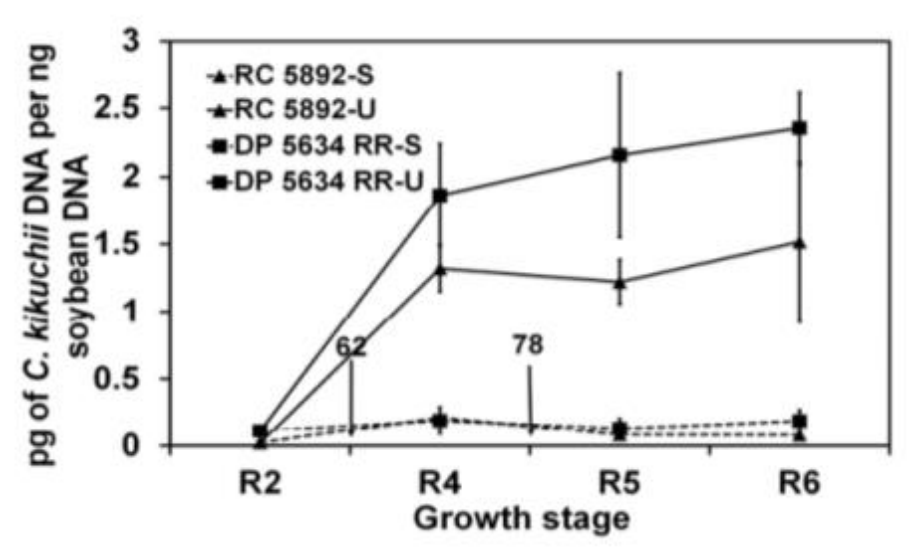
- **S11-20337GT**
- **LA13006**
- **S15-10434C**
- **S13-3851C**
- **R15-2422**
- **S15-3847RY**
- **S16-14558**
- **S16-11644**
- **S11-17025**
- **S15-10879**
- **S15-5904RY**
- **R12-6751RR**
- **S13-1059C**
- **S14-15146R**
- **S16-8156**
- **S16-14687**
- **Progeny 4930LL**
- **R11-7999**
- **S13-10590C**
- **S12-4718**
- **S11-9618RR2**
- **S13-2743C**
- **R07-6669**
- **S13-1955C**
- **R11-171**
- **UA 5615C**
- **S15-16886C**
- **R13-4638RY**
- **R13-9687**
- **REV 51A56**
- **R04-342**
- **UA 5014C**
- **UARK-288**

Commercial Cultivar Susceptibility

- **R15-1150**
- **S11-20195GT**
- **S15-17812C**
- **R10-298**
- **R15-818**
- **S13-1805C**
- **S16-3739**
- **S15-17815C**
  - **Overwhelmingly susceptible**

Identifying Resistance Factors

- Disease appears R5 onwards, rarely earlier
- Varietal and nutritional differences
- What plant attributes affect resistance?



Modified from Chanda et al., 2014

Plant Triggers

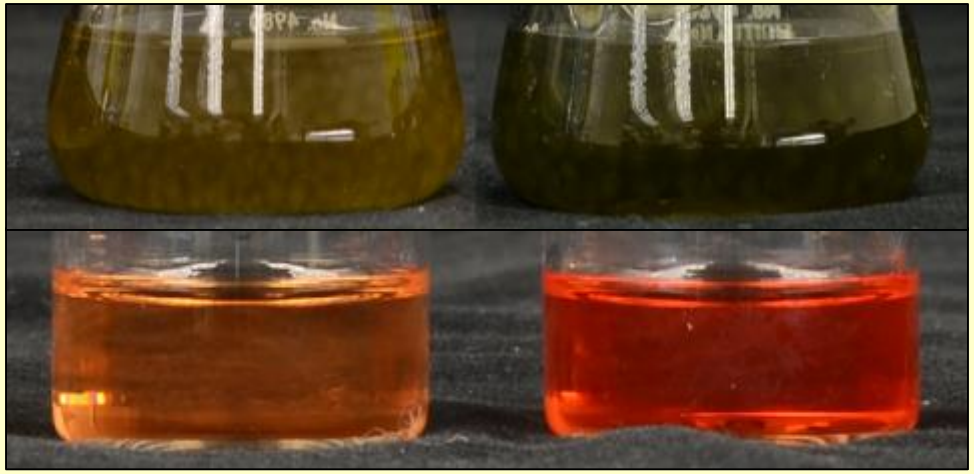
H_2O

37^oC

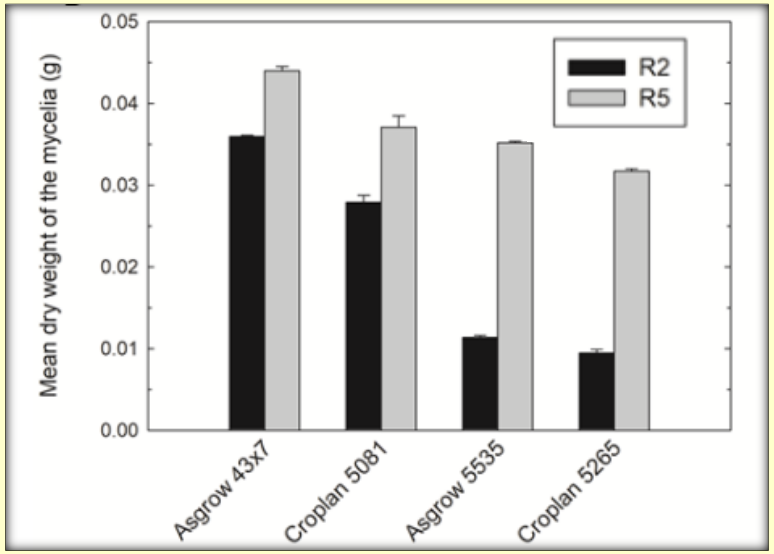
Slide: Marija Zivanovic

Growth Stage Triggers

- *C.* cf. *flagellaris* grown in extracts
- Much higher toxin production at R5
  - Disease onset not likely entirely environmental



R2 **R5**



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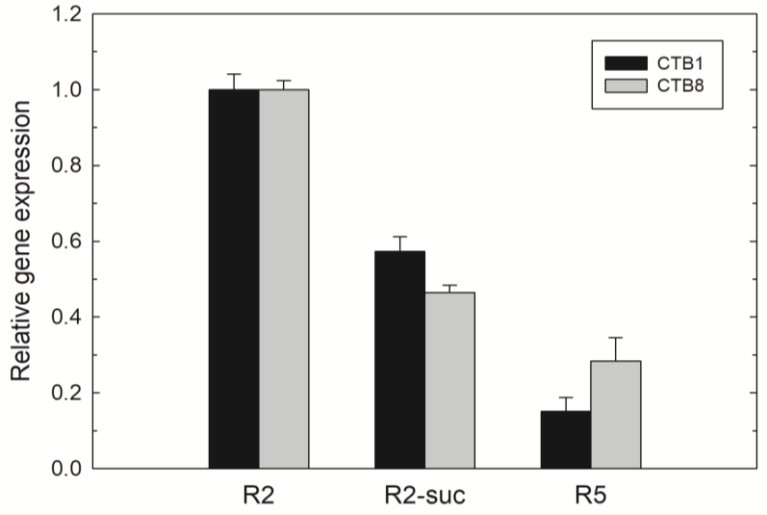
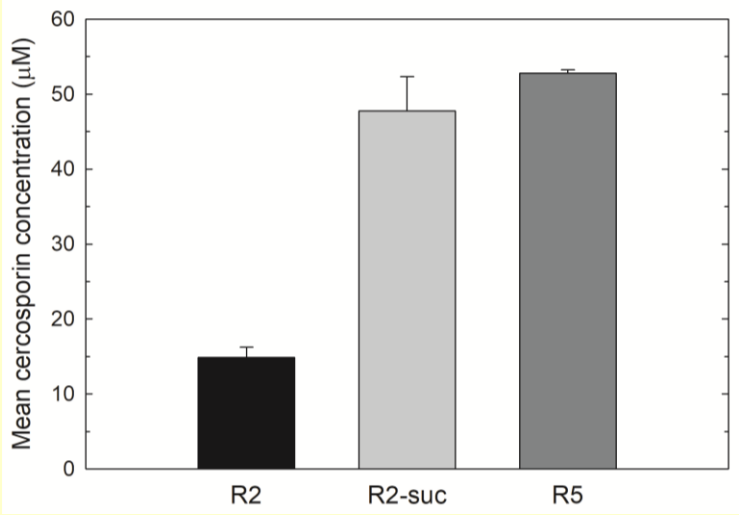
Plant Triggers

Hormones

Sugars





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Continuing and Future Work

- Move breeding lines forward, continue commercial variety testing
- Quantify pathogenicity triggers *in planta*
- Correlate field and laboratory results: leaf disk assay, variety trials, pathogenicity triggers
- Yield loss estimations
- Pathogen population and fungicide resistance

Cercospora Collection

- 1,000 isolates from 16 locations across 7 states
- Catalogued and backed up for future projects

- Population Determination
- Fungicide Resistance Test (QoI (Pyracla), DMI (Tetra), MBC (TP-M), SDHI (Bosc))

Collaborators

- Dr. Trey Price
- Dr. Thanos Gentimis
- Dr. Blair Buckley
- Dr. Tom Allen
- Dr. John Rupe
- Dr. Guy Padgett
- Dr. Xin-Gen Zhou
- Dr. Pengyin Chen
- Dr. Terry Spurlock
- Dr. Ed Sikora
- Dr. Heather Kelly
- Dr. Leandro Mozzoni
- Marija Zivanovic
- Bob Holland
- Dr. Liliana Florez
- Dr. Moldir Orazaly
- David Moseley
- Dr. Sara Thomas-Sharma
- Clark Robertson
- Evangeline Smith
- Ethan Hunt
- Michael Clubb
- Dr. Zhi-Yuan Chen

Variety Keys

1	UA 5014C	5	AR	
2	UA 5213C	5.2	AR	
3	Osage	5.6	AR	
4	UA 5612	5.6	AR	
5	AG46X6	4.6	Asgrow	4670RR2Y
6	UA 5615C	5.6	AR	
7	R09-430	5.2	AR	
8	R09-1589	4.9	AR	
9	R13-1019	5.7	AR	R09-4798
10	AG47X6	4.7	Asgrow	48A76
11	R11-2354	6	AR	
12	R11-7999	5.7	AR	R11-1192
13	UA 5414RR	5.6	AR	
14	UA 5715GT	5.7	AR	
15	AG53X6	5.3	Asgrow	CZ5375RY
16	R11-89RY	5.4	AR	
17	R10-197RY	5.6	AR	
18	UA 5814HP	5.8	AR	
19	UA 4805	4.8	AR	
20	DG5580	5.5	Delta Grow	55R68
21	S11--20337	4	MO	
22	S12-2418	4	MO	
23	S12-3782	4	MO	
24	S11-17025	5	MO	
25	REV56R63	5.7	Terral	P5752RY
26	S11-20124	5	MO	
27	LA560512	5	LA	
28	39RY43	3.9	Dyna-Gro	CZ4181RY
29	DG4967LL	4.9	Delta Grow	
30	Croplan5265	5.2	Croplan	Croplan5081

A Croplan 5081
B Asgrow 5535
C Croplan 5265