Improved diagnostic assays for SDS

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Which is SDS?

BSR

SDS

Dean Malvick, University of M
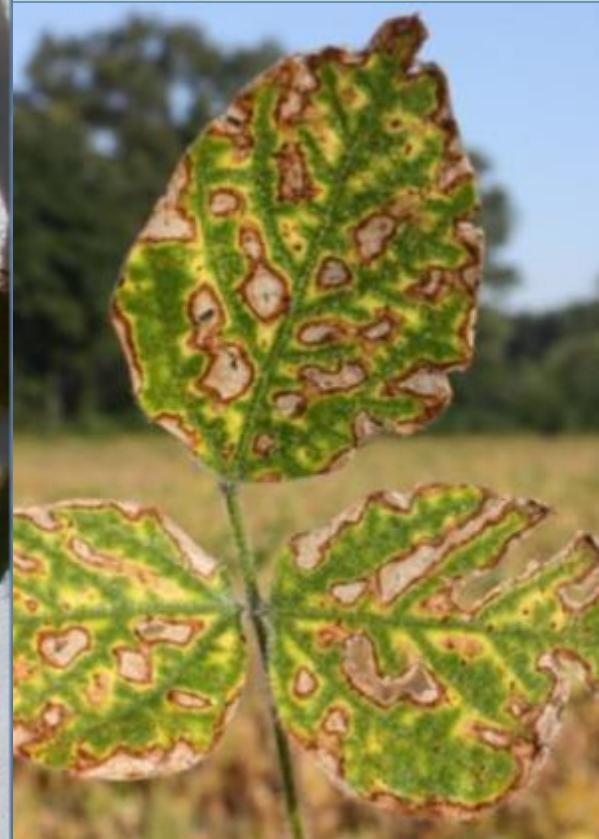
**BSR: Brown stem rot**

Sudden Death Syndrome (SDS)
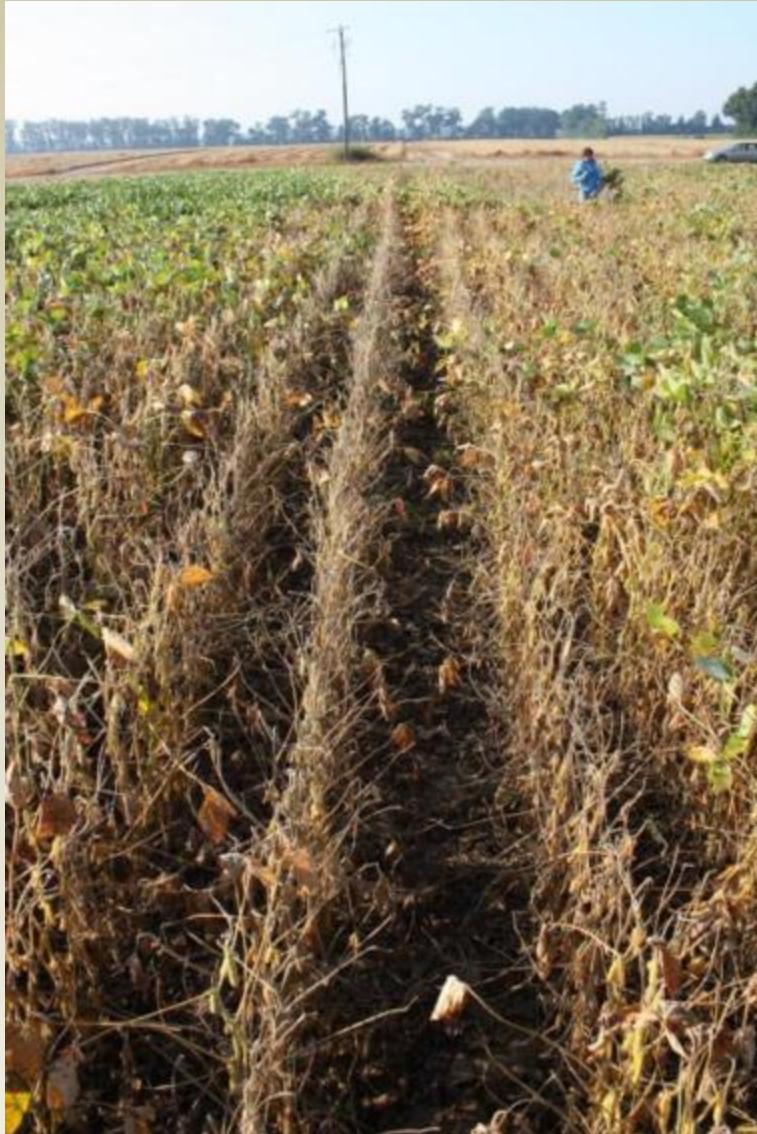
In recent years, SDS has become a significant concern in the Mid-West

Soybean SDS – Michigan

- 2009 - Confirmed SDS
- 2010 - Confirmed SDS
- 2011 - Confirmed SDS
- 2012 - Confirmed SDS
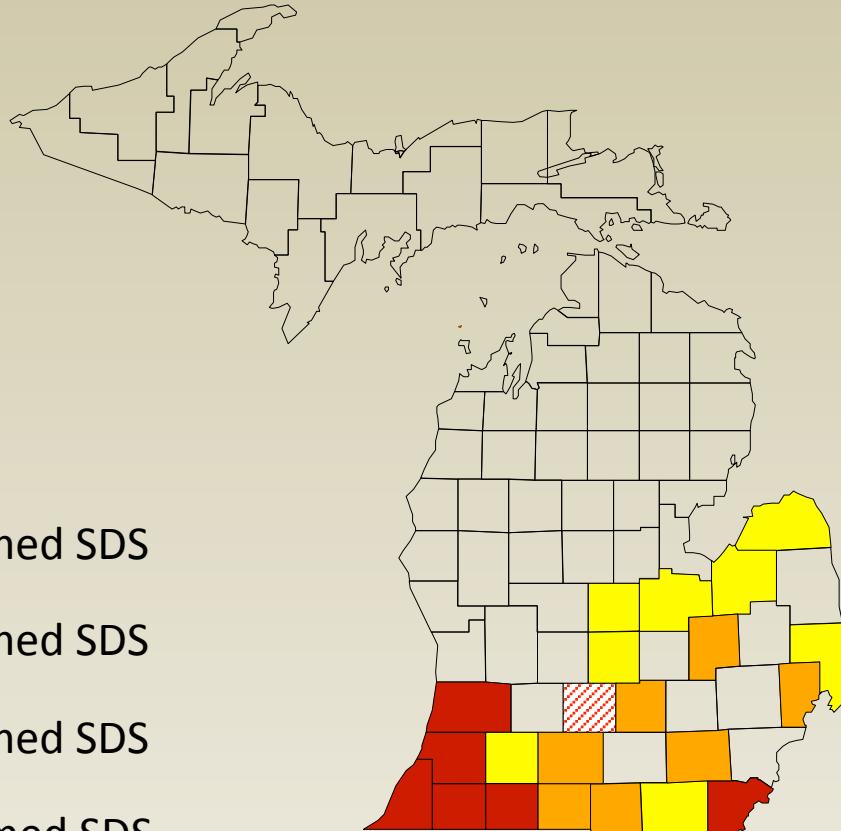
Prevalence and severity gradient from north to south

Fusarium virguliforme life cycle

Complex Soilborne Disease:
- **Root rot**
- **Foliar symptoms**

Why molecular tools?

- Disadvantages of conventional methods:
    - Low sensitivity (plant and soil isolation)
    - Difficult to identify colonies (slow growth rate)
    - Variable colony morphology
    - Time consuming

- Need for a rapid, quantitative, sensitive and reproducible assay: qPCR
   - Diagnostic assay
    - Research tool

Real-time qPCR assay

- **Hydrolysis probe chemistry**
  - R: reporter fluorescent molecule
 - Q: fluorescent quencher molecule
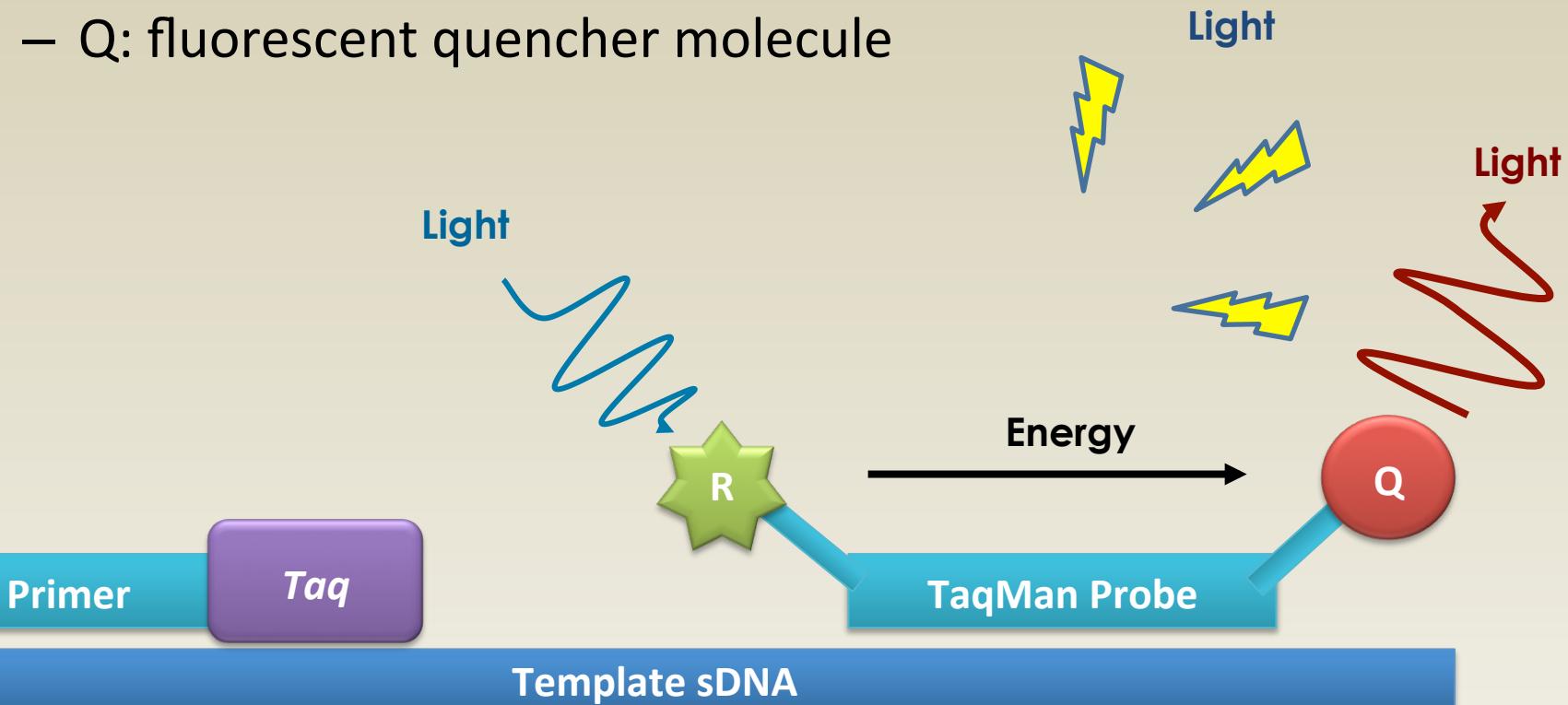
qPCR Serial Dilution Standard Curve
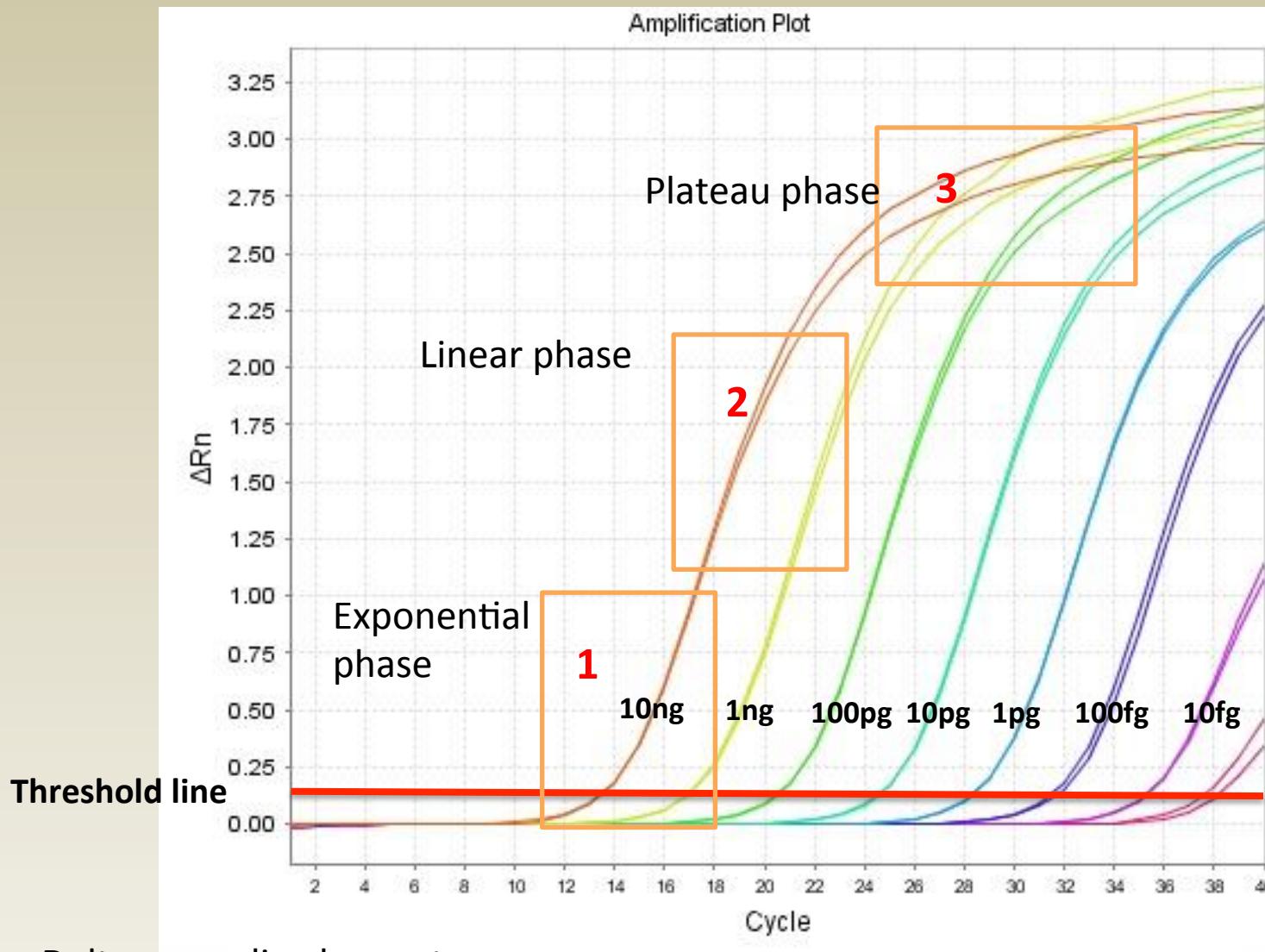
ΔRn: Delta normalized reporter

Unknown Sample Quantification

Standard Curve

$y = -3.547x + 36.098$
$R^2 = 0.998$

Ct (cycle threshold)

DNA quantity (fg)

1fg, 10fg, 100fg, 1pg, 10pg, 100pg, 1ng, 10ng

30 pg
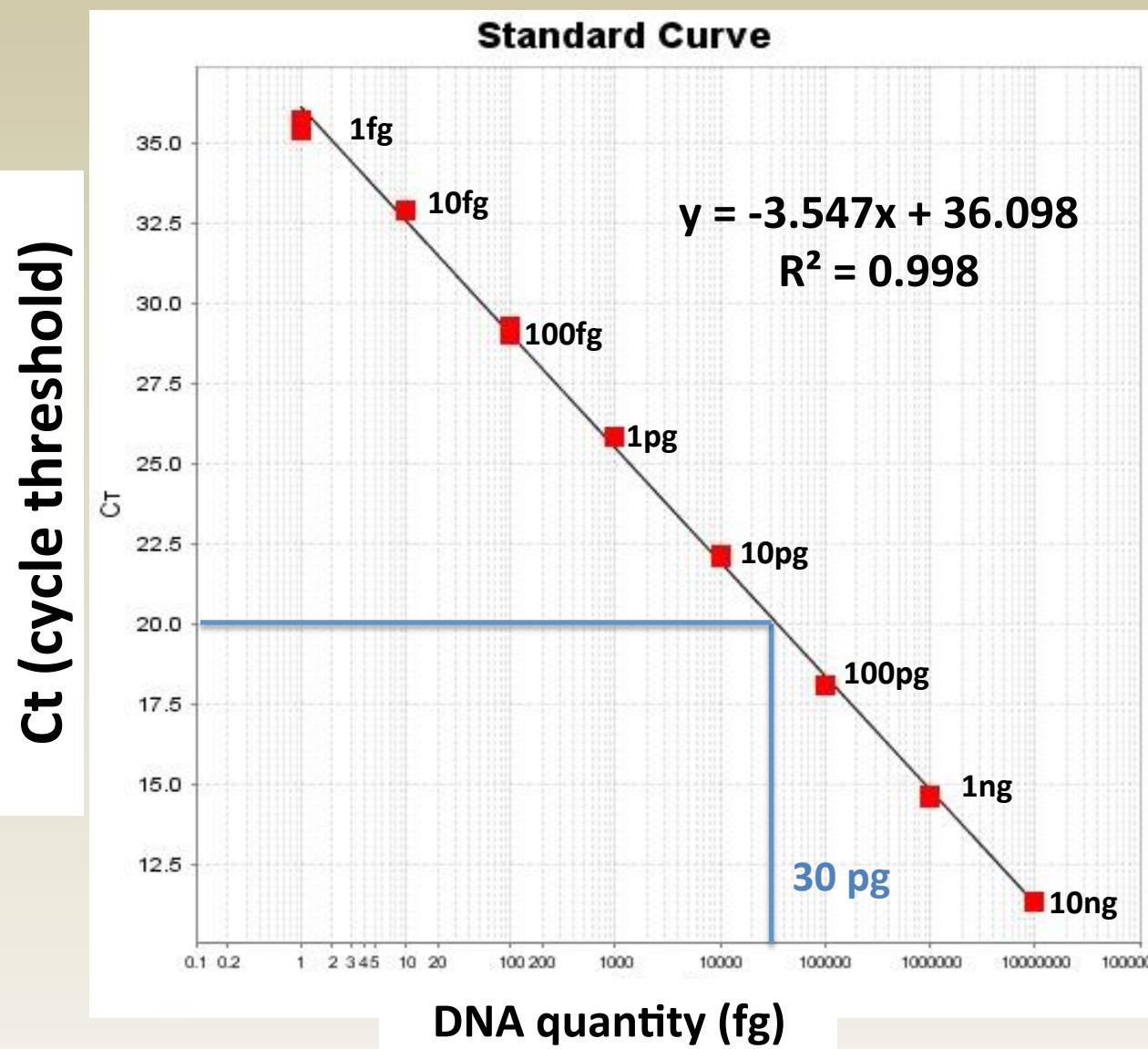
F. virguliforme diagnostic assays

Assay	Specific	Sensitivity	Loci
Gao et al. 2004	?	90fg	mtDNA SSU
Li et al. 2008	?	100fg	mtDNA SSU
Mbofung et al. 2011	Yes	25pg	Tox-1
Malvick and Westphal (unpub.)	-	-	-
Fakhoury et al. (unpub.)	-	-	-
Chilvers et al. (unpub.)	-	-	rDNA

F. solani f.sp. *glycines* - phylogenetics

IGS rDNA + locus 44
5016 bp
1 of 4 MPTs
151 steps
CI = 0.92
RI = 0.98

5 steps

100

100

100

100

100

100

100

100

100

100

100

36877
31949
43824
43825
46170
46171
46173
46175

F. crassistipitatum

22158
22275
31104
31157
36023

F. cuneirostrum

31776
31793
31096
34549
31950

F. tucumaniae

22276
31156

F. phaseoli

22678
31757
31762
34938

F. brasiliense

22825
31041
32392
34436
36896
36605

F. virguliforme

SDS Fusaria

BRR Fusaria

Aoki et. al. 2011

Target locus

Ribosomal DNA
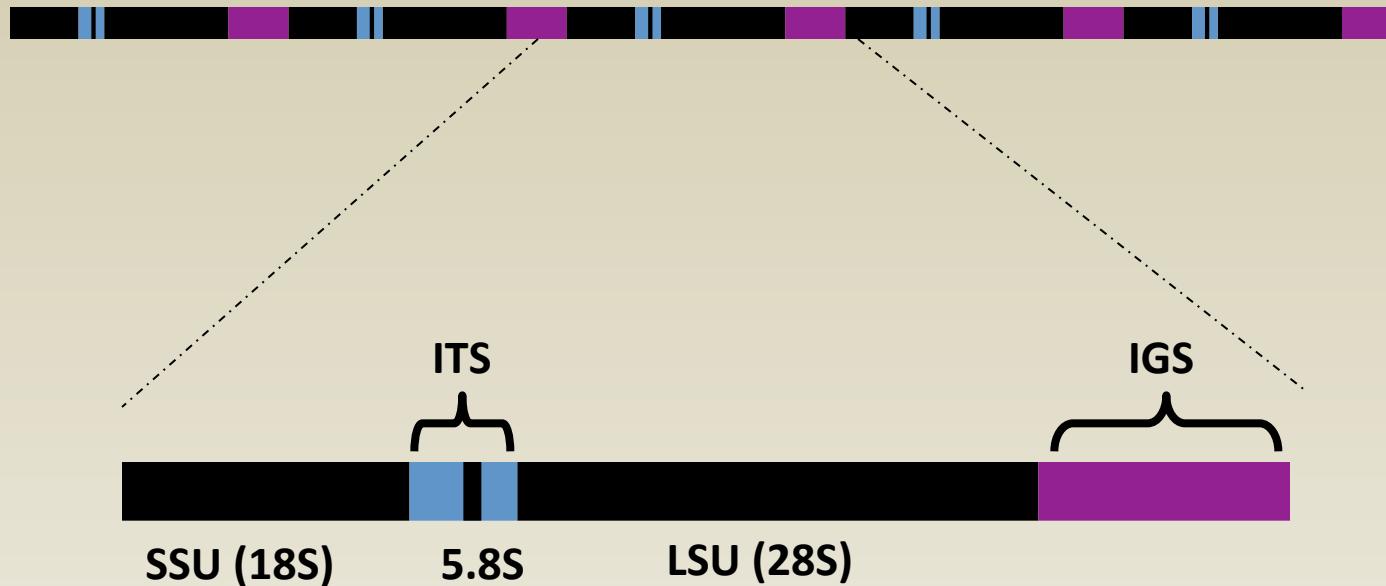
Assay test panel

Fusarium spp.		Others
F. oxysporum	*F. virguliforme* (5)	*Pythium sp.*
F. graminearum	*F. tucumaniae* (2)	*P. gregata* (A&B)
F. solani	*F. phaseoli* (2)	*P. sojae*
F. sambucinum	*F. brasiliense* (2)	*S. sclerotiorum*
F. avenaceum	*F. cuneirostrum* (1)	*R. solani*
F. acuminatum	*F. eumartii* (1)	
F. tricinctum	*F. javanicum (2)*	
F. torulosum	*F. crassistipitatum (1)*	Soybean
F. equiseti		Non-infested Soil
F. cerealis		Lambda phage

Detection of *F. virguliforme* in soil

$$y = -3.875 \log 10(x+1) + 38.775$$
$$R^2 = 0.9812$$

10 conidia/0.5 g soil

Ct Value

Log10(Conidia+1)
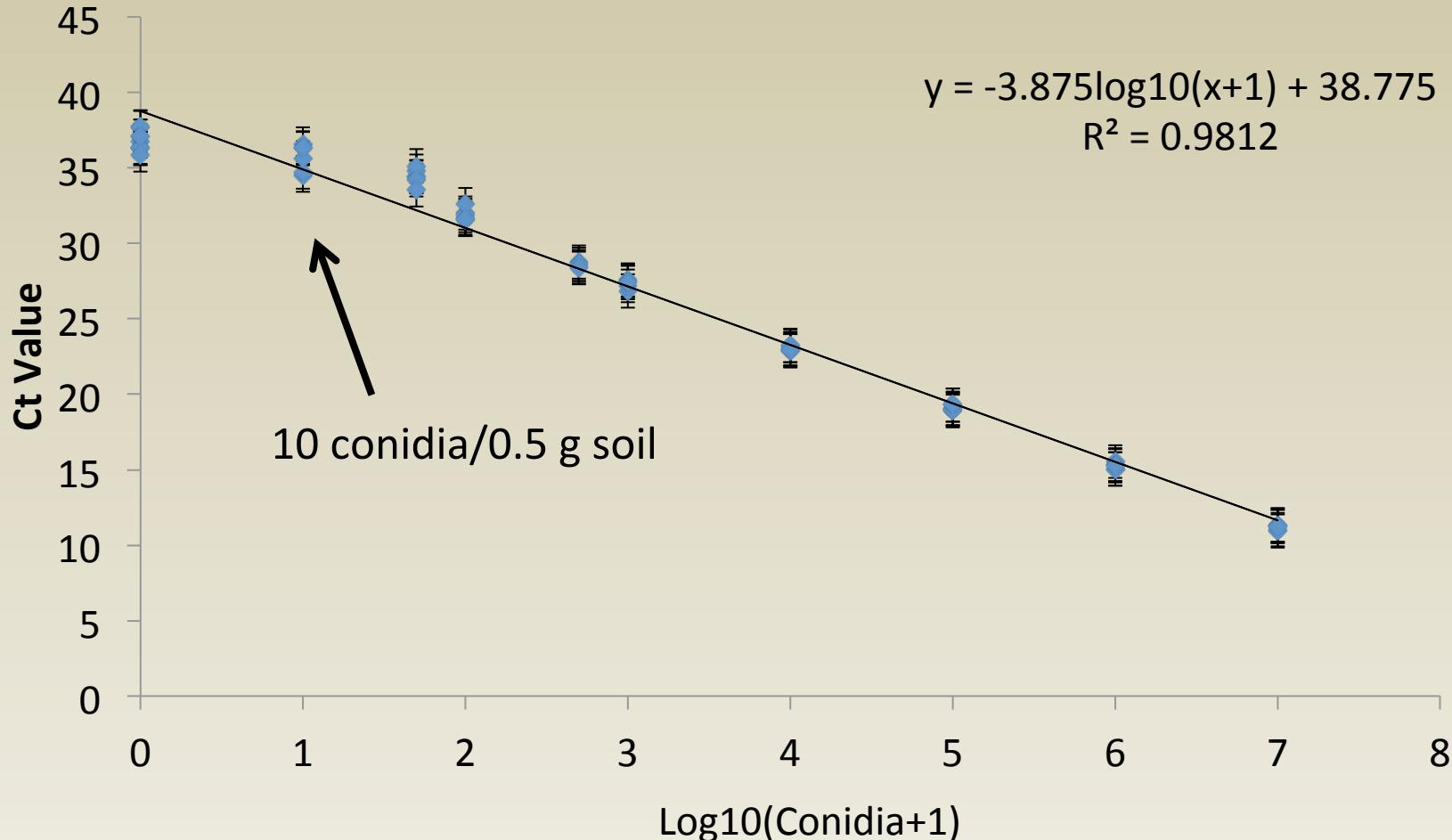
Phenotyping SDS resistance

Soybean germplasm screening (SIU)

- Estimate **disease incidence** (DI) from 0 to 100%

- Rate **disease severity** (DS), scale from 0 to 9

- **Disease index (DX)** = DI x DS / 9

DS= 0   **DS= 3**   **DS= 5**   **DS= 9**

Images: Bradley Serven

Commercial and MSU lines

ORIGINAL PAPER

Separate loci underlie resistance to root infection and leaf scorch during soybean sudden death syndrome

S. Kazi · J. Shultz · J. Afzal · J. Johnson · V. N. Njiti ·
D. A. Lightfoot

Disease index (Aug-22-11)
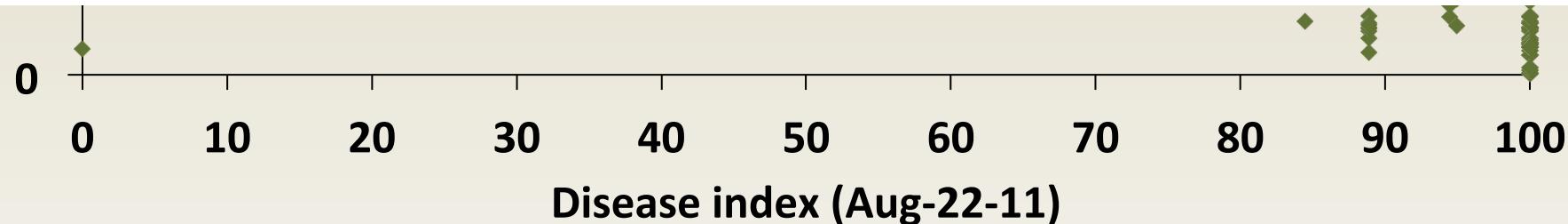
Application of qPCR

Step 1

Sample Collection
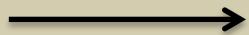
Step 2

Sample Processing
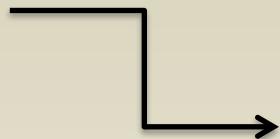
Step 3

DNA Extraction
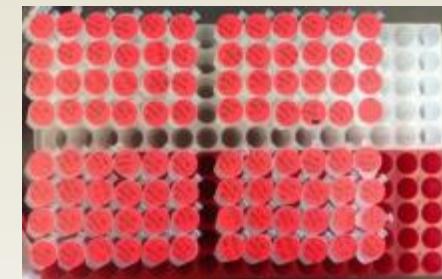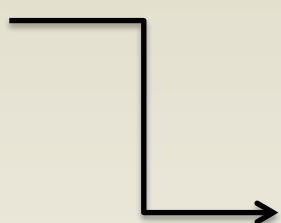
Application to mapping population

$y = 0.0166x + 4.2756$
$R^2 = 0.3129$
$p < 0.001$
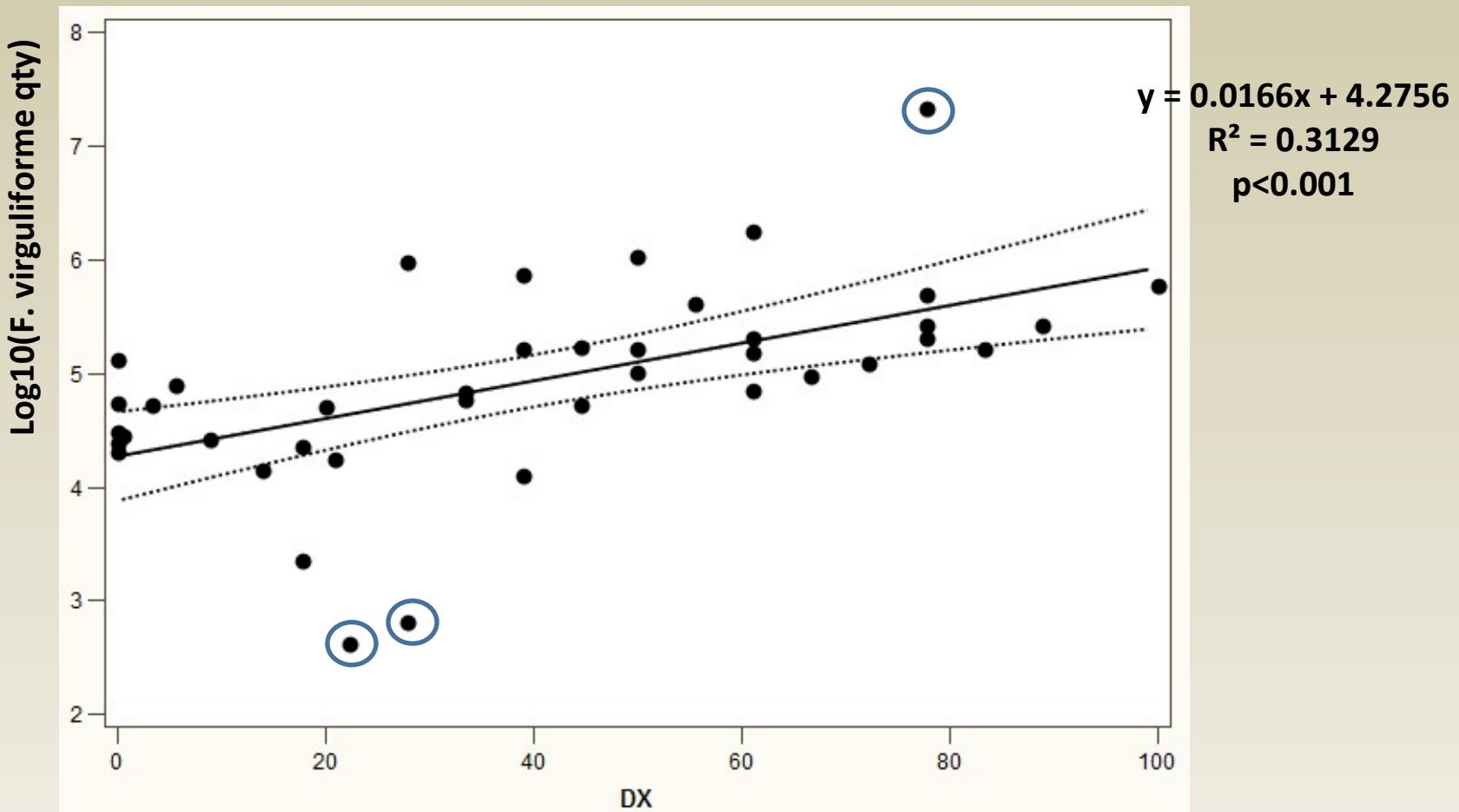
With outliers excluded, linear regression is still significant (P< 0.001)

# *F. virguliforme* time series study

Inoculated location

Time points 1-9 (V4 to R8)
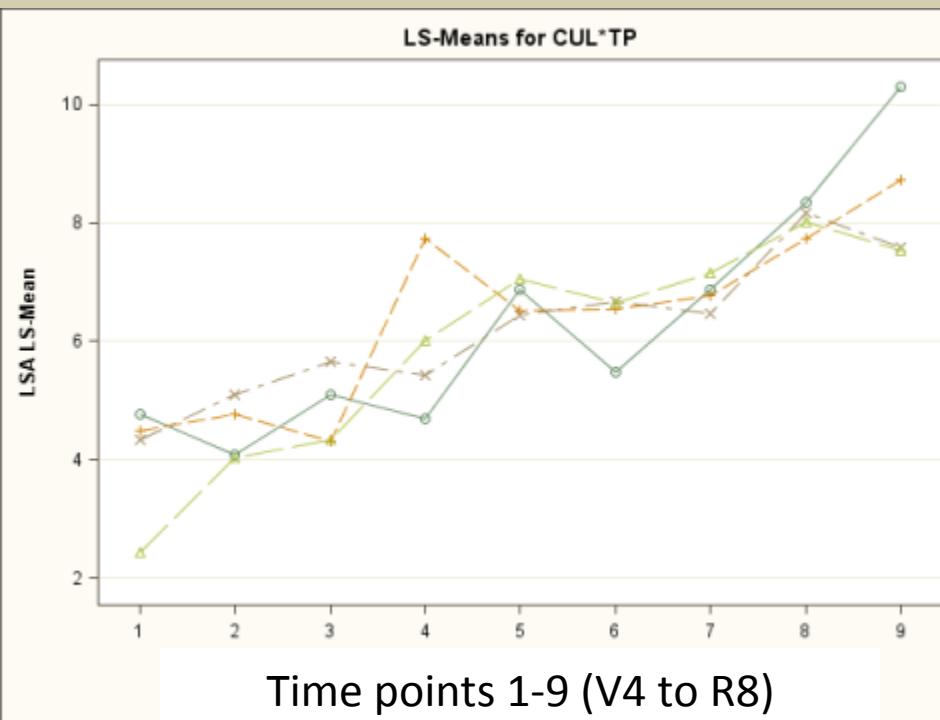
Naturally infested location

Time points 1-9 (V4 to R8)
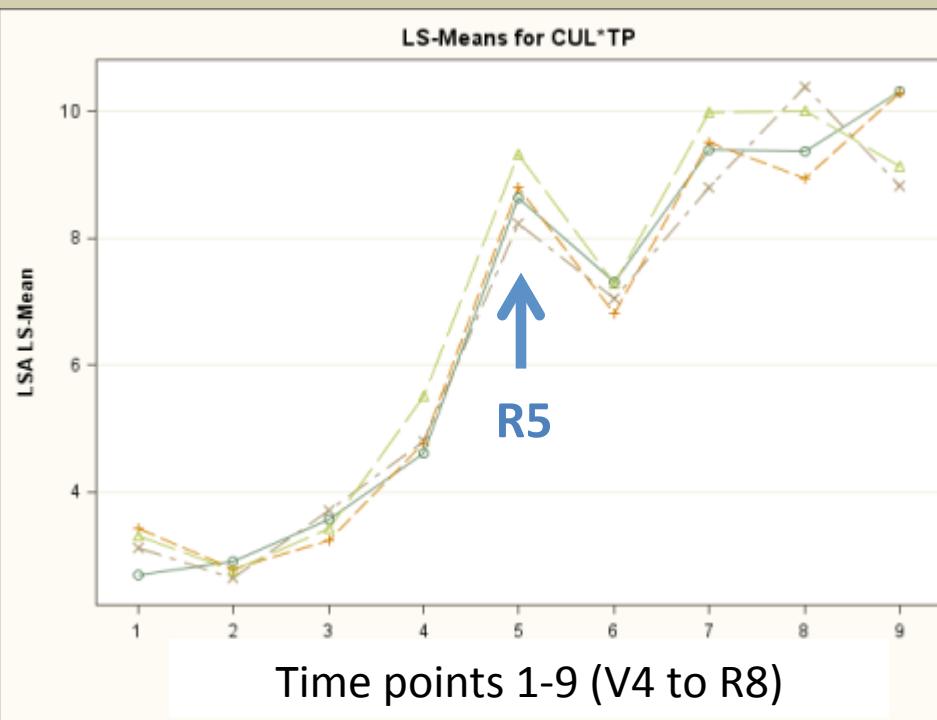
Dry beans – *F. virguliforme*?

# *F. virguliforme* and dry beans
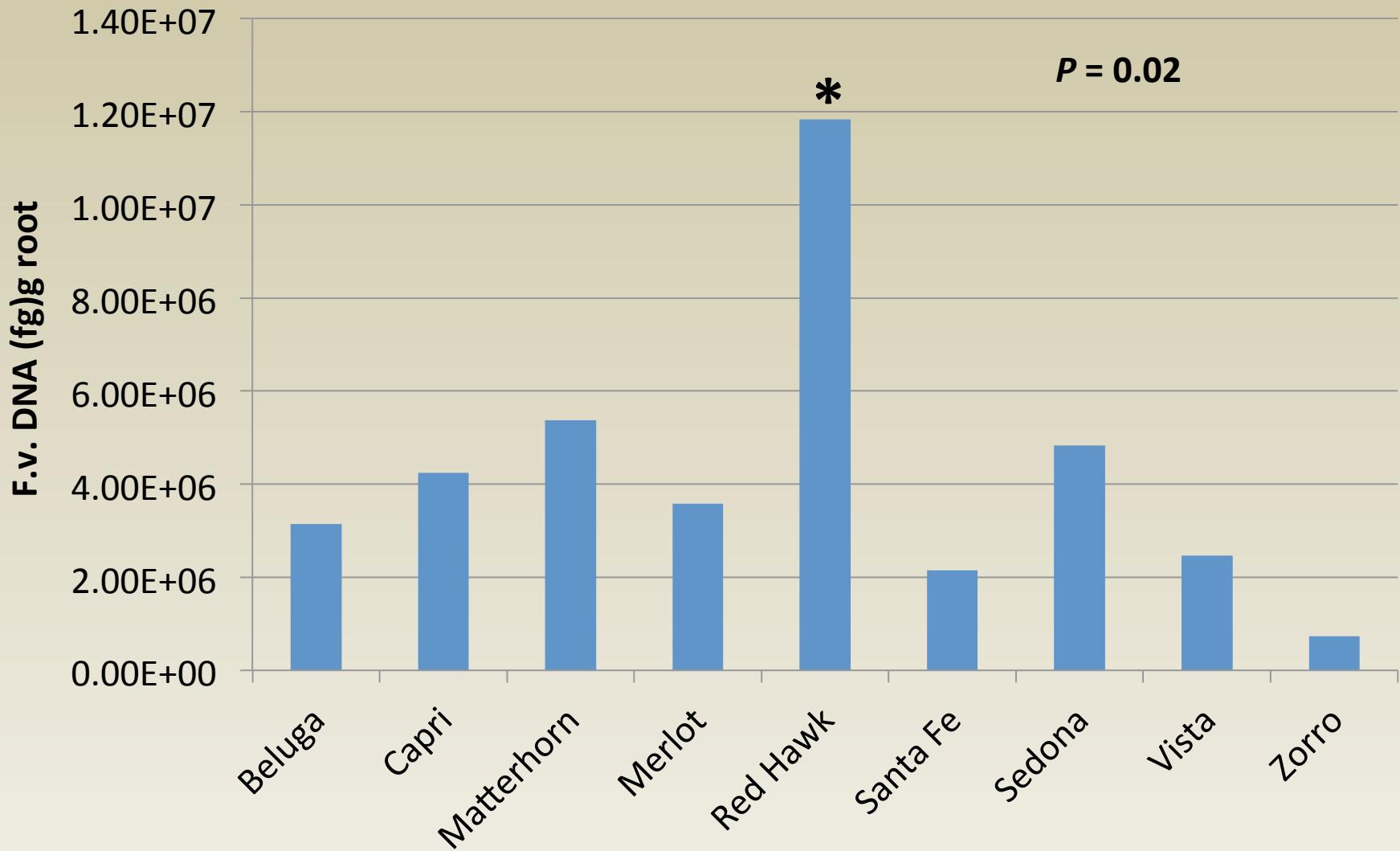
F.v. and root lesion nematodes on dry beans

Time series studies:
- **Field**
- **Greenhouse**

# Next steps: Validating existing assays
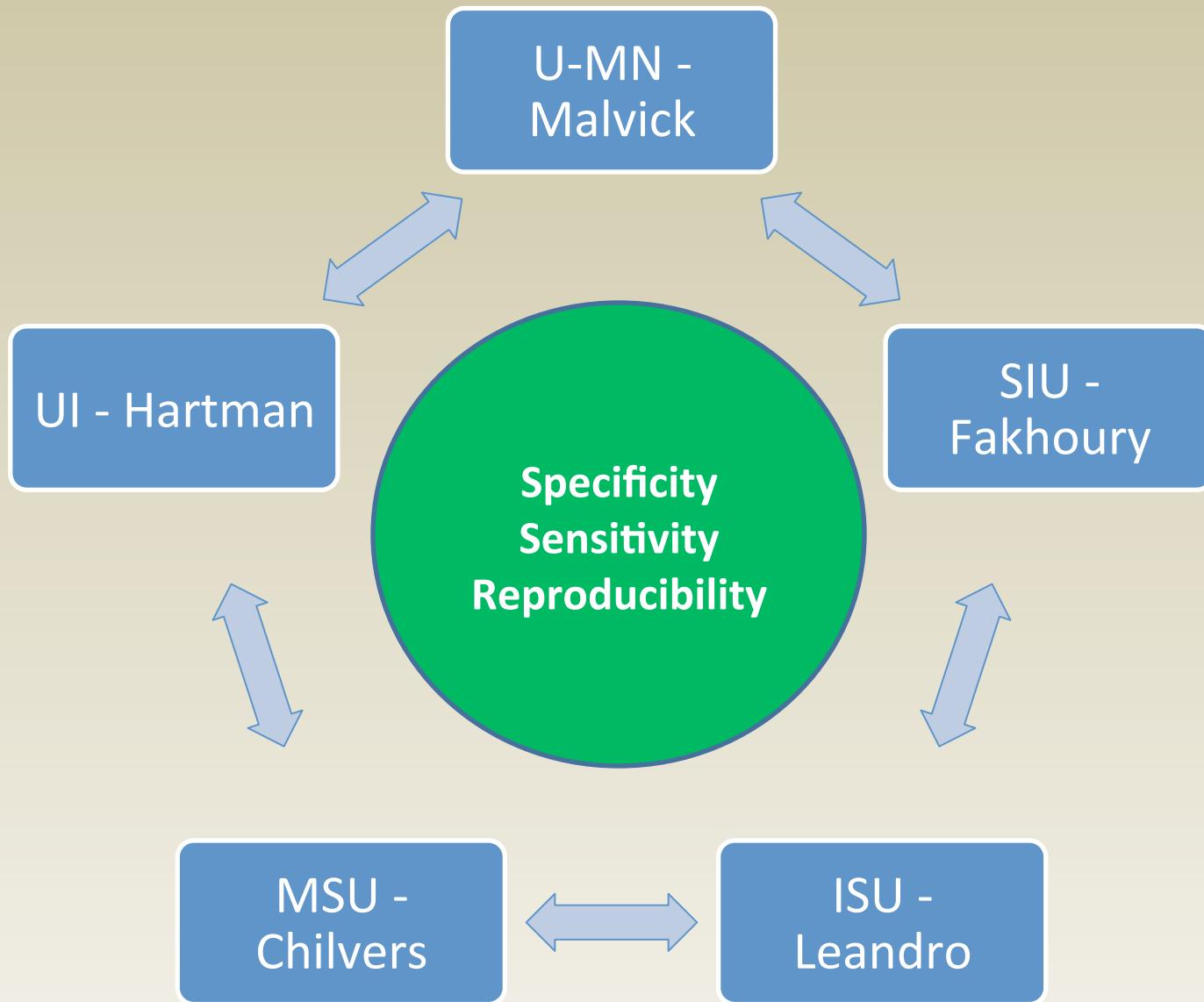
Summary: Molecular tool applications

- Early detection, prior to symptom development

- Phenotype of root infection for breeding

- APHIS and Diagnostic labs

- Etiology and epidemiology research

- Relationship between SCN and *F. virguliforme*

- Soil, risk prediction...disease mitigation

Acknowledgements

- **MI Farmers & Agribusiness**
- **MSUE Extension Educators**
- **Fred Warner – MSU diagnostic lab**
- **John Boyse and Randy Laurenz**
- **Chilvers lab members:**
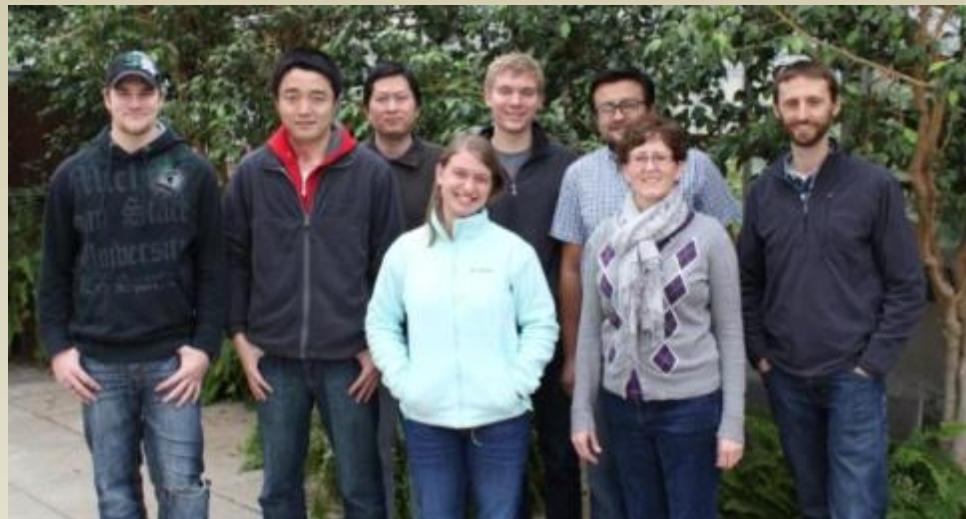
Not pictured: Stephanie (u.grad) & Dan (rot. student)

Questions?