

Screening and Identifying Soybean Lines for Charcoal Rot Resistance in Non-Field Environments

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Outline

- Introduction
- Inoculation techniques
 - Cut stem mycelial plug inoculation
 - Seedling radicle inoculation
- Evaluations
 - Select set of soybean entries to compare field and non-field test results
 - Regional entries to compare field and non-field test results
- Summary

Introduction

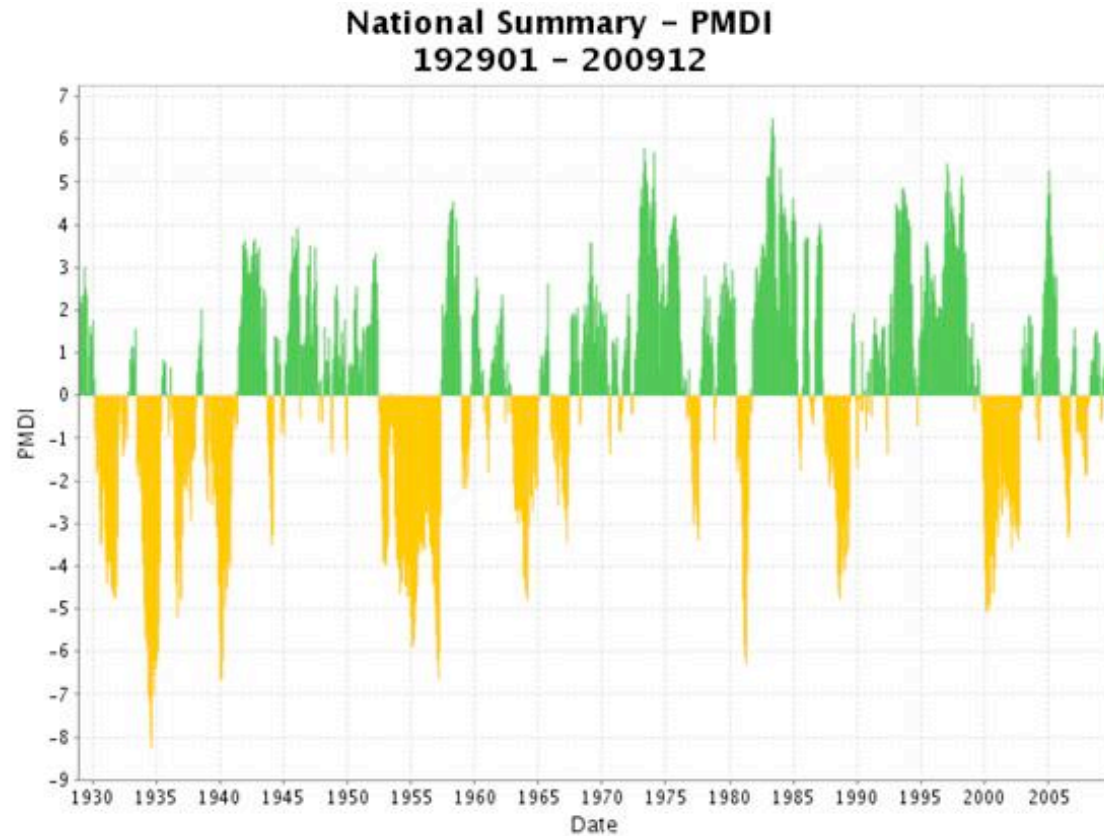
- *Macrophomina phaseolina*
 - *Macrophoma phasiolina*
 - *Botryodiplodia phaseoli*
 - *Rhizoctonia bataticola*
 - *Sclerotium bataticola*
- Wide host range (440 plus species)
 - Includes most US-grown crops
- Distribution
 - Everywhere (tropics to subtropics)
- First reported in the USA in NJ in 1890 (sweet potato)
- First reported in IL in 1939 (many crops including soybean)



Drought Favors Charcoal Rot ?

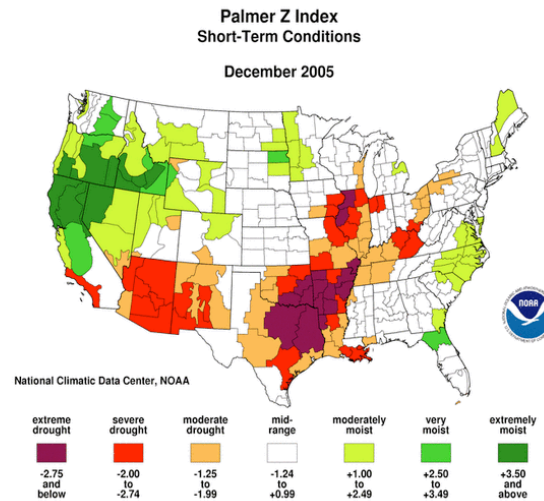
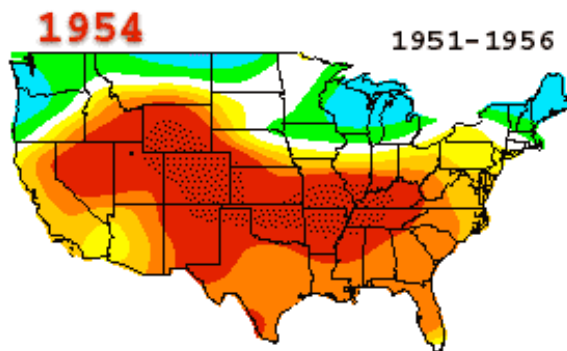
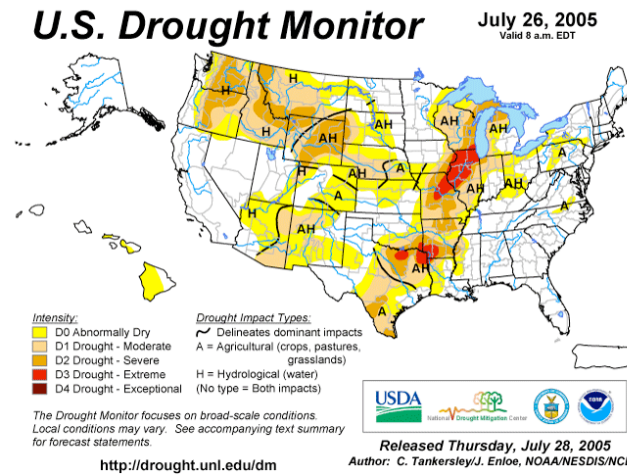
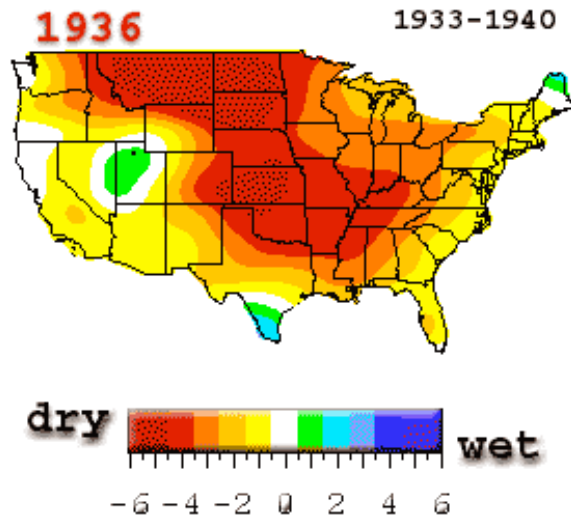
National Oceanic and Atmospheric Administration National Climatic Data Center

Palmer Drought Severity Index (PDSI): measure the duration and intensity of the long-term drought based on measurements of precipitation, air temperature, and local soil moisture; values range from -6.0 (extreme drought) to +6.0 (extreme wet)



Drought Favors Charcoal Rot

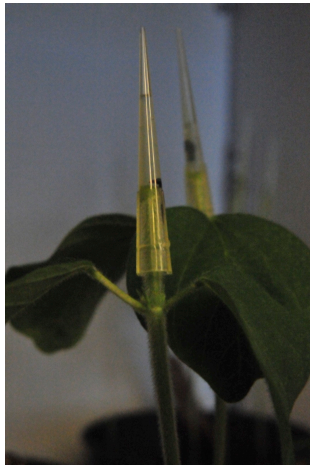
National Oceanic and Atmospheric Administration National Climatic Data Center



Objectives

- Develop screens that provide reliable and repeatable differences in responses of soybean entries when inoculated with *M. phaseolina*
- Make screens relatively simple to use and be able to accommodate large number of entries
- Compare tests results with known field results
- Utilize these techniques to discover, characterize, and develop partially resistant germplasm

Cut Stem Mycelial Plug Inoculation



Inoculate 2-3
wk-old plants



Plugs from
2-3 d-old
cultures



Record lesion length over time

Cut Stem-Mycelial Plug Inoculation



Compare Cut Stem Mycelial Plug Inoculation with Field CFUI Evaluations*

- 22 lines – most had been field-tested and ranked for susceptibility
- One isolate *M. phaseolina* (Mp) from Illinois
- Experimental unit (EU) was four plants of each entry
- EUs were arranged in an RCBD design with 6 blocks
- V2-V3 plants were inoculated with a plug cut from the margin of an actively growing PDA culture with a 10-200 µl pipette tip placed on the tip of the stem cut 2.5 cm above the unifoliolate node
- Inoculated plants incubated at 30C and 16 h photoperiod
- Length of necrosis measured at 7, 9, and 14 days after inoculation
- Area under the disease progress curve (AUDPC) was analyzed
- Two tests of the same entries with different randomizations

*Ranks and classification from Mengistu, et al. 2007. Crop Science 47: 2453-2461.

Summary of Greenhouse Results and Comparison with Field Measurements

Entry	AUDPC*		CFUI Rank & classification**
Pharoah	263	a	2 (S)
Croton	247	ab	9 (MS)
LS98-3032 (SIU)	239	ab	3 (S)
LS98-0358 (SIU)	220	a-c	1 (S)
LS94-3207 (SIU)	217	a-c	4 (S)
LS98-0265 (SIU)	217	a-c	12 (MS)
LS92-1088 (SIU)	215	a-d	11 (MS)
Stressland	197	b-e	5 (S)
DT99-17483	196	b-e	16 (MR)
DT98-7553	184	c-e	13 (MS)
LS98-1430 (SIU)	183	c-e	MR
Manokin	183	c-e	6 (S)

Continued

Summary (continued)

Entry	AUDPC*		CFUI Rank & classification**
DT99-16864	182	c-e	17 (MR)
Spencer	182	c-e	
LS98-3257 (SIU)	180	c-e	MR
DT99-17554	175	c-e	15 (MR)
AP 4882	171	de	7 (S)
Pana	165	ef	
LS98-2574 (SIU)	164	ef	MR
GX98-0609	160	ef	8 (MS)
N98-7265	157	ef	10 (MS)
DT 97-4290	133	f	14 (MR)

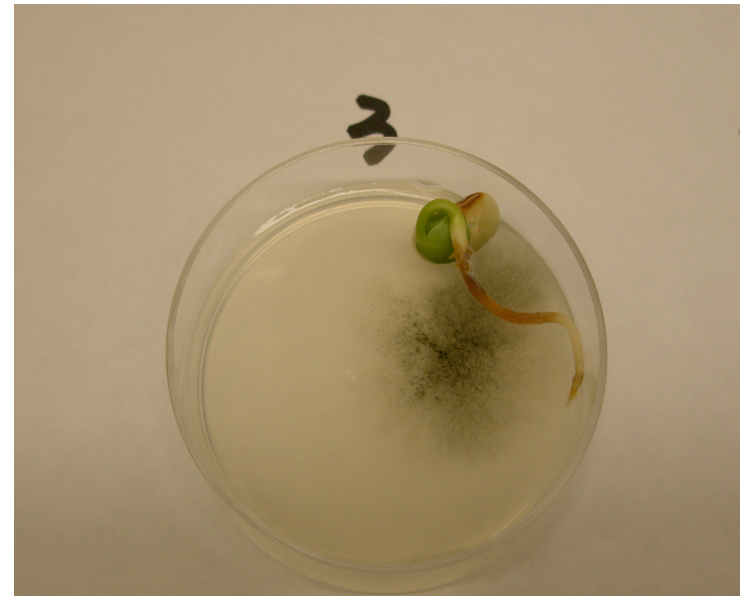
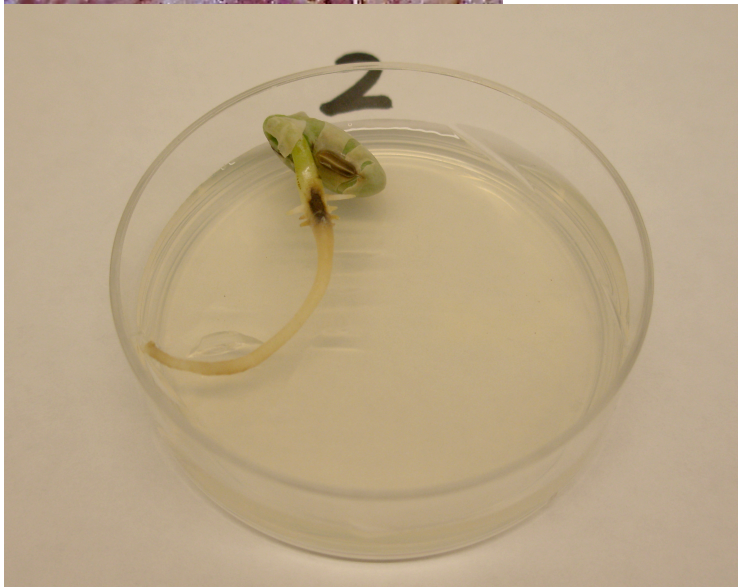
*Spearman's rank correlation r between greenhouse tests = 0.62, $P = 0.001$.

**Ranks and classification from Mengistu, et al. 2007. Crop Science 47: 2453-2461.

Seedling Radicle Inoculation

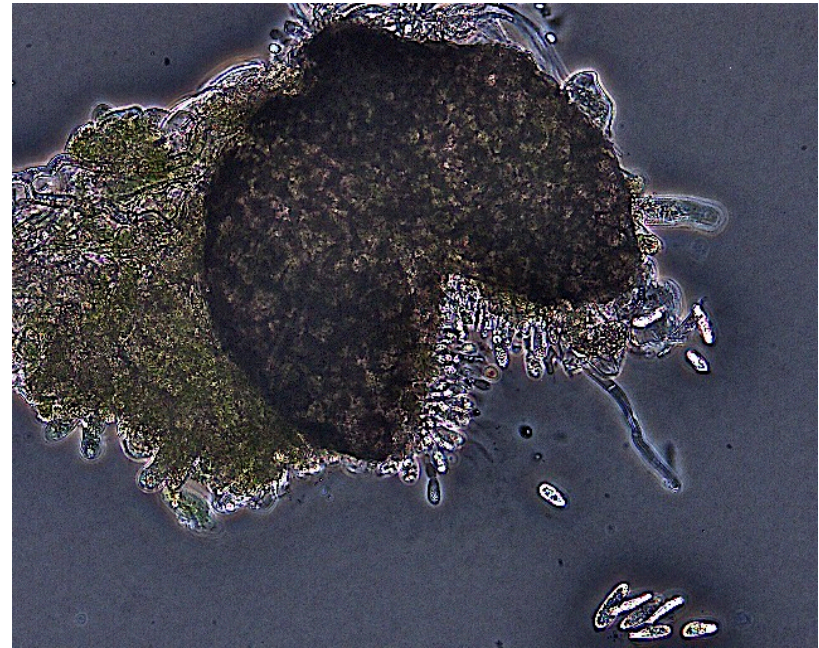
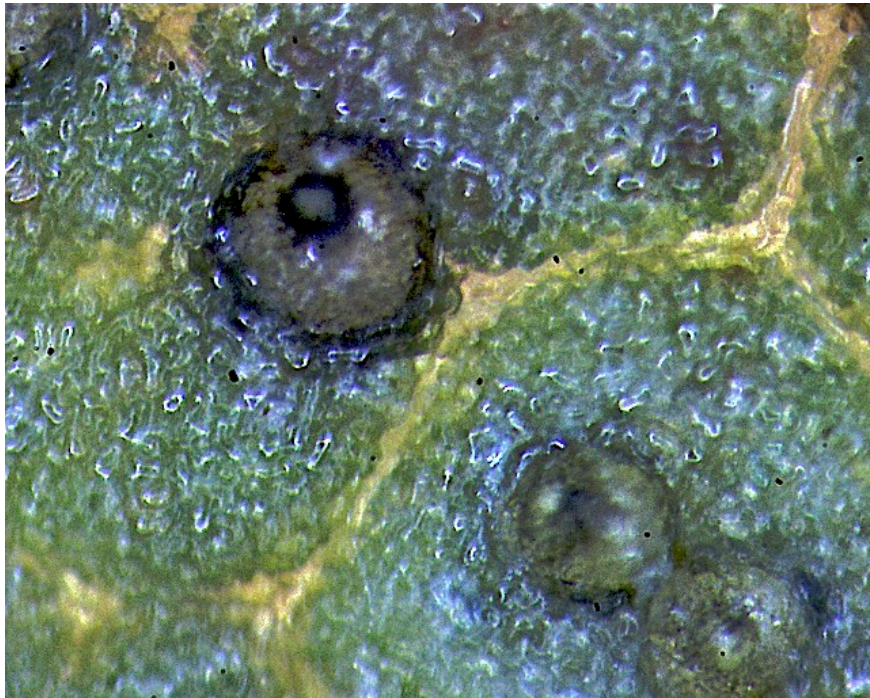


- Surface disinfect seeds
- Allow them to germinate 2-3 days
- Inoculate with a mycelial plug or conidia
- Interested in conidia to adjust dosage and quantify inoculum



Production of Conidia for Inoculum

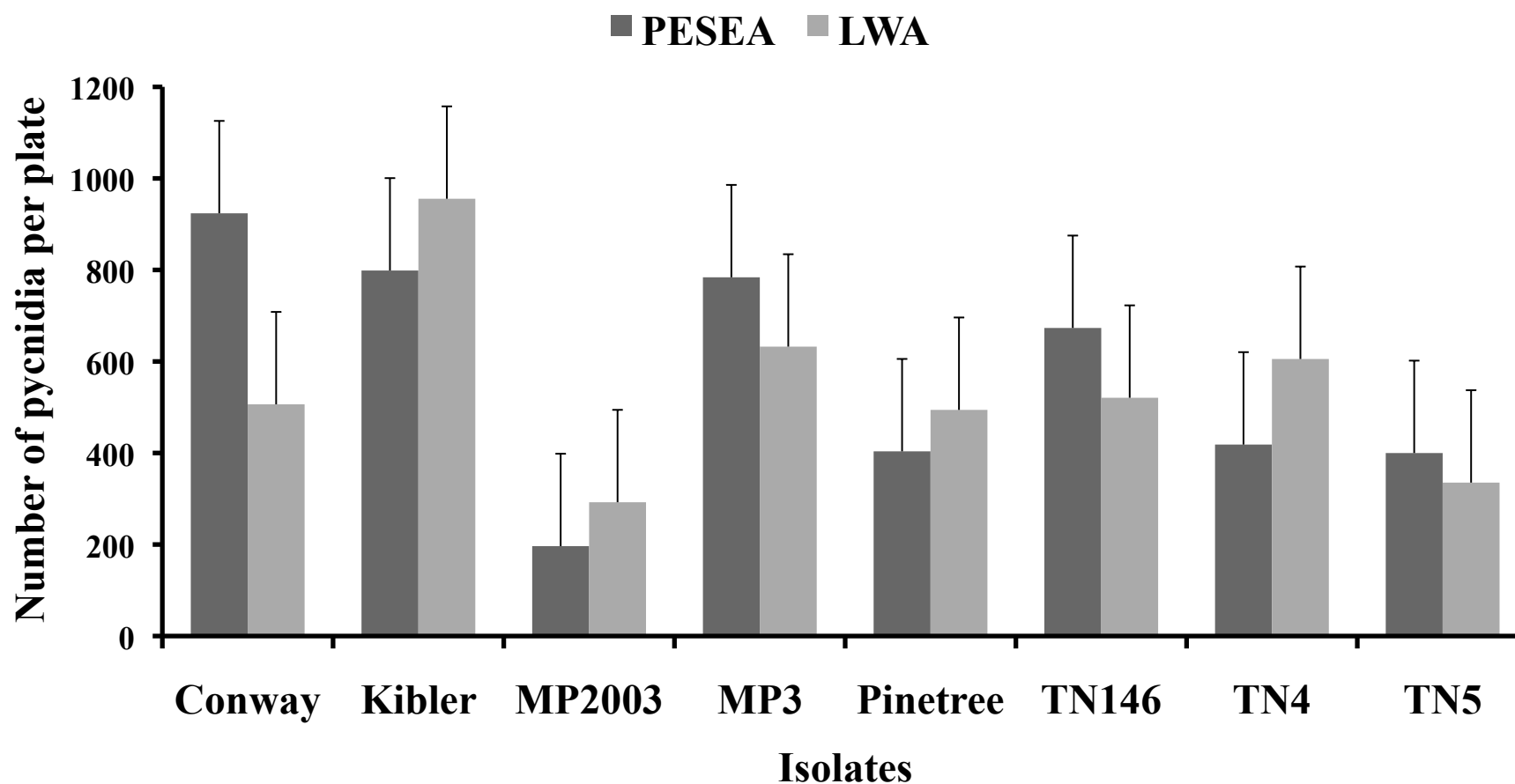
- Conidia of *M. phaseolina* isolates have been produced in culture in the past especially from isolates of other hosts
- Eight different *M. phaseolina* isolates tested
- Several different media were tested including using sterile soybean leaflets on water agar and peanut butter extract saturated filter paper placed on agar amended with soynut butter extract (PESEA)



Pycnidia and conidia of
Macrophomina
phaseolina



Production of *M. phaseolina* Pycnidia on Filter Paper (PESEA) and Leaf Water Agar (LWA)



Vertical bars = $LSD (0.05) = 202$

Isolate Differences in Conidia Production Based on 80 Pycnidia

Isolate	Number of conidia x 10 ⁴	Location	Year isolated	Contributor
MP2003	4.3 a	Urbana, IL	2003	G. Hartman
Pinetree	3.7 b	Pinetree, AR	1998	J. Rupe ¹
MP3	3.1 c	Champaign, IL	1998	J. Manandahar ²
TN4	2.7 cd	Jackson, TN	2006	A. Mengistu ³
TN146	2.3 d	Neosho, TN	2006	A. Mengistu
Kibler	1.6 e	Kibler, AR	2008	J. Rupe
TN5	1.3 e	Ames, TN	2006	A. Mengistu
Conway	0.6 f	Conway, AR	2004	J. Rupe

¹University of Arkansas, Fayetteville, AR; ²Dairyland Seed Company, Gibson City, IL; ³ USDA-ARS, Jackson, TN.

Germination and Infectivity of Conidia Produced on PESEA

- Tested conidia germination of eight isolates
- Conidia from the Pinetree isolate was used to test infectivity on soybean radicles
- Charcoal rot susceptible line LS98-0358 and the partial resistant line DT97-4290 radicles were inoculated
- 1 μ l drop containing 500 conidia followed by 3 d incubation at 30° C
- The length of each lesion (mm) length from 20 radicles of each soybean genotype was measured

Germination and Infectivity of Conidia Produced on PESEA

- There were no significant differences among the eight isolates for percent germination of conidia (mean germination 83%)
- There were significant ($P < 0.01$) differences in lesion length produced on line LS98-0358 (2.9 mm) compared to line DT97-4290 (0.5 mm)

Multiple Evaluations of the 19 Soybean Entries Used in the Multistate Trial

- Radicle Inoculation
 - 2 cm long radicles were inoculated with a 1 μ l drop containing 500 conidia
 - 3 days of incubation at 30°C
 - Lesion length measurements, CFUs, QPCR
- Cut stem inoculation
 - Inoculated with agar plugs on cut stem
 - Lesion lengths measured over time

Sorted Ranks

Radicle Length	Lesion Length	CFU	QPCR	Cut stem
DP-3478	LS98-3257	LS98-3257	LS98-3257	LS92-1088
Croton	LS98-0719	LS98-0719	LS98-0719	Pharoah
DP4546	DT99-16864	LS98-2574	LS98-2574	LS98-0358
Pharoah	LS98-0358	LS92-1088	DT99-16864	DT99-16864
R01-581F	DP-3478	DT99-17554	DT99-17554	DP4546
DT97-4290	AG4866	DT99-16864	DR5806	LS98-0719
LS98-1430	DT99-17554	DR5806	LS92-1088	DT99-17483
DT98-7553	LS98-1430	LS98-0358	AG4866	DT99-17554
LS98-3257	LS92-1088	AG4866	DT99-17483	R01-581F
DT99-17554	LS98-2574	LS98-1430	LS98-0358	DR5806
LS98-2574	DR5806	DT99-17483	DP105	DP105
DT99-17483	DP4546	DP-3478	LS98-1430	LS98-1430
LS98-0358	DP105	DP105	R01-581F	Croton
AG4866	R01-581F	Pharoah	DT98-7553	AG4866
DR5806	DT99-17483	DT97-4290	DP-3478	LS98-2574
LS98-0719	Pharoah	DT98-7553	DT97-4290	DP-3478
DP105	DT98-7553	R01-581F	Pharoah	LS98-3257
DT99-16864	DT97-4290	DP4546	DP4546	DT98-7553
LS92-1088	Croton	Croton	Croton	DT97-4290

Selected Comparison of Partial Resistance vs. Susceptible Soybean Genotypes

Genotype	Cut stem (AUDPC)	Radicle conidia inoculation (ng DNA)	TN CFU	MO CFU	SIU CFU	AR CFU	KS CFU
DT97-4290	175 (1)	3.5 (4)	2467 (5)	1462 (8)	446 (10)	134 (7)	330 (1)
LS98-0358	336 (14)	8.7 (7)	30067 (10)	1660 (10)	1202 (13)	473 (15)	670 (8)

Total tested in common at each location was 15 entries

Summary I

- Cut stem mycelial plug inoculation provided repeatable differences in responses of soybean entries
- Conidia of *M. phaseolina* isolates were produced, infective, and used in seedling radicle inoculation evaluation, and also provided repeatable differences in responses of soybean entries
- Both techniques were relatively simple to use and able to accommodate large numbers of entries
- Both techniques compared relatively well to known field results
- These techniques should assist to discover, characterize, and develop partially resistant soybean germplasm

Summary II

Pros

- Eliminates maturity differences
- Control environment – each experiment is the same
- Direct measurement of infection/colonization
- No escapes
- Allows for ease and rapid comparison between isolates

Cons

- Not natural – resistance mechanisms could be circumvented
- Controlled environment is needed to complete the test
- Lack of strong resistance makes separation more difficult