

Multi-environment soybean cultivar evaluation for charcoal rot resistance

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ACKNOWLEDGEMENTS

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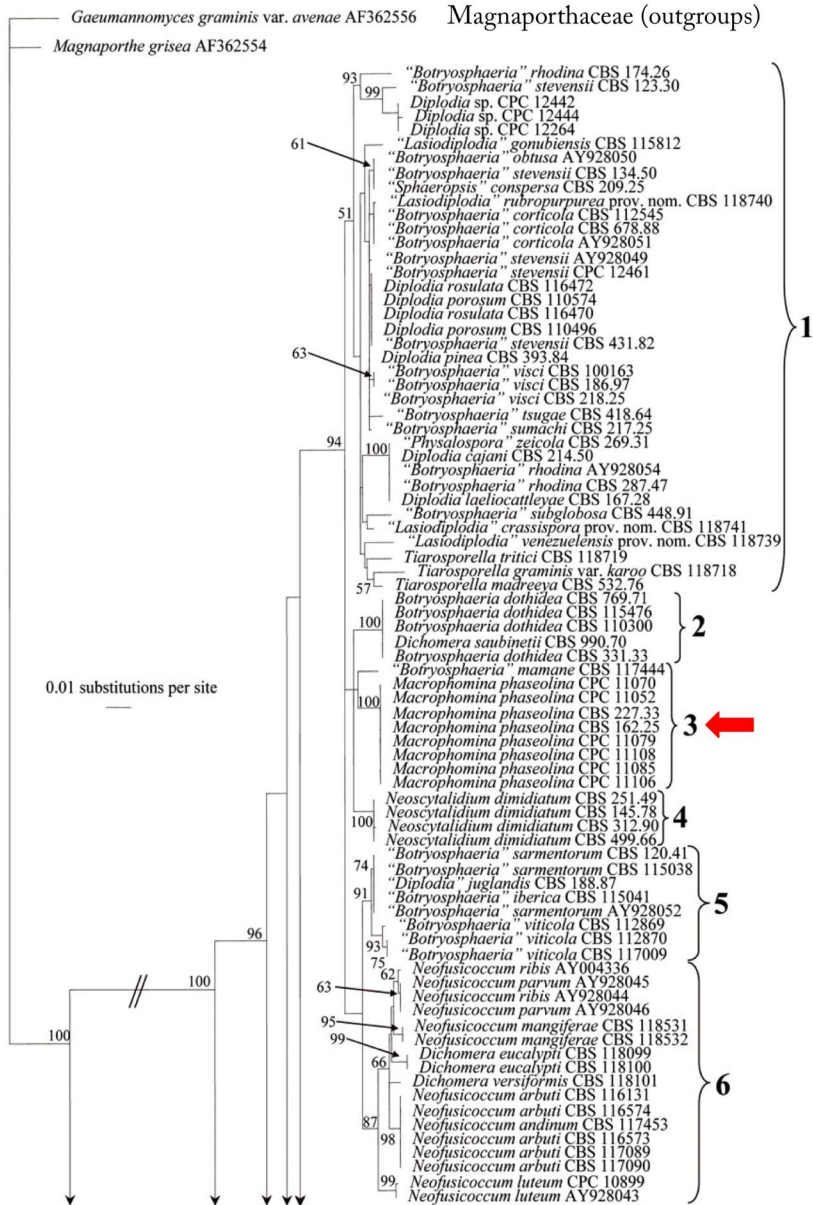
OUTLINE

1. Introductory material
2. Multi-environment soybean cultivar screening
 - 2a. Severity estimators (RSS, root CFUs)
 - 2b. Yield and tolerance
3. Other methods:
 - 3a. Pathogen populations in the soil
 - 3b. Effect of host genotype upon soil populations



Lineages of the *Botryosphaeriaceae* (Pezizales) fungi (numerous ascomycete plant pathogens)

Macrophomina phaseolina



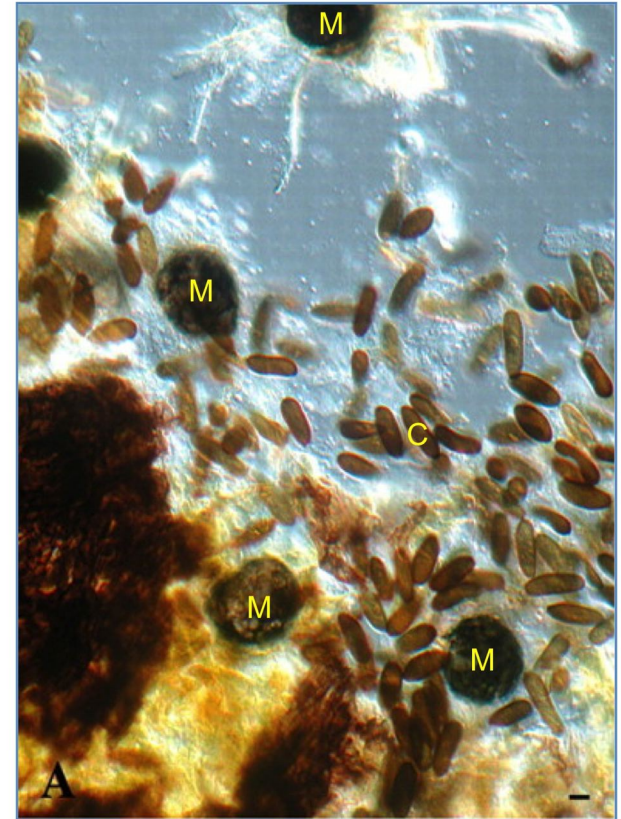
Diplodia



Botryosphaeria dothidea



Neofusicoccum



MICROSCLEROTIA and CONIDIA produced by *M. phaseolina*

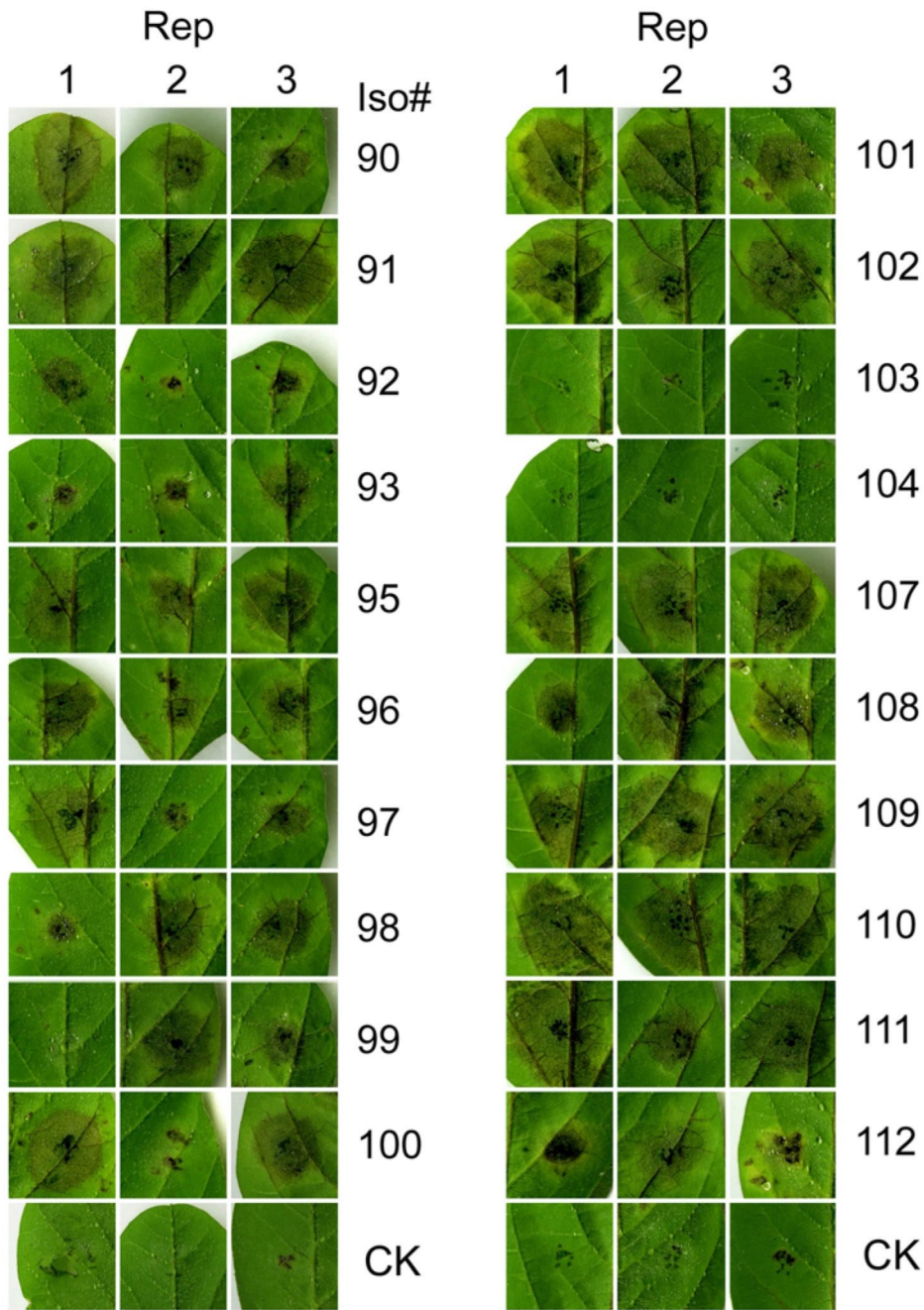


Guignardia

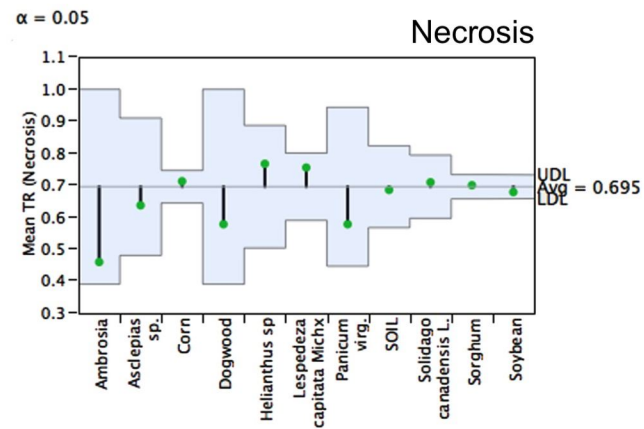
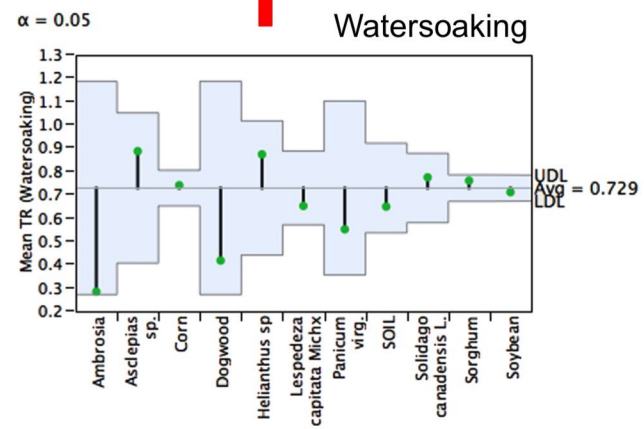
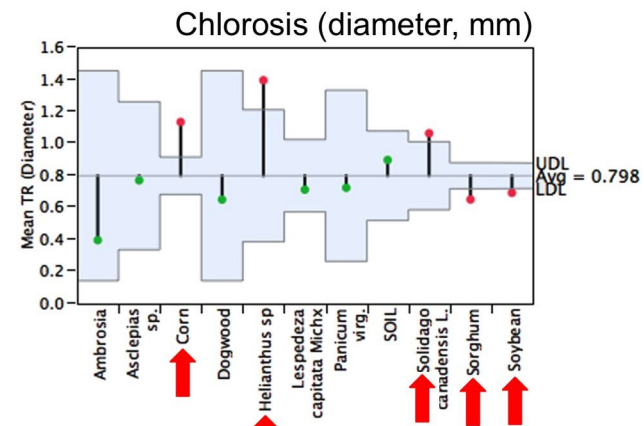


Phyllosticta

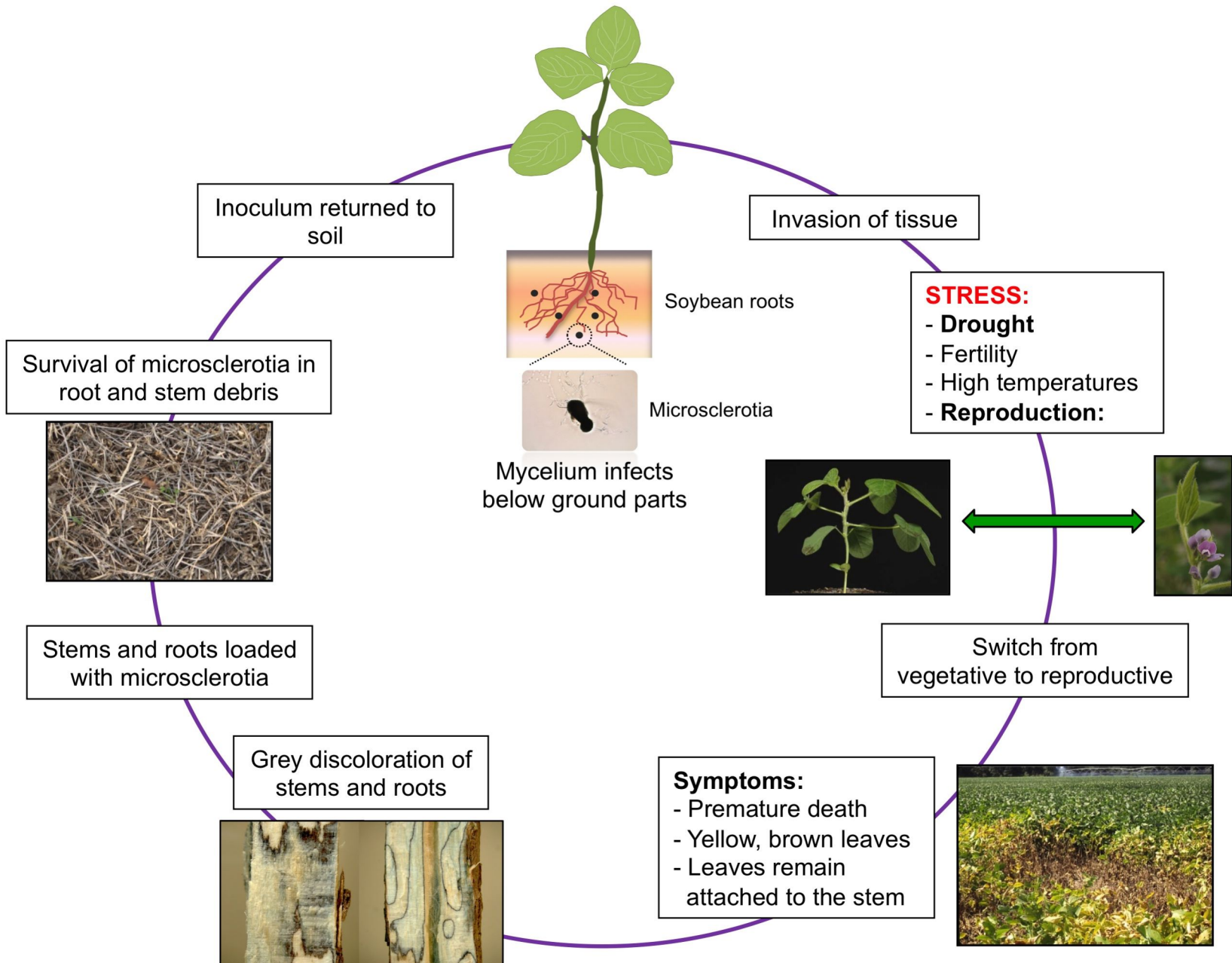




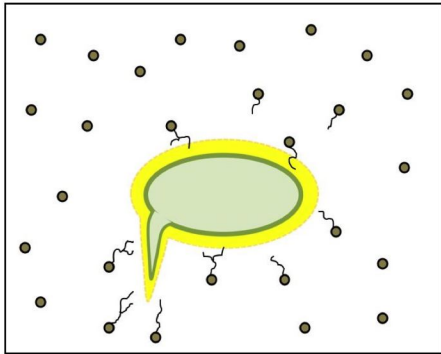
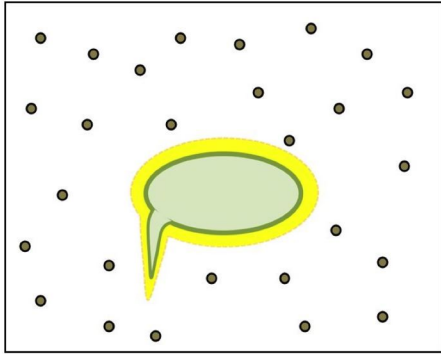
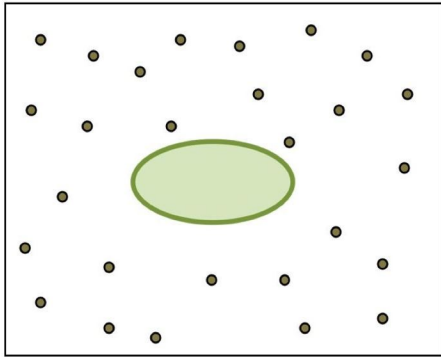
Detached soybean leaves (1 wk. trifoliolate, cv. 'Pharoah')



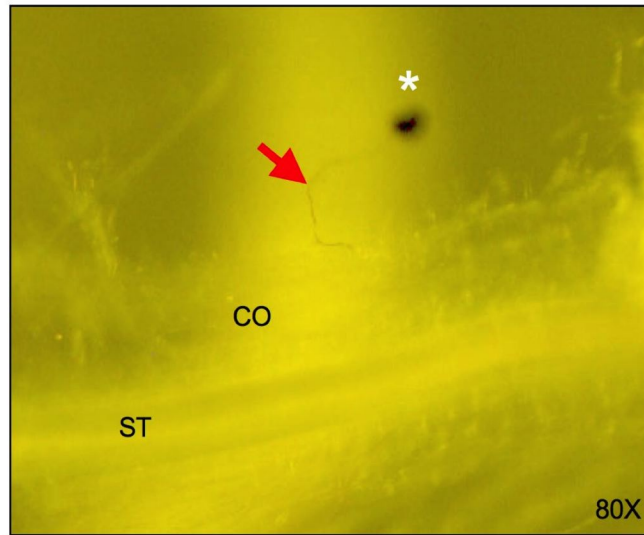
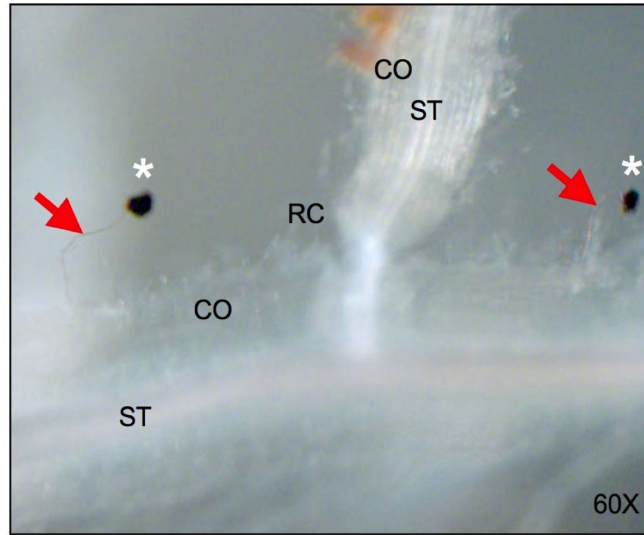
$\alpha = 0.05$



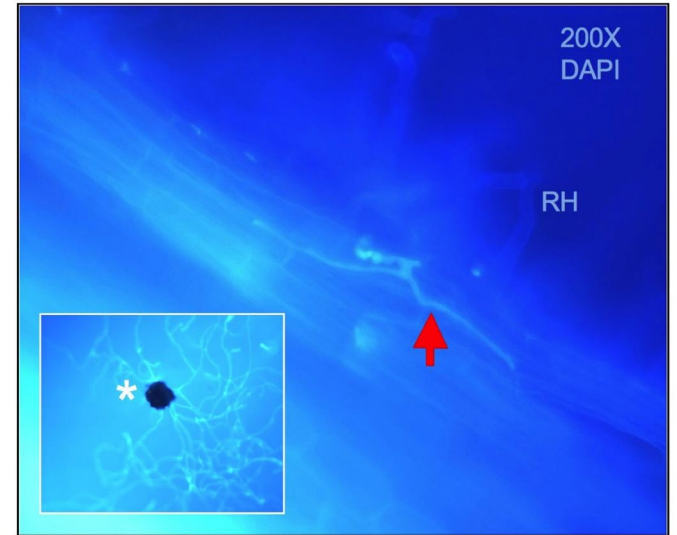
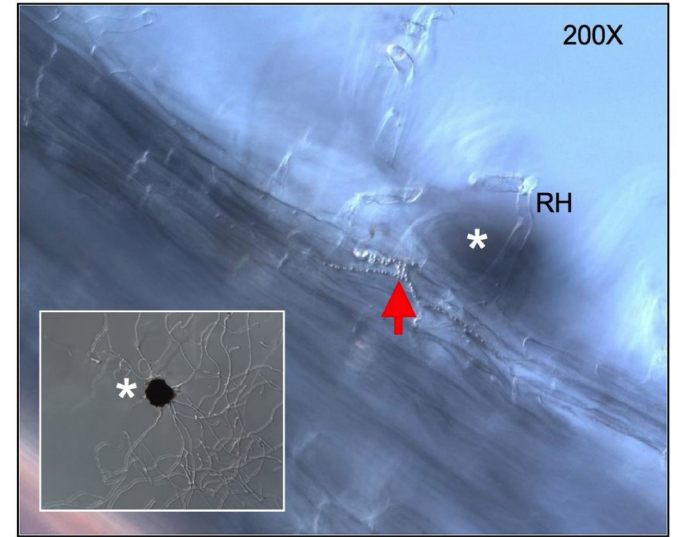
Disease cycle of charcoal rot in soybean (*Macrophomina phaseolina*)



**MICROSCLEROTIA
SURROUNDING A
GERMINATING
SEED**



SOYBEAN SEEDLING ROOT (V1)
* = MICROSCLEROTIA;
CO = CORTEX;
RC = ROOT CRACK; **ST** = STELE;
HYPHAE = red arrow



SOYBEAN SEEDLING ROOT (V1)
* = MICROSCLEROTIA;
RH = ROOT HAIR;
RUNNER HYPHAE = red arrow

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CONSTRAINTS AND PRIMARY OBJECTIVE

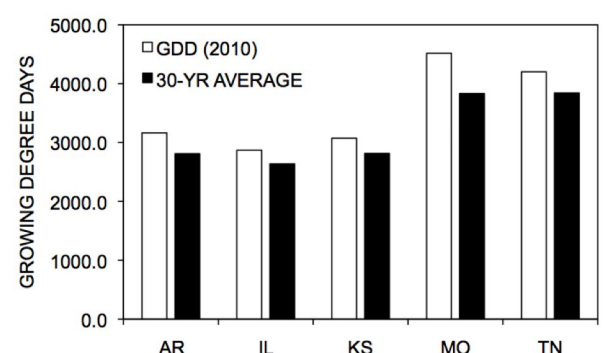
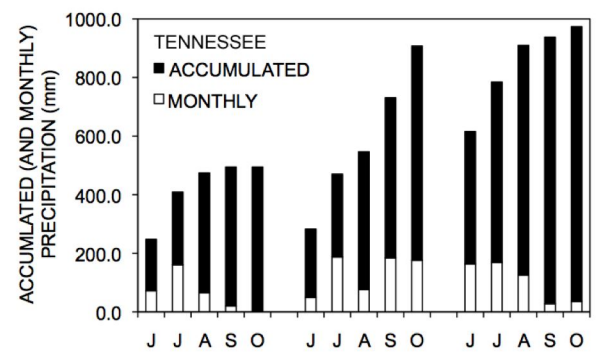
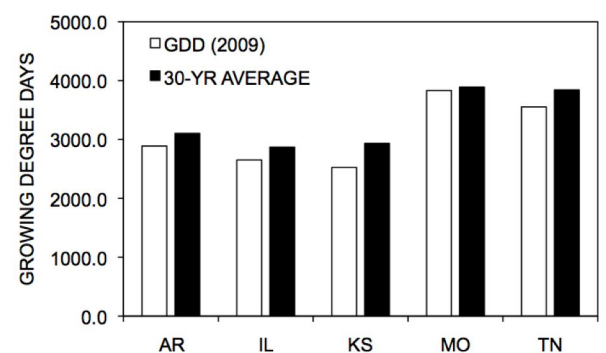
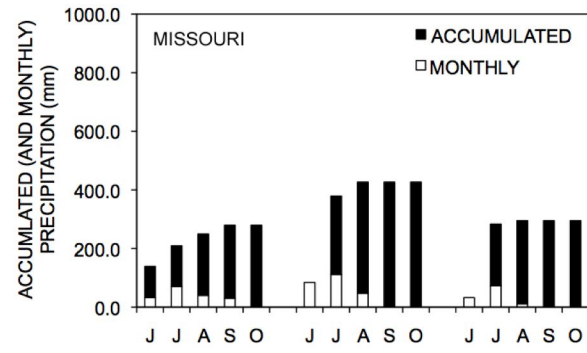
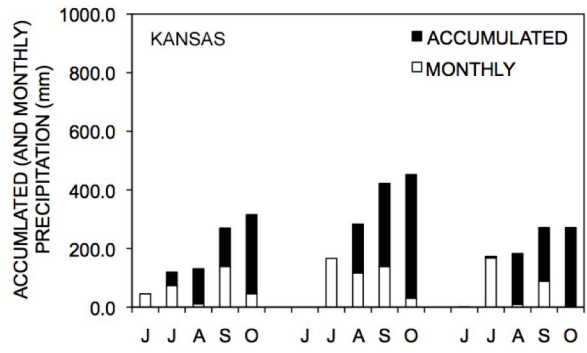
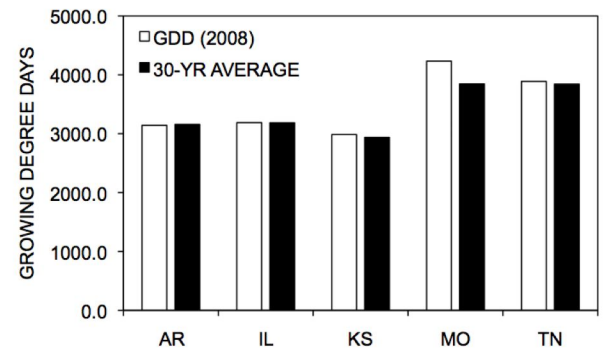
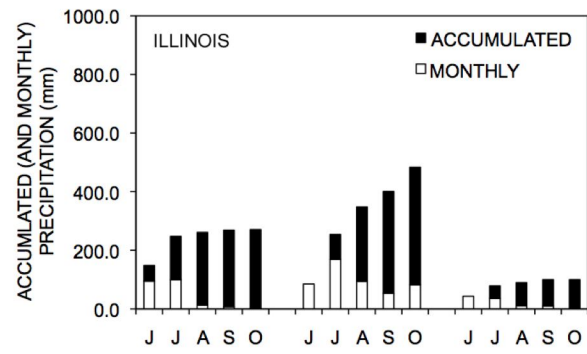
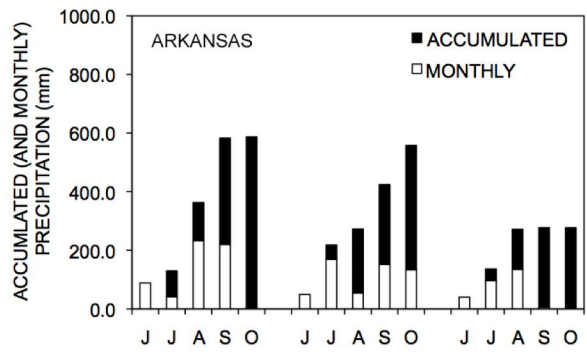
“Charcoal rot cultivar evaluation using adapted and exotic sources of resistance.” (USB #8247/9247)

Constraint #1 Field screening methods not standardized across locations (e.g. states).

Constraint #2 Charcoal rot is favored by heat and drought; environmental conditions vary widely between test locations.

Constraint #3 Lack of “standard” susceptible and resistant checks.

Performance Measure: Identify and confirm the reactions of soybean varieties and lines with resistance across multiple environments.



Precipitation (monthly and accumulated) and growing degree days for the five locations used in the multi-environment test.

EXPERIMENTAL PLOTS AND GENOTYPES

Field plots were established from 2008 to 2010 in five locations for a total of fifteen environments:

- (1) **Rowher, Arkansas** (Herbert silt loam)
- (2) **Carmi, Illinois** (Armiesburg silty clay loam)
- (3) **Columbus, Kansas** (Cherokee silt loam)
- (4) **Portageville, Missouri** (Tipton sandy loam)
- (5) **Jackson, Tennessee** (Dexter fine-silty loam)

Seeds were treated with ApronMaxx + Moly (0.8 ml/kg mefanoxam + fludioxinil + molybdenum) prior to planting.

The experiments were conducted at the same locations each year and genotypes were planted in two to four rows (3.0 × 6.1 m) in randomized complete block design with three to six replications depending upon environment and seed availability.



- ★ ROHWER, ARKANSAS (Herbert silt loam)
- ★ CARMI, ILLINOIS (Armiesburg silty clay loam)
- ★ COLUMBUS, KANSAS (Cherokee silt loam)
- ★ PORTAGEVILLE, MISSOURI (Tiptonville sandy loam)
- ★ JACKSON, TENNESSEE (Dexter fine-silty loam)

Genotypes, locations, years and replications tested in the regional charcoal rot field screening trial.

MG	Genotype	Arkansas			Illinois			Kansas			Missouri			Tennessee			Charcoal rot resistance?
		08	09	10	08	09	10	08	09	10	08	09	10	08	09	10	
3	Croton ^w	3 ^x	4	4	4	6	5	3	4	3	4	4	3	3	4	3	CR susceptible
3	Hamilton ^y	-	-	4	-	-	4	-	-	-	-	-	-	-	4	3	--
4	DP4546	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
4	LS98-0719	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
4	LS98-1430	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
4	LS98-2574	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
4	LS98-3257	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
4	DK4866 ^y	3	4	-	4	6	-	3	4	3	4	4	3	3	4	3	--
4	DP3478 ^y	-	4	4	-	6	5	-	4	3	-	4	3	3	4	3	--
4	DT97-4290	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	Moderately resistant
4	LS98-0358	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	CR susceptible
5	DP105 ^y	3	4	-	-	-	-	-	-	-	-	-	-	-	-	-	--
5	DP5806	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
5	DT98-7533	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	Moderately resistant
5	DT99-1686	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	Moderately resistant
5	DT99-1748	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	Moderately resistant
5	DT99-1755	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	Moderately resistant
5	LS92-1088	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	?
5	Pharoah	3	4	4	4	6	5	3	4	3	4	4	3	3	4	3	Susceptible
5	R01-581F ^y	-	4	4	-	6	-	-	-	3	-	4	3	-	-	-	--

^wCroton = MG 3.9; ^xThe number of replications are indicated in each cell of table;

^yGenotypes that were not tested in each environment were excluded from the detailed analyses.

Plots near Columbus, Kansas.



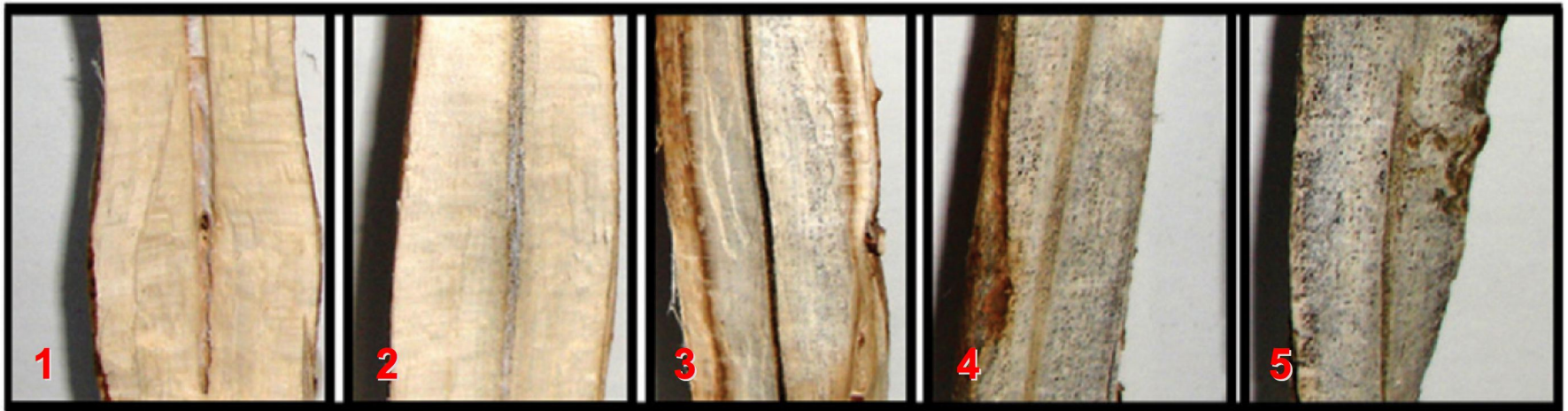
1. ROOT AND STEM SEVERITY (RSS)

RSS was assessed on ten randomly selected plants for each plot at the R7 growth stage.

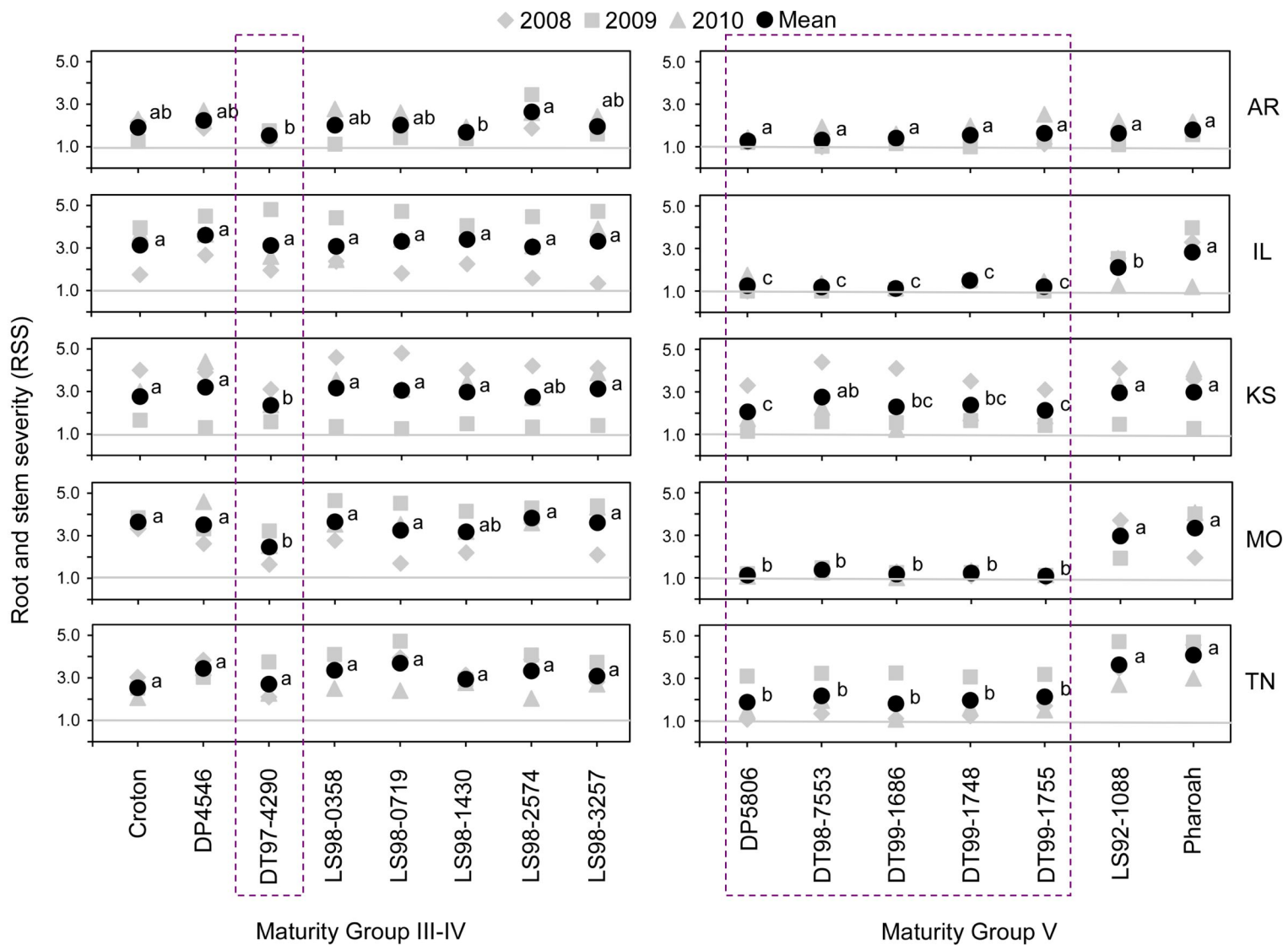
Stem and taproots were split longitudinally and visually rated for the level of gray discoloration and microsclerotia covering vascular and cortical tissue.

No discoloration

Gray discoloration &
numerous microsclerotia



← R → ← MR → ← MS → ← S →



L = location;
Y = year;
G = genotype

L	< 0.0001	Y*G	0.0044
Y	0.0029	L*G	< 0.0001
G	< 0.0001	L*Y*G	< 0.0001
L*Y	< 0.0001	Model	< 0.0001

L	< 0.0001	Y*G	0.0427
Y	0.0002	L*G	< 0.0001
G	< 0.0001	L*Y*G	0.0101
L*Y	< 0.0001	Model	< 0.0001

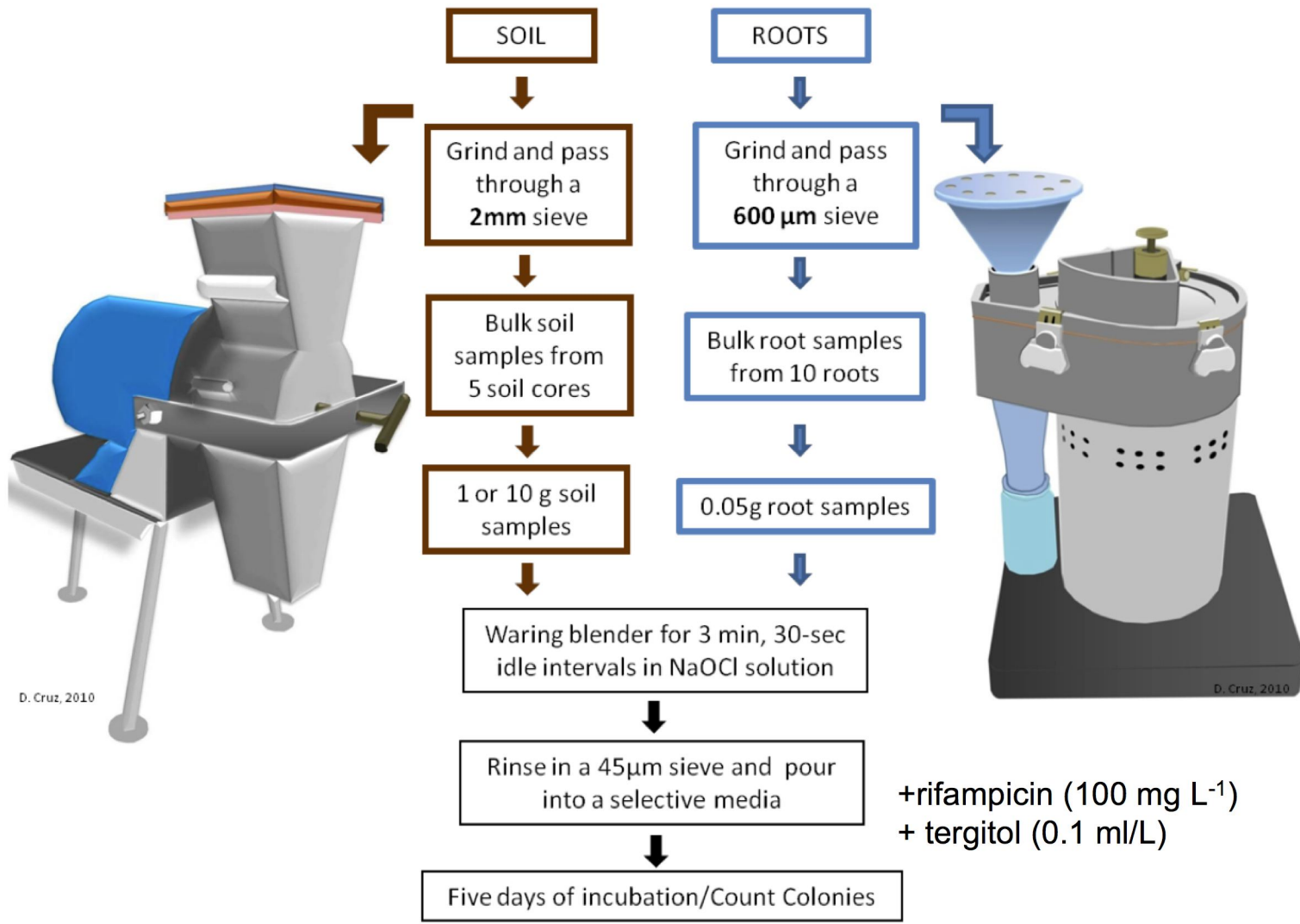
2. ROOT COLONY FORMING UNITS (CFU)

Samples that were collected for RSS were also used to determine CFUs.

Samples for CFU were taken from the lower stem and root, including lateral and fibrous roots of each plant, by excising the stem and roots just below the cotyledonary node.

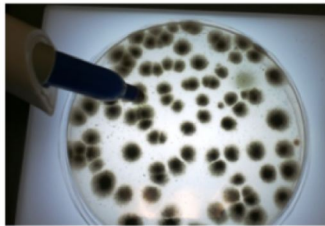
Ten stem parts and roots from each plot were thoroughly washed and rinsed in water to remove soil, air dried, and stored at 25°C.

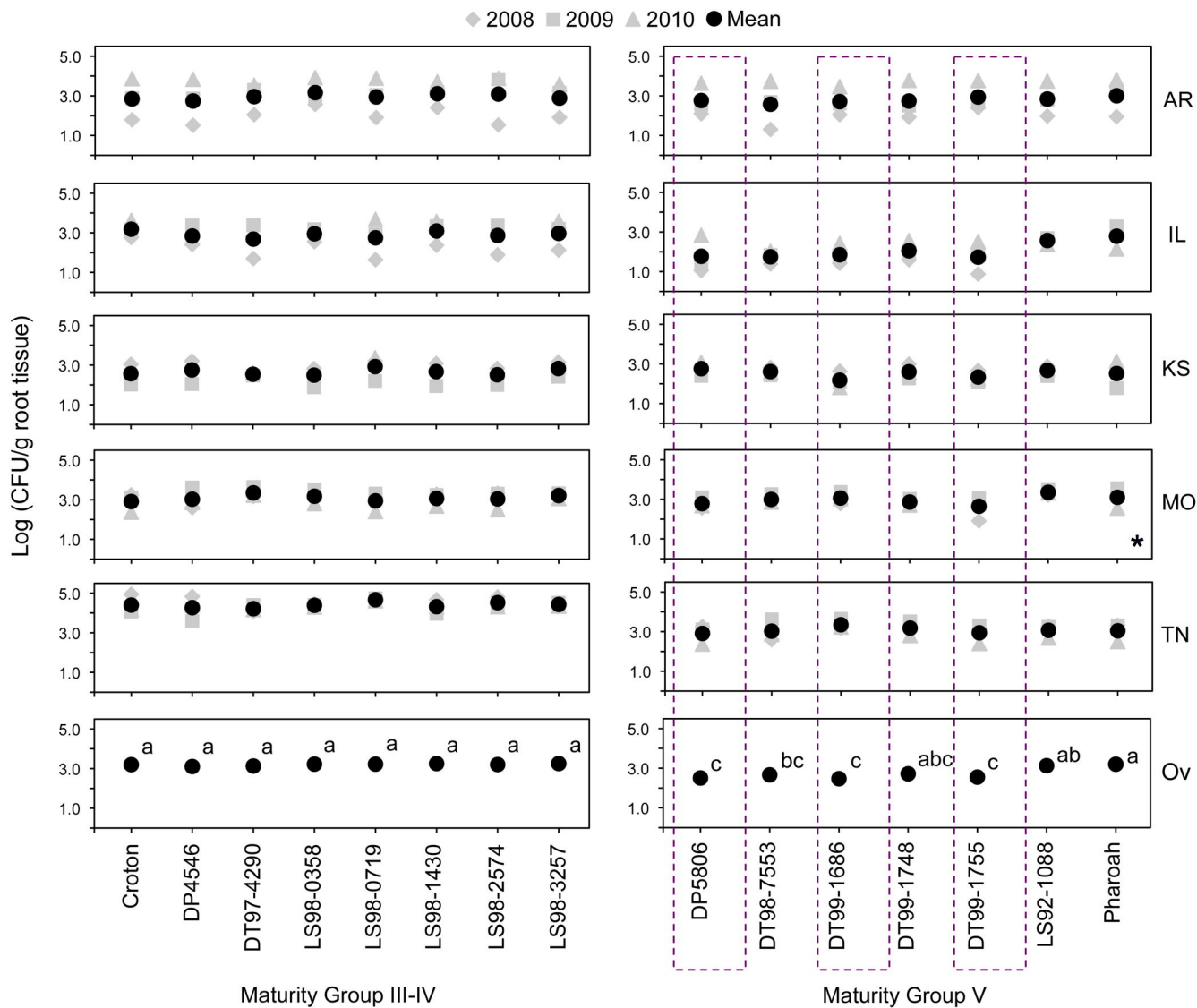




D. Cruz, 2010

D. Cruz, 2010



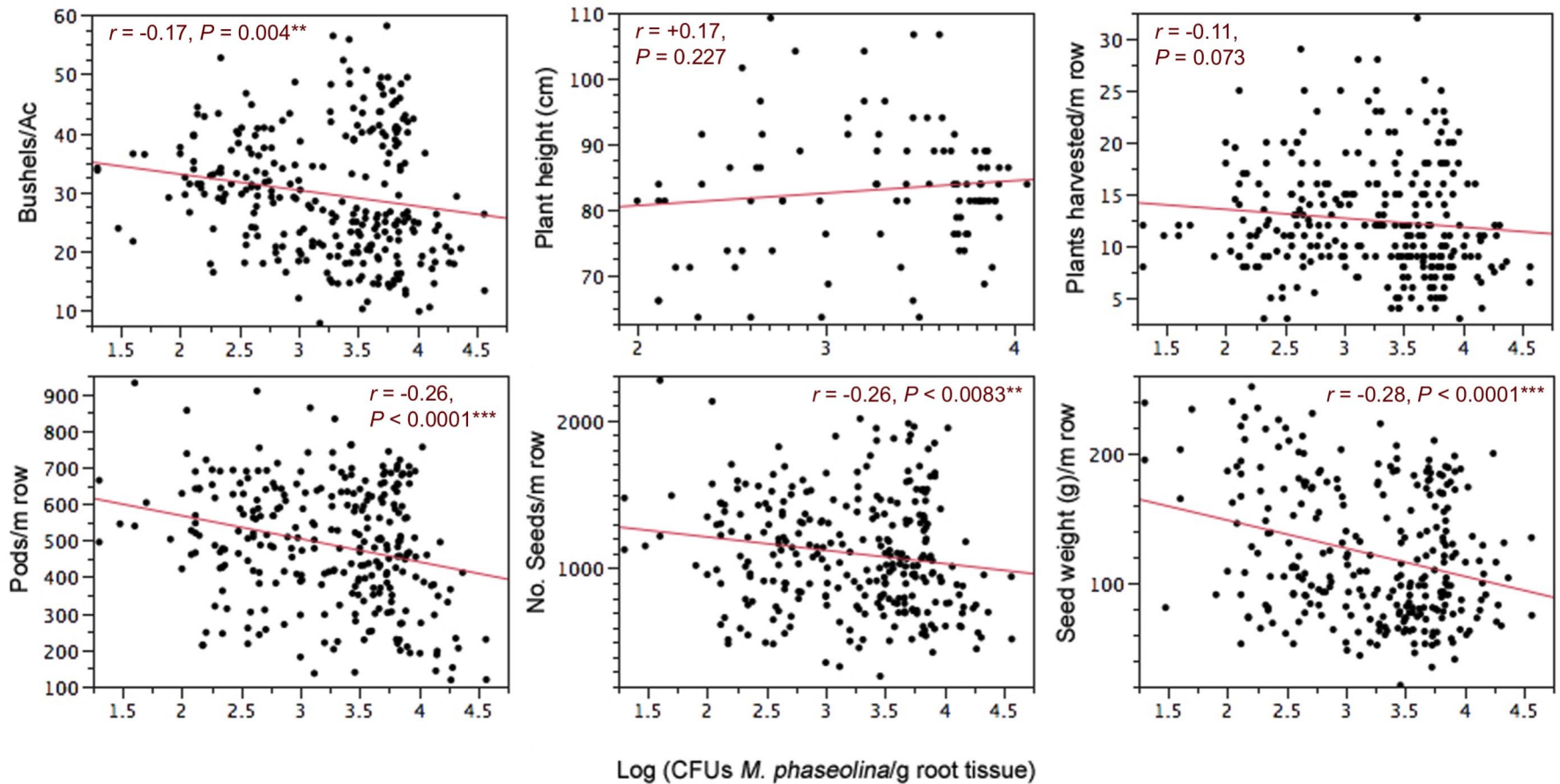


L = location;
Y = year;
G = genotype

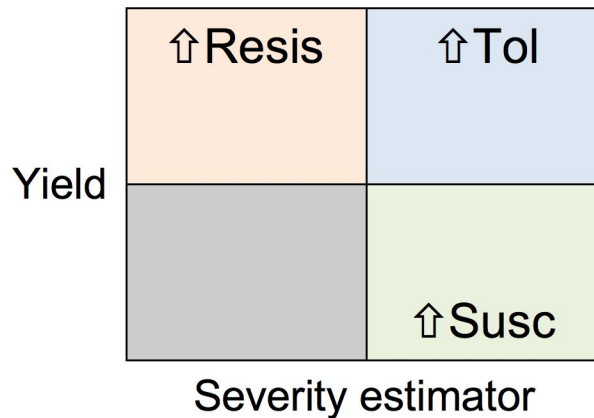
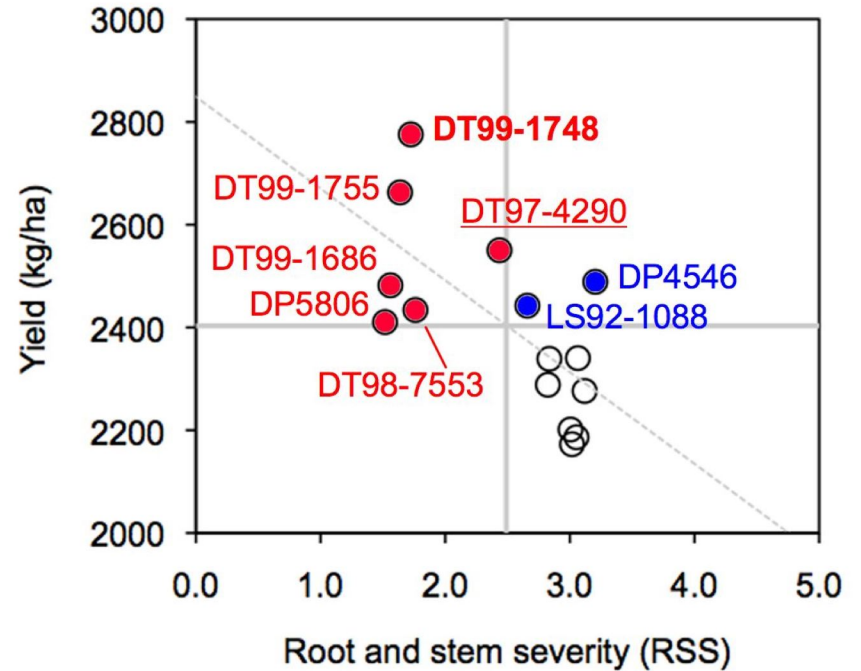
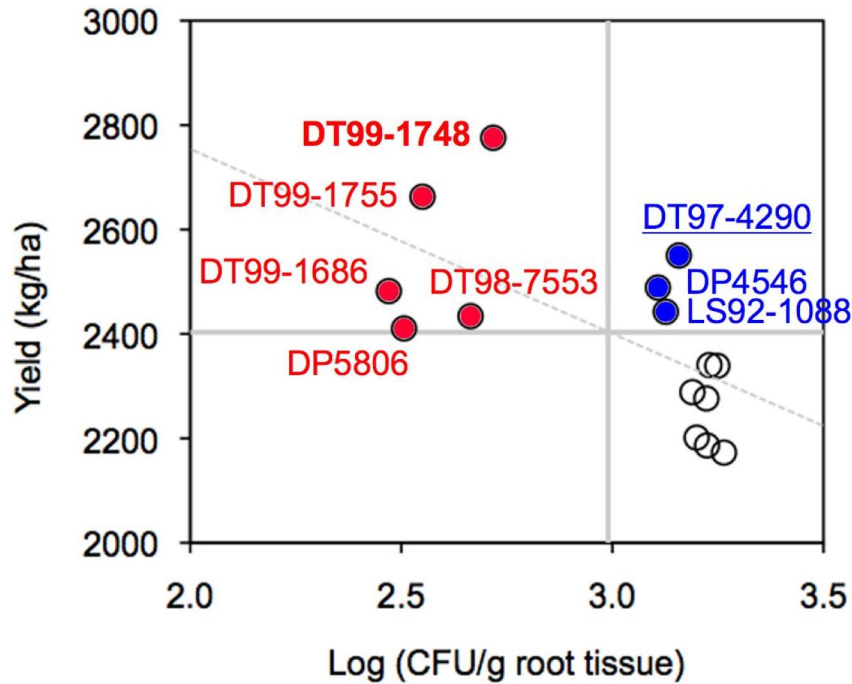
L	< 0.0001	Y*G	0.0106
Y	< 0.0001	L*G	0.0871
G	0.6863 ✗	L*Y*G	0.075
L*Y	< 0.0001	Model	< 0.0001

L	< 0.0001	Y*G	0.0026
Y	< 0.0001	L*G	< 0.0001
G	< 0.0001	L*Y*G	< 0.0001
L*Y	< 0.0001	Model	< 0.0001

Yield response ("**drag**") of soybean genotypes over a six year period under charcoal rot disease pressure in southeast Kansas.



Relationship between yield and severity parameters for test genotypes across 15 environments



Tolerance Index

$$\text{T.I.} = 1 - \frac{\left[\frac{\text{RANK}_{\text{severity}} + \text{RANK}_{\text{yield}}}{2} \right]}{\text{HIGHEST RANK}} = \text{Proportional Value}$$

RANK_{severity} = the lowest severity is ranked "1" and highest ranked "n".
Use root and stem severity value (RSS).

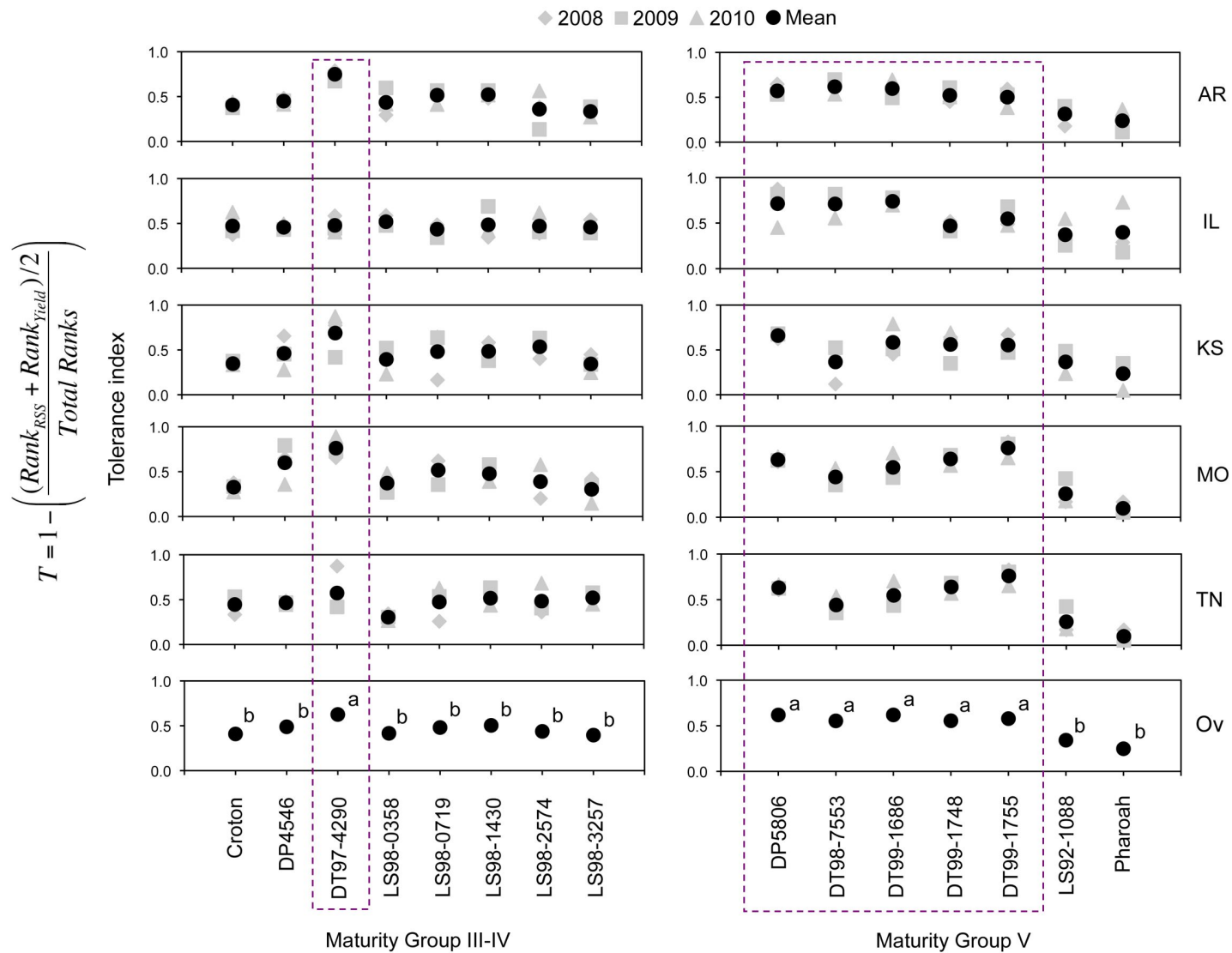
RANK_{yield} = the highest yield is ranked "1" and lowest ranked "n".

HIGHEST RANK = largest *n*th rank for both categories (should be the same number)

Proportional Value = High numbers are more tolerant than lower numbers.

Categories (based upon Ward's hierarchical clustering across 15 environments)

1 = 0.00 to 0.24, **2** = 0.25 to 0.48, **3** = 0.49 to 0.71, **4** = > 0.72



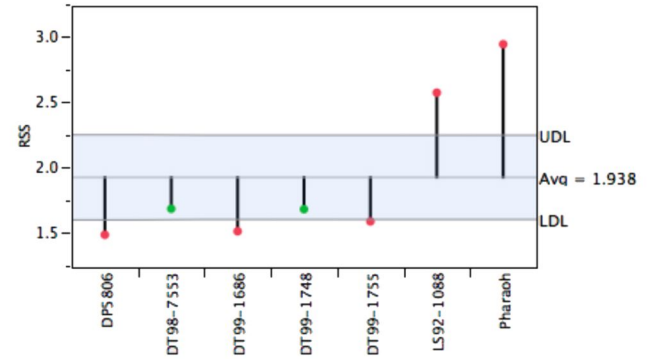
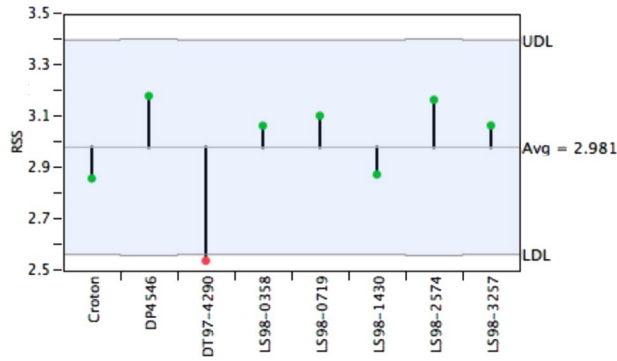
L = location;
Y = year;
G = genotype

L	0.0001	Y*G	0.0007
Y	< 0.8746	L*G	< 0.0001
G	< 0.0001	L*Y*G	< 0.0001
L*Y	< 0.9993	Model	< 0.0001

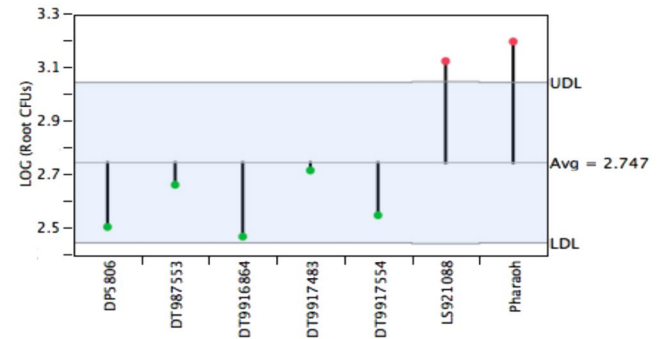
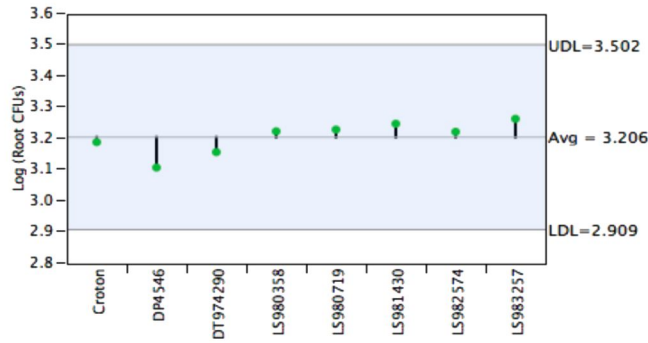
L	0.9861	Y*G	< 0.0001
Y	0.7318	L*G	< 0.0001
G	< 0.0001	L*Y*G	< 0.0001
L*Y	< 0.9663	Model	< 0.0001

Analysis of means for MGIII-IV and MG V genotypes

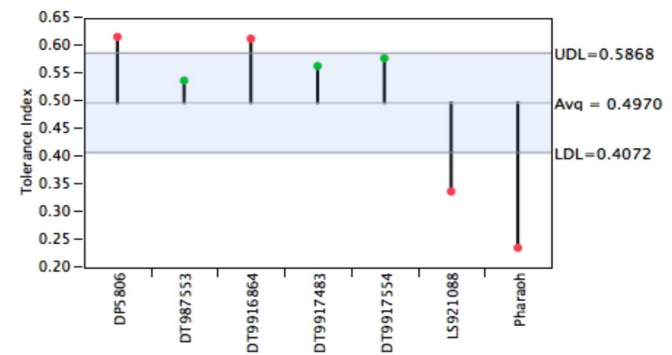
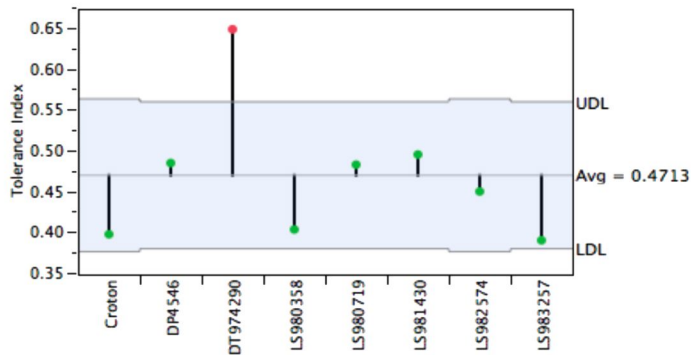
RSS



CFUs



Tolerance



Maturity Group III-IV

Maturity Group V

Resistance determination of genotypes tested in the regional charcoal rot field screening trial.

MG	Genotype	Charcoal rot resistance?	2008 - 2010 study ^z	Tolerance index ^a
3	Croton	CR susceptible	MR	2
3	Hamilton ^y	?	MR	--
4	DP4546	?	MS	2-3
4	LS98-0719	?	MS	2-3
4	LS98-1430	?	MR	3
4	LS98-2574	?	MS	2
4	LS98-3257	?	MS	2
4	DK4866 ^y	?	MS	--
4	DP3478 ^y	?	MR	--
4	DT97-4290	Moderately resistant	MR	3
4	LS98-0358	CR susceptible	MS	2
5	DP105 ^y	?	MR	--
5	DP5806	?	MR	3
5	DT98-7533	Moderately resistant	MR	3
5	DT99-1686	Moderately resistant	MR	3
5	DT99-1748	Moderately resistant	MR	3
5	DT99-1755	Moderately resistant	MR	3
5	LS92-1088	?	MS	2
5	Pharoah	Susceptible	MS	1
5	R01-581F ^y	?	MR	--

^xThe number of replications are indicated in each cell of table; ^yGenotypes that were not tested in each environment were excluded from the detailed analyses; ^zResistance based upon RSS scale (Mengistu et al., 2007); ^aTolerance index (1 = low tolerance, 4 = high tolerance; "--" = not calculated).

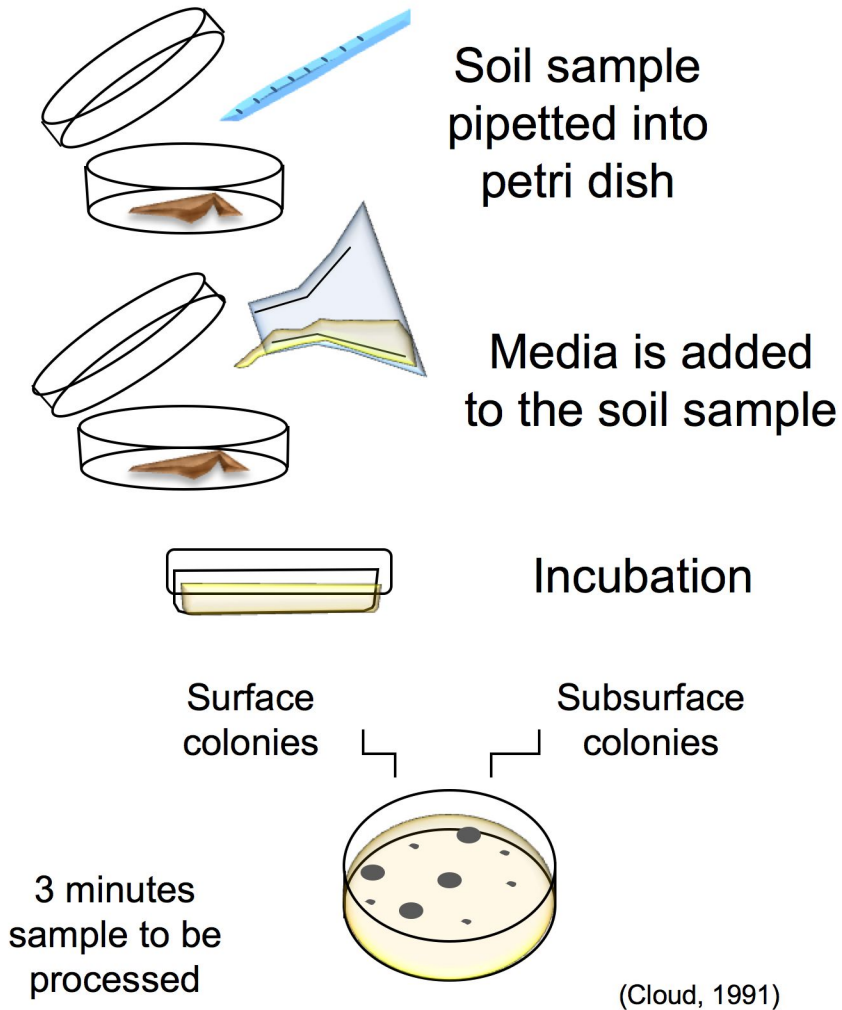
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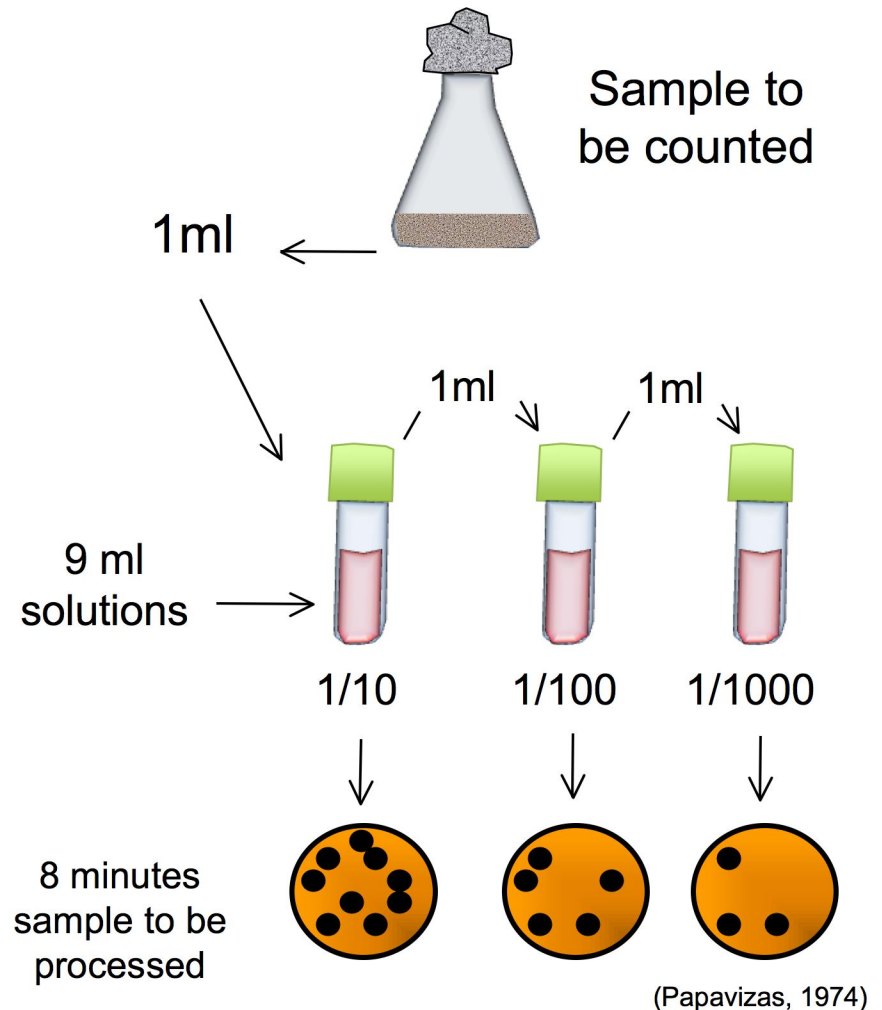


Methods for quantification and detection of *M. phaseolina* from soil

Direct counting method



Serial dilution method



Linear relationships between soil populations of *M. phaseolina*, severity (RSS and PHSD), root colonization (CFUs), and yield (kg/ha) across the Portageville, Missouri and Columbus, Kansas test sites.

		RSS	Root CFU	PHSD	Yield
Pre-soil	ρ	+0.195	+0.338	+0.092	+0.504
	P	0.0228*	< 0.0001***	0.2864	< 0.0001***
Post-soil	ρ	+0.217	-0.089	+0.235	-0.270
	P	0.0113*	0.3026	0.006**	0.0015**

"Pre-soil" = soil populations of *M. phaseolina* at planting

"Post-soil" = soil populations at harvest

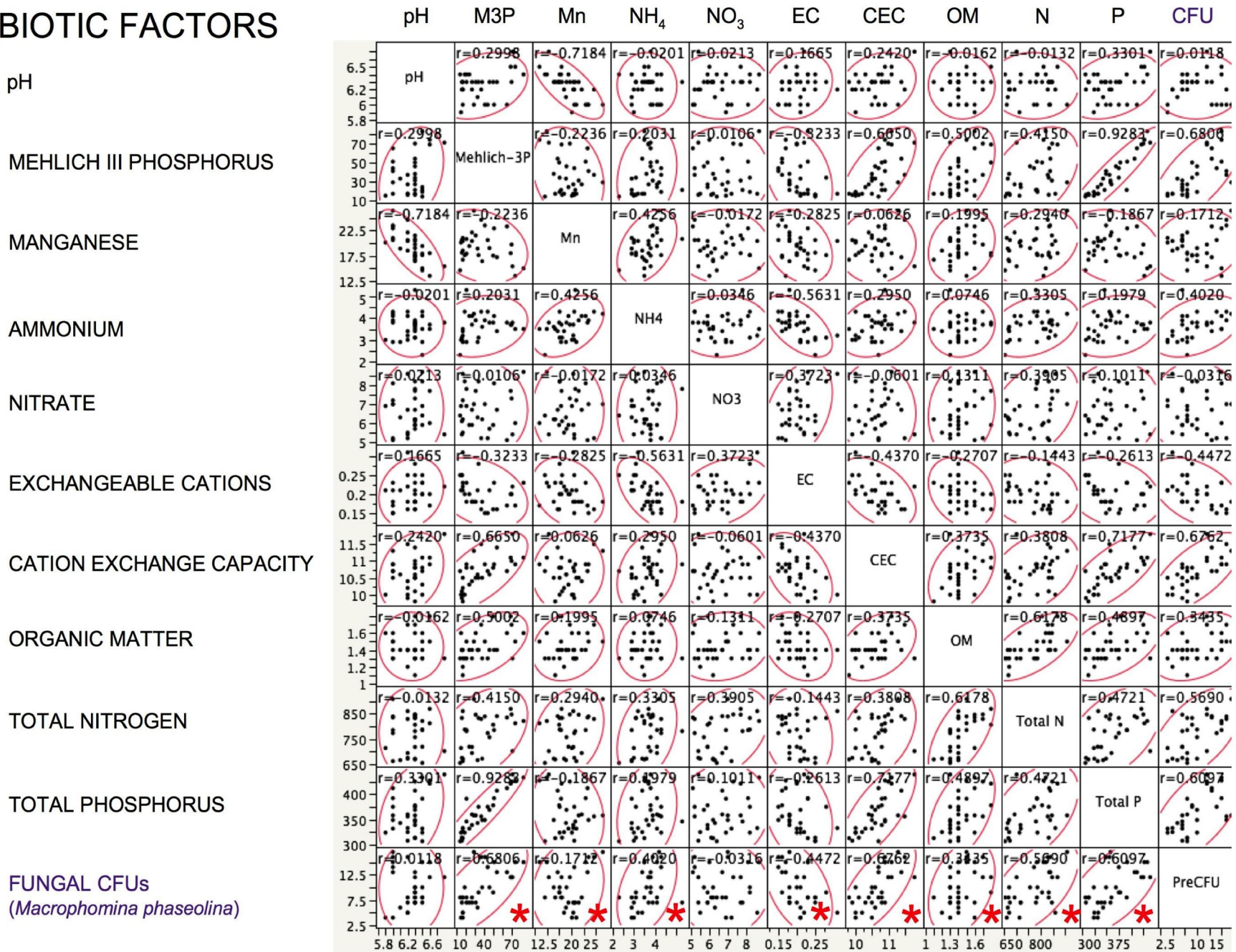
"RSS" = root and stem severity

"Root CFU" = root populations of *M. phaseolina* at R7 stage

"PHSD" = proportion (or percent) stem height discoloration

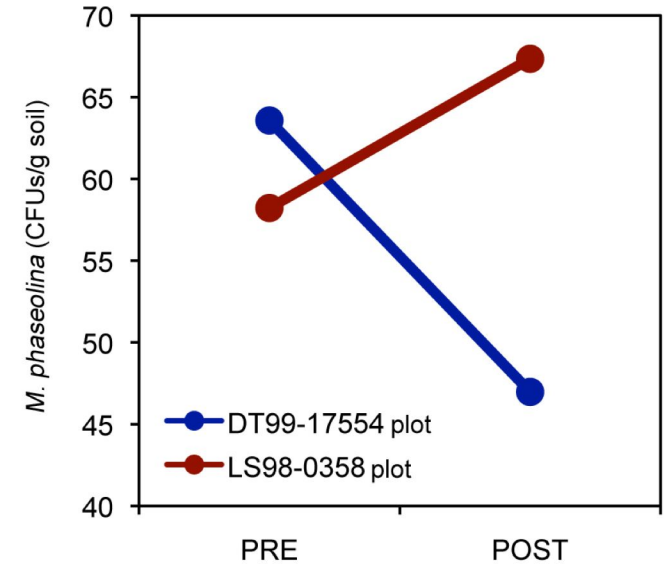
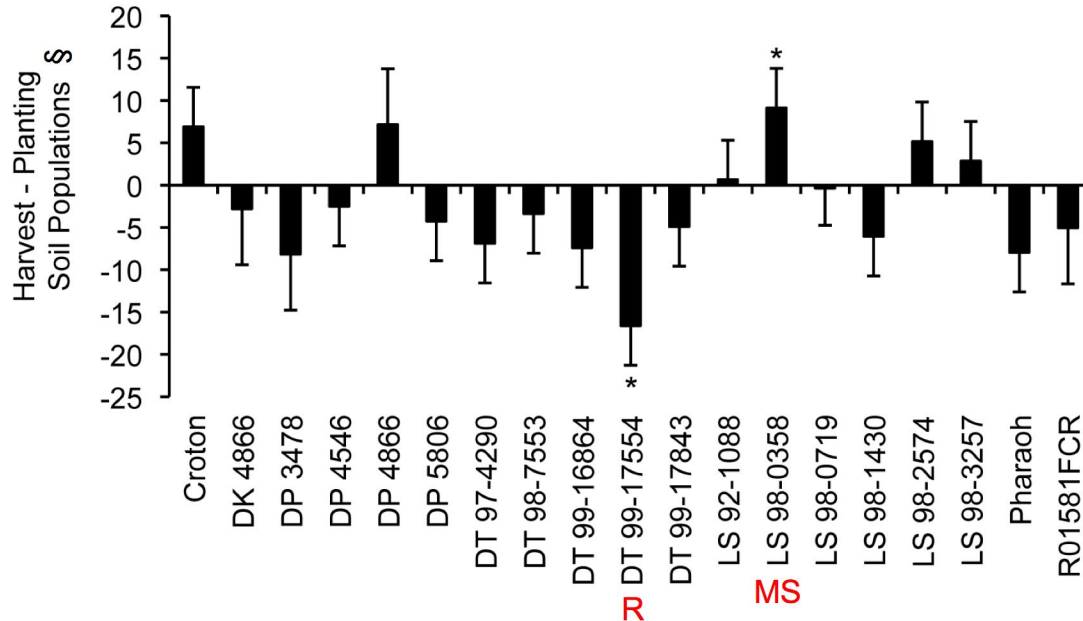
"Yield" = kg/ha

ABIOTIC FACTORS



*Correlations significant when $r > 0.33$ (DF = 32 - 1 = 31)

Does soybean genotype influence *M. phaseolina* populations in the soil?



*Significantly different for zero at $P < 0.05$

§Mean of the 2008 and 2009 seasons (Portageville, MO and Columbus, KS test sites)

"Pre" = *M. phaseolina* soil populations at planting

"Post" = *M. phaseolina* soil populations at harvest

Post - Pre:

> 0 = *M. phaseolina* soil population at harvest > at planting

0 = *M. phaseolina* soil population at harvest = at planting

< 0 = *M. phaseolina* soil population at harvest < at planting

SUMMARY

1. Significant **location*year*genotype** interactions exist for RSS, root CFUs, PHSD (not shown), and yield.
 - ◆ Training was held at Jackson, Tennessee for severity measures in order to minimize variability in data collection.
 - ◆ Environmental variation was high; more data analysis in progress.
2. Genotype differences were more apparent in MG V entries compared to MGIII-IV.
 - ◆ This may be due to a planting date effect; more analysis here.
 - ◆ No genotype differences for root CFUs in MGIII-IV across env.
3. A **tolerance index** is proposed that incorporates **severity** (RSS) and **yield**.
 - ◆ Severity and yield are balanced in the formula, but this does not have to be so.
 - ◆ Root CFUs are highly variable and less reliable than RSS, PHSD.

SUMMARY (cont)

4. **ANOM** for RSS, root CFUs, and tolerance index could be used to identify test entries with superior (and inferior) performance across environments.

5. Soil pathogen populations across two locations (4 env.) were associated with:
 - 5a. **severity** (generally +)
 - 5b. **yield** (pre + (?), post -).

6. Soil pathogen populations were influenced by **host genotype**.
 - 5a. DT99-17855 (R), < MP at harvest
 - 5b. LS98-0358 (MS), > MP at harvest

