

SDS qPCR assay

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What makes a good qPCR assay?

1. Specific

- Only amplify target pathogen DNA

2. Robust

- Consistent and efficient
- Tolerate to poor DNA quality

3. Sensitive

- Lower limit of detection

4. Validated

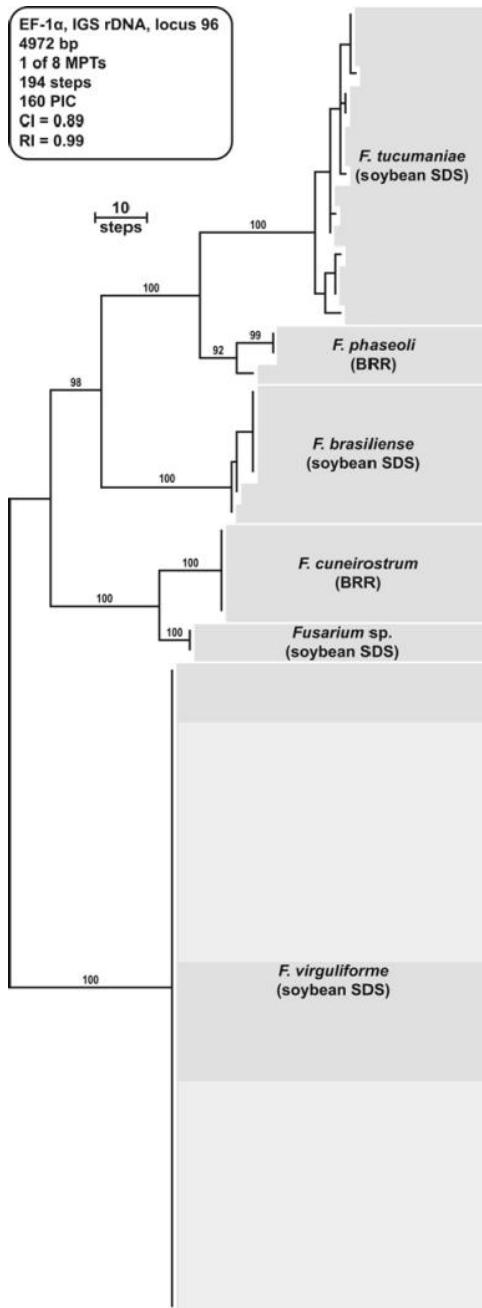
MIQE guidelines

Why improve?

Previously published assays were either not specific or lack of sensitivity

Assays	Specific	Sensitivity	Loci
Gao et al. 2004	No	90fg	mtDNA SSU
Li et al. 2008	No	100fg	mtDNA SSU
Mbofung et al. 2011	Yes	25pg	FvTox-1

EF-1 α , IGS rDNA, locus 96
4972 bp
1 of 8 MPTs
194 steps
160 PIC
CI = 0.89
RI = 0.99



Choice of locus

Closely related non-target species

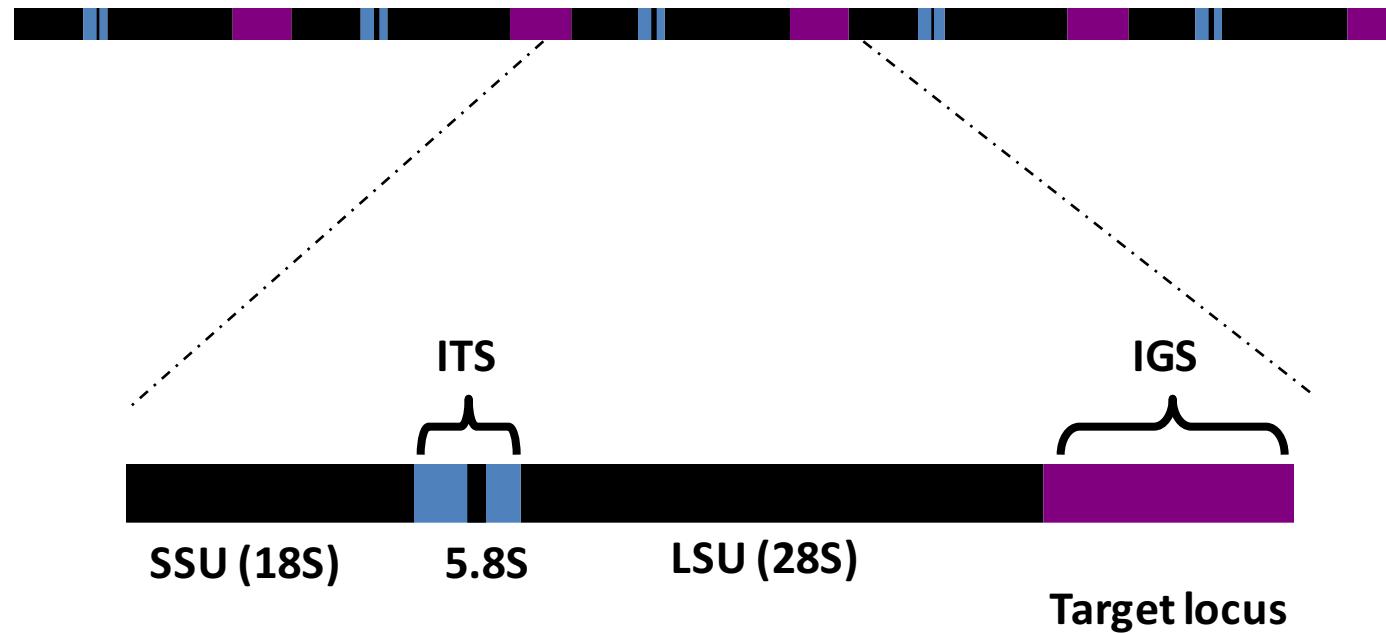
Phylogenies guided assay design

Target species
– *Fusarium*
virguliforme

O'Donnell et al. 2010

qPCR Locus Selection

Ribosomal DNA (multicopy)



ITS: Internal transcribed spacer

IGS: Intergenic spacer

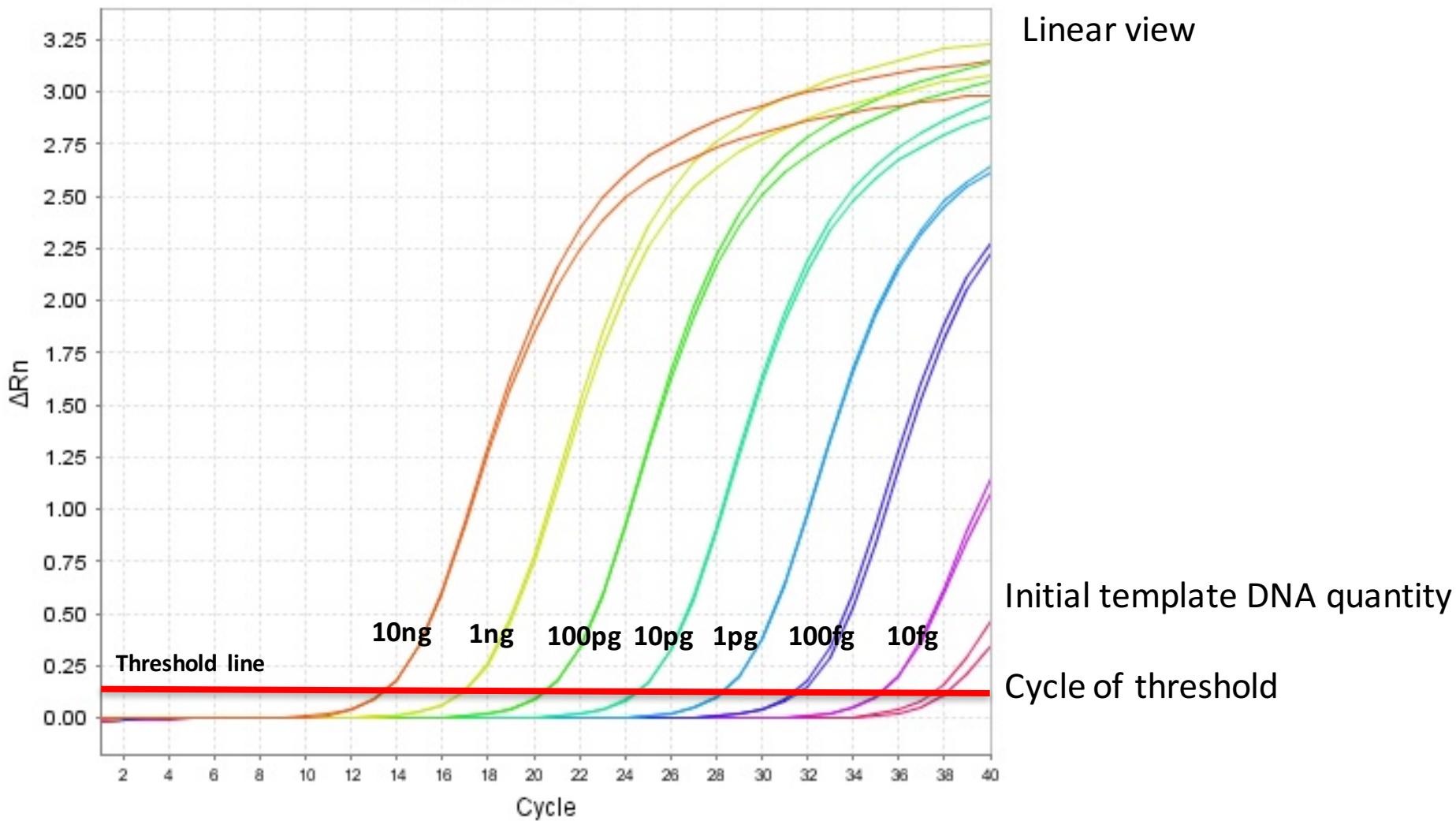
Primers and probes

F. virguliforme intergenic spacer

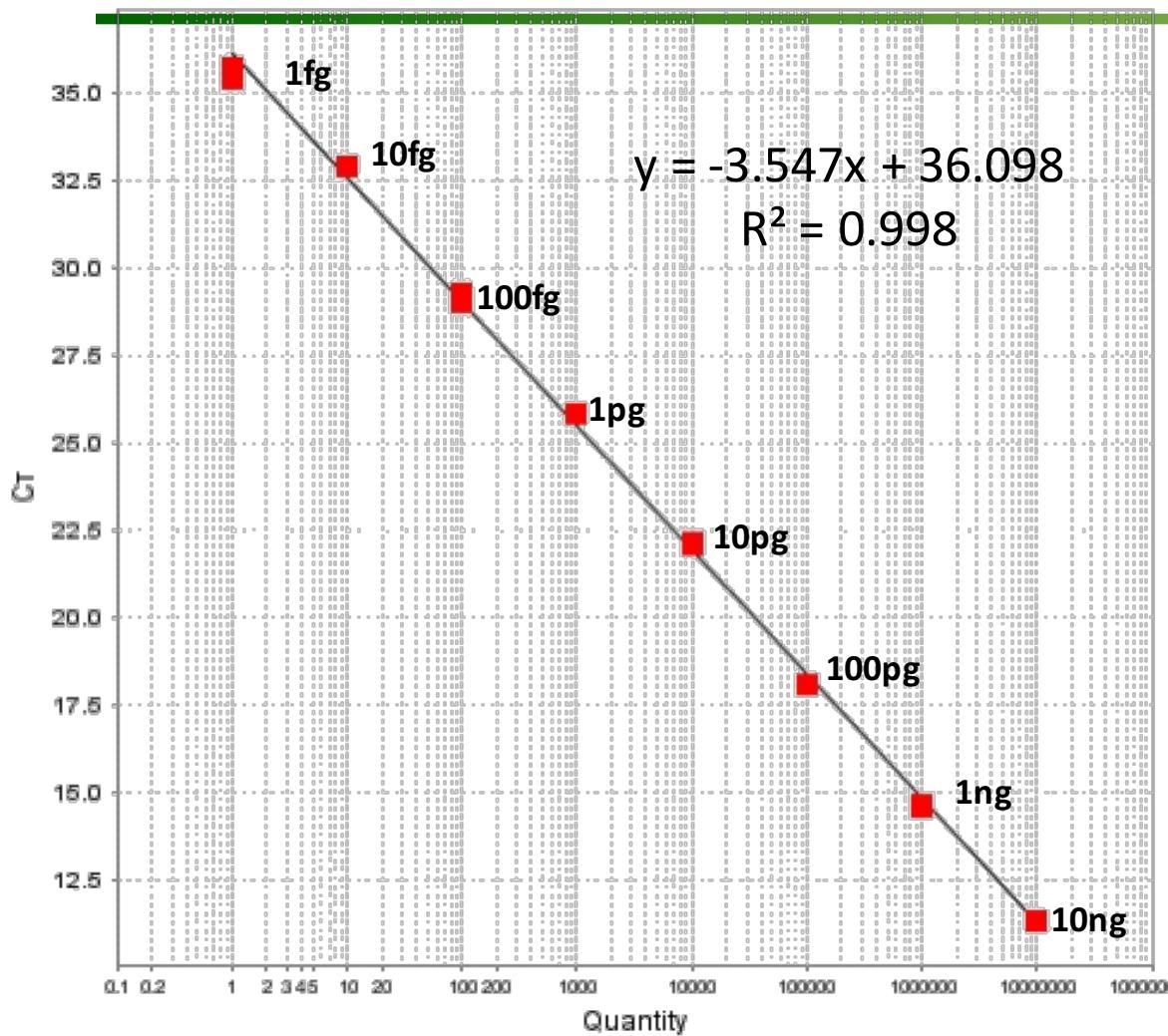


Names	Sequences (5'-3')
F6-3	GTAAGTGAGATTAGTCTAGGGTAGGTGACT
R6	GGGACCACCTACCCCTACACCTACT
FvPrb-3	6FAM-TTTGGTCTAGGGTAGGCCG-MGBNFQ

F. virguliforme qPCR standard curve



Standard curve and efficiency



Calculation of PCR efficiency:

Assume:

- 1), 100% PCR efficiency
- 2), 1:10 serial dilution

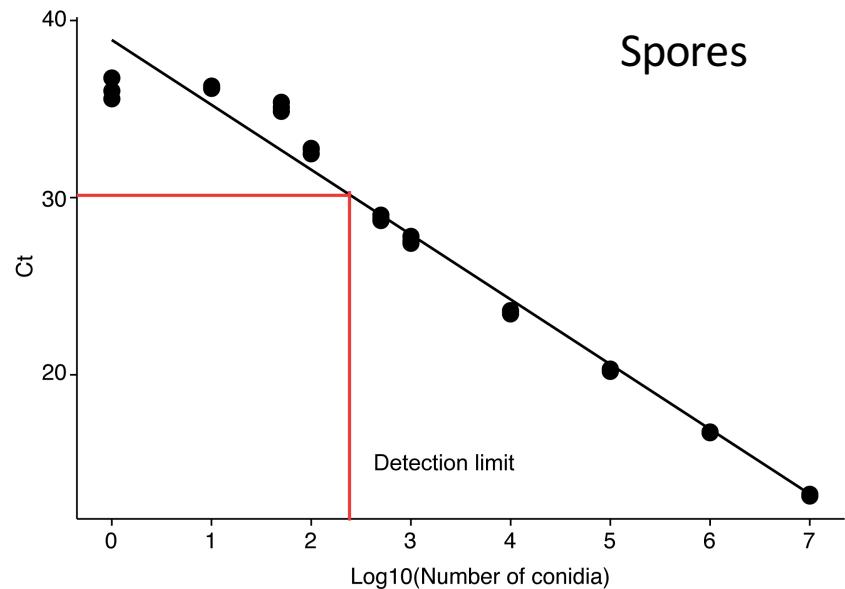
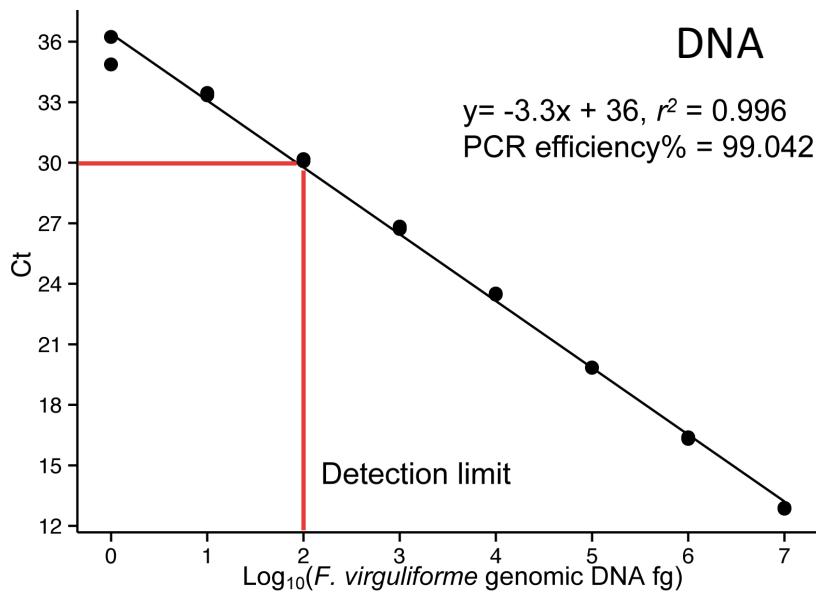
$$2^n = 10 \text{ fold}$$

$$n = \log_{10}/\log_2 = 3.32$$

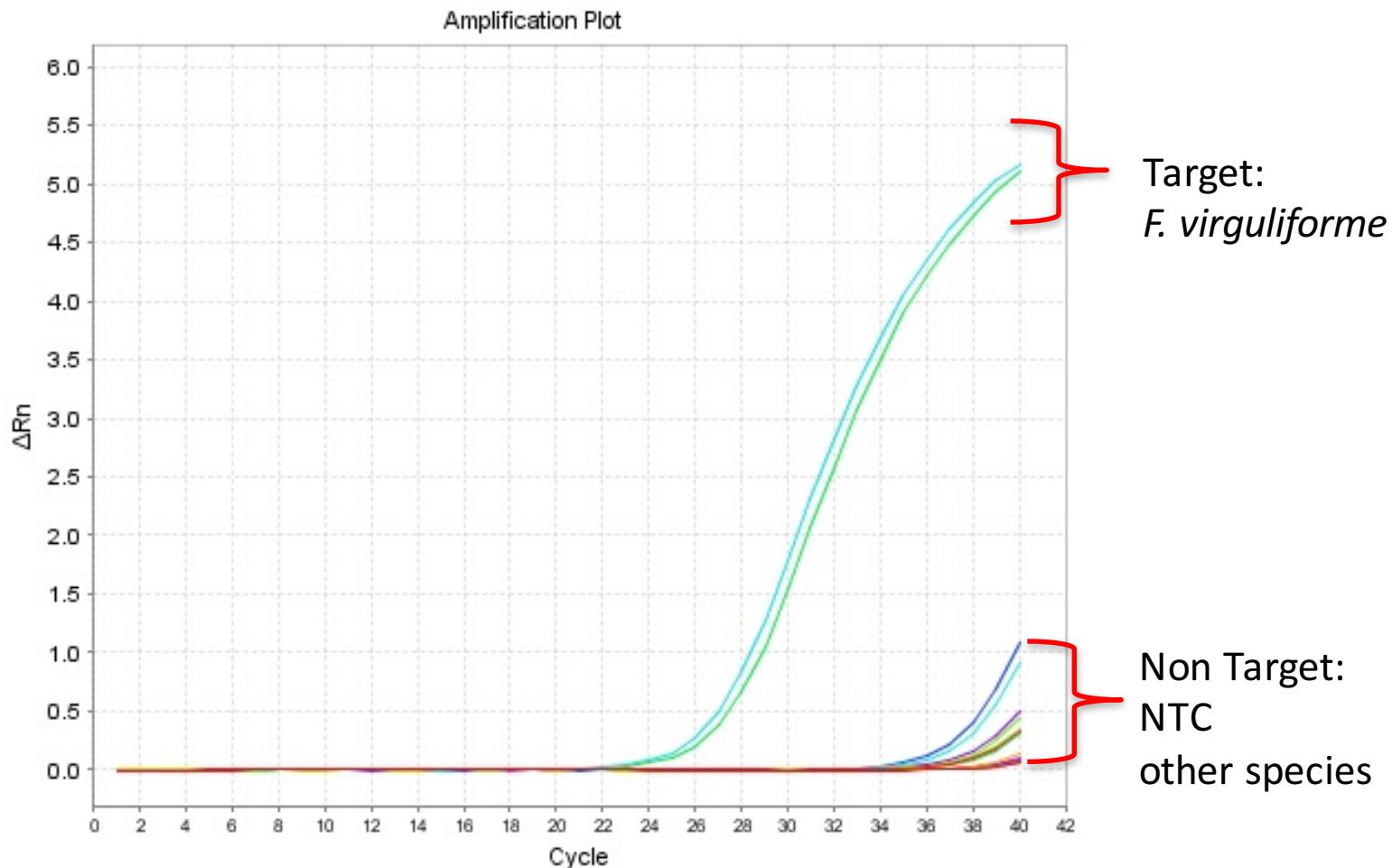
$$\text{PCR efficiency} = -3.32/\text{slope}$$

Sensitivity test

- Lower limit of detection
 - Genomic DNA
 - Amount of pathogens in plant tissue

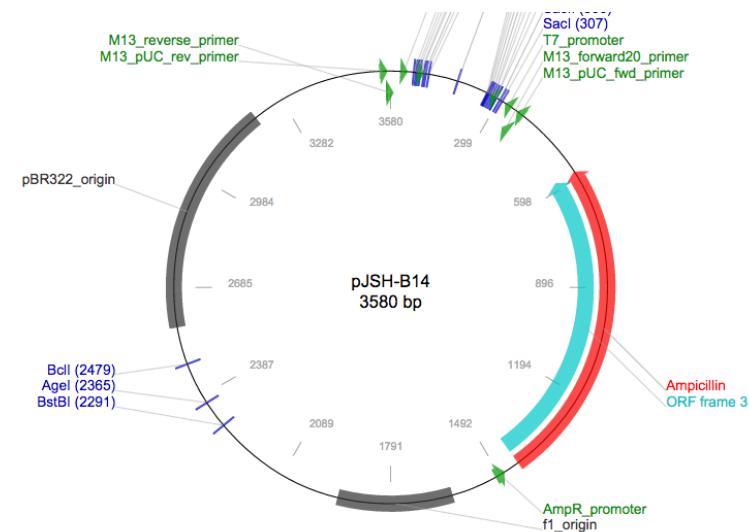


Specificity test



HHIC probe

- Linearized plasmid used as an exogenous control to increase negative call veracity
- Indicator for the effect of PCR inhibitors



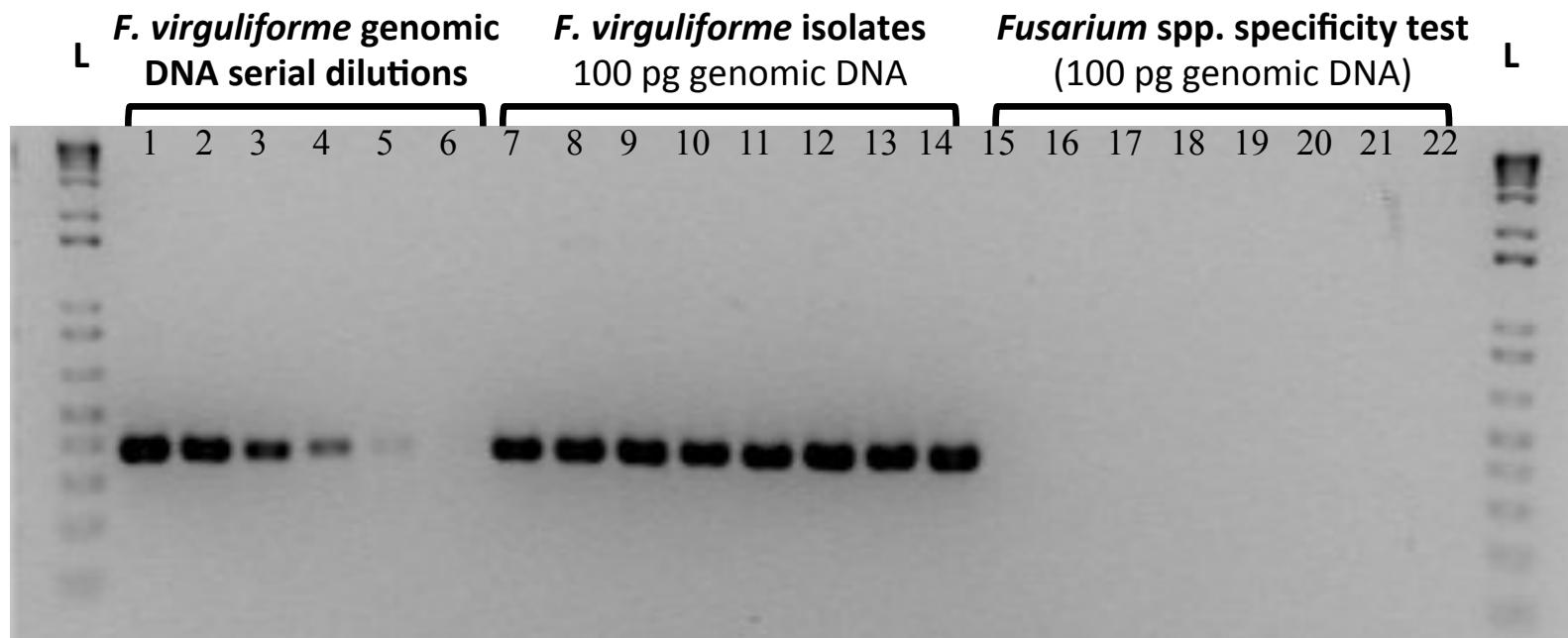
Comparison with other qPCR assays

Round-robin with 6 *F. virguliforme* quantification assays tested at 5 labs

Assay	Specificity (%)	Inclusivity (%)	False Positives (%)	False Negatives (%)	Sensitivity Range across labs
Chilvers Lab	92 b	93 a	8.1	7.5	0.05
B	68 c	92 a	31.7	8.5	0.05 to 0.5
C	46 d	92 a	54.2	8.5	0.05 to 5.0
D	97 a	83 b	2.7	17.5	0.5 to 50.0
E	40 e	92 a	60.2	10	0.05 to 5.0
F	93 b	88 a	6.4	11.6	0.05 to 5.0

Gel image of PCR results

Fusarium virguliforme conventional PCR assay



End-point PCR

Application Case

Treatments:



Fluopyram
($\mu\text{g}/\text{seed}$)

250

150

75

37.5

0

0

Base seed
treatment:

+

+

+

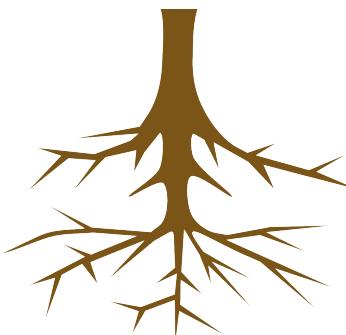
+

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- Design: 6 TRT \times 5 REP
- Repeated on cultivars: VarA, VarB
- Sampling: 5 time points
- Naturally infested field: Decatur, MI

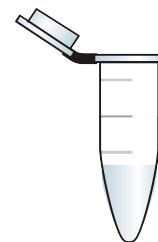
Pathogen quantification



Dry roots



Grind roots



DNA extraction



qPCR quantification

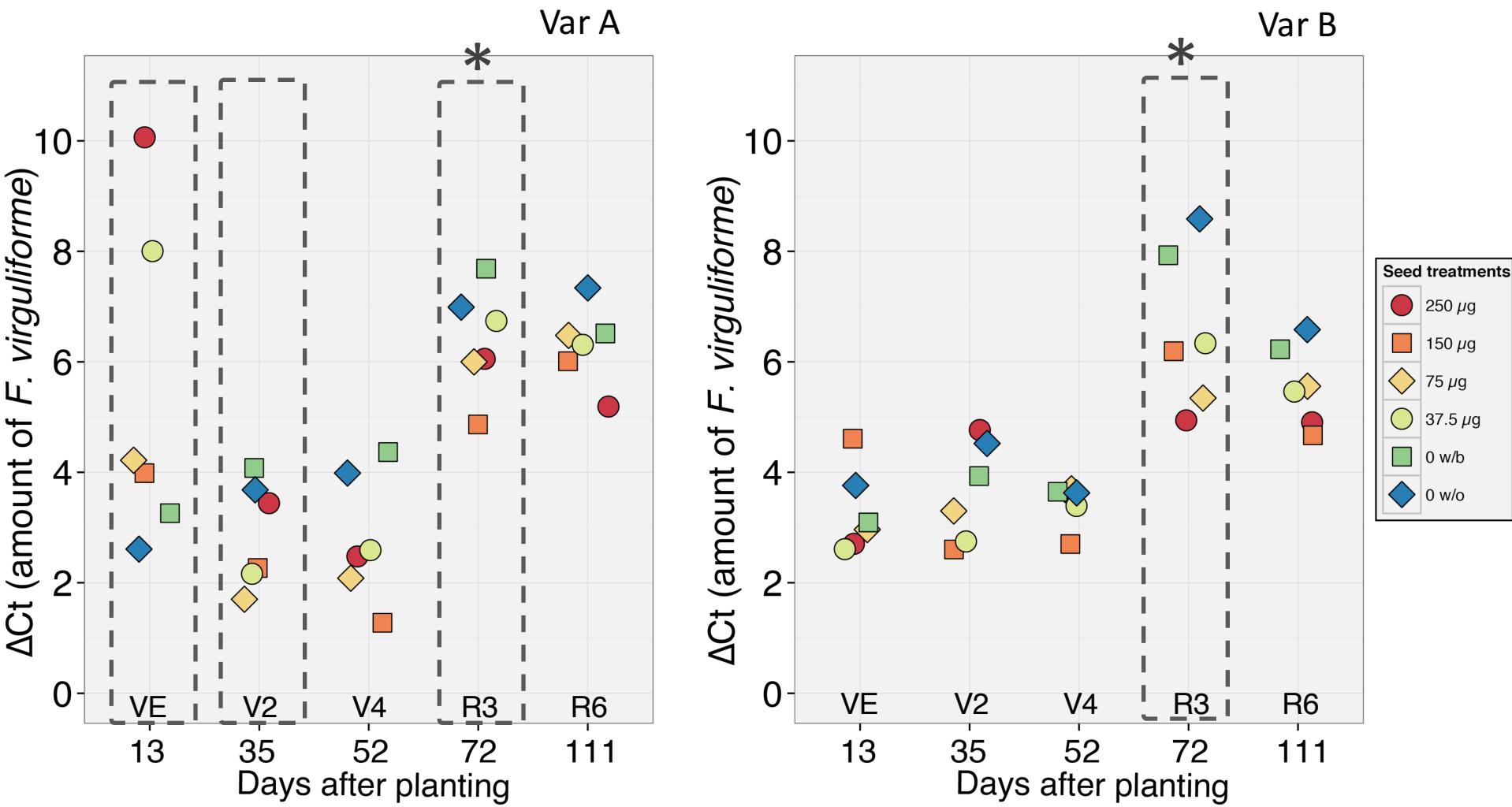
ΔCt method:

$$\Delta Ct = \text{SoyCt} - \text{FvCt}$$

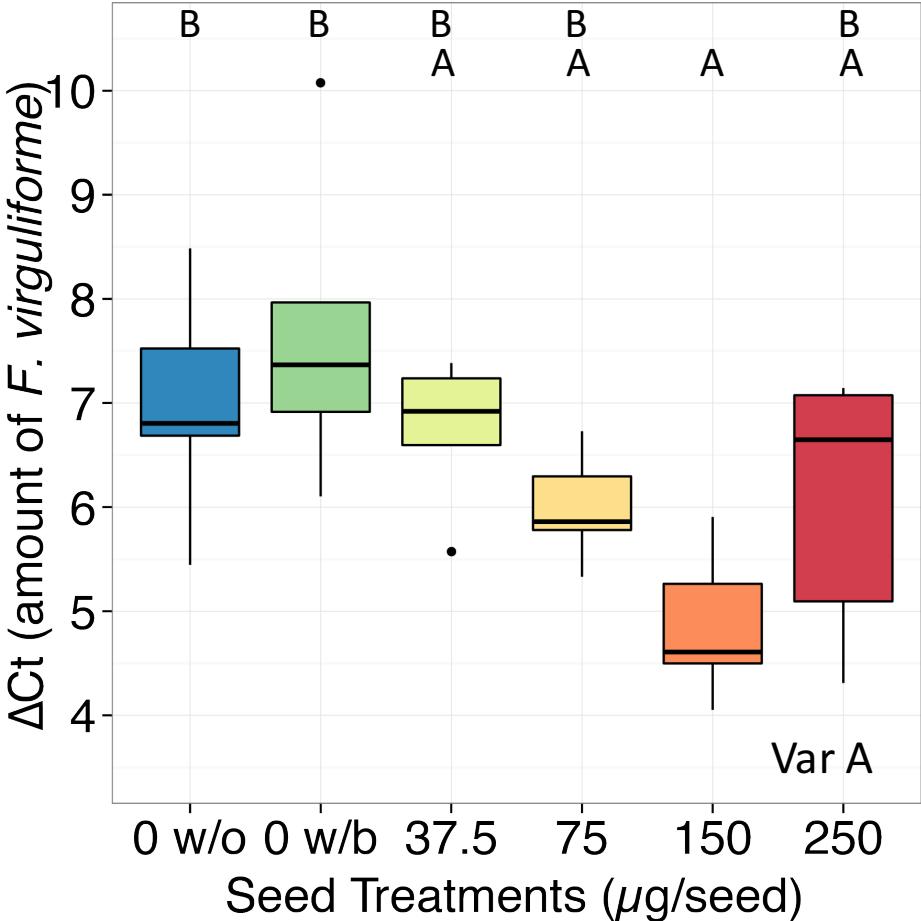
FvCt: Ct value of qPCR assay quantifies *F. virguliforme* rDNA IGS

SoyCt: Ct value of qPCR assay quantifies soybean beta-tubulin gene

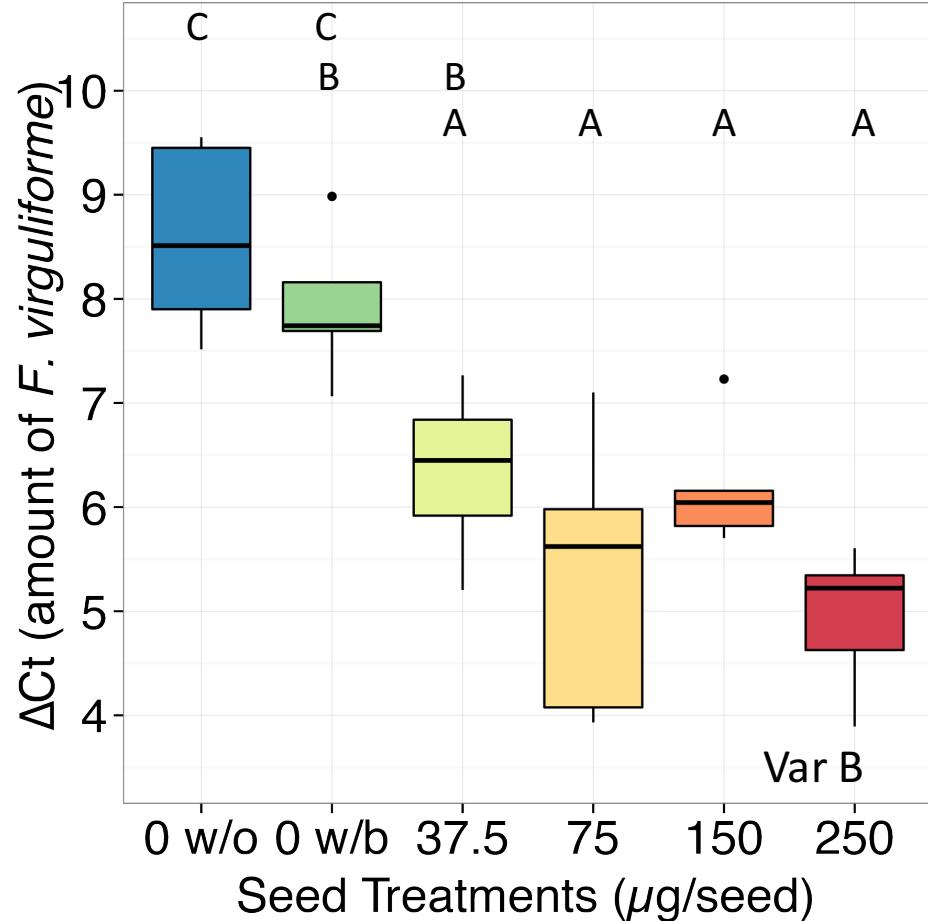
Temporal *F. virguliforme* root colonization



R3 Growth Stage

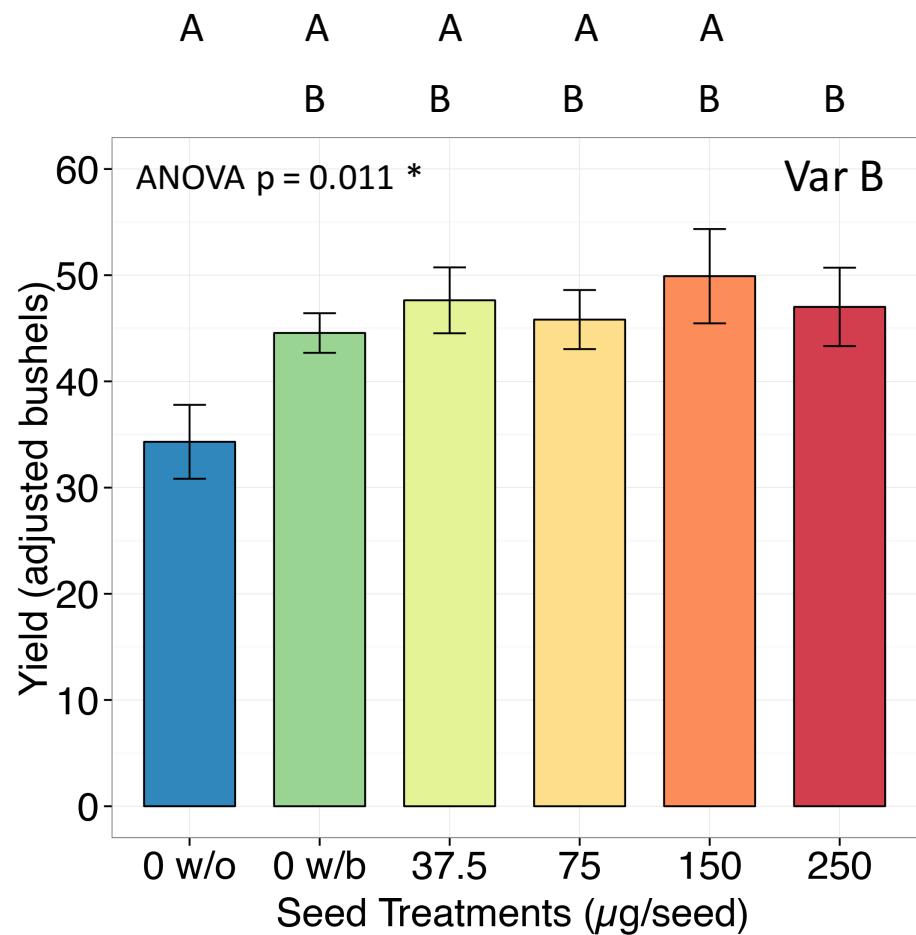
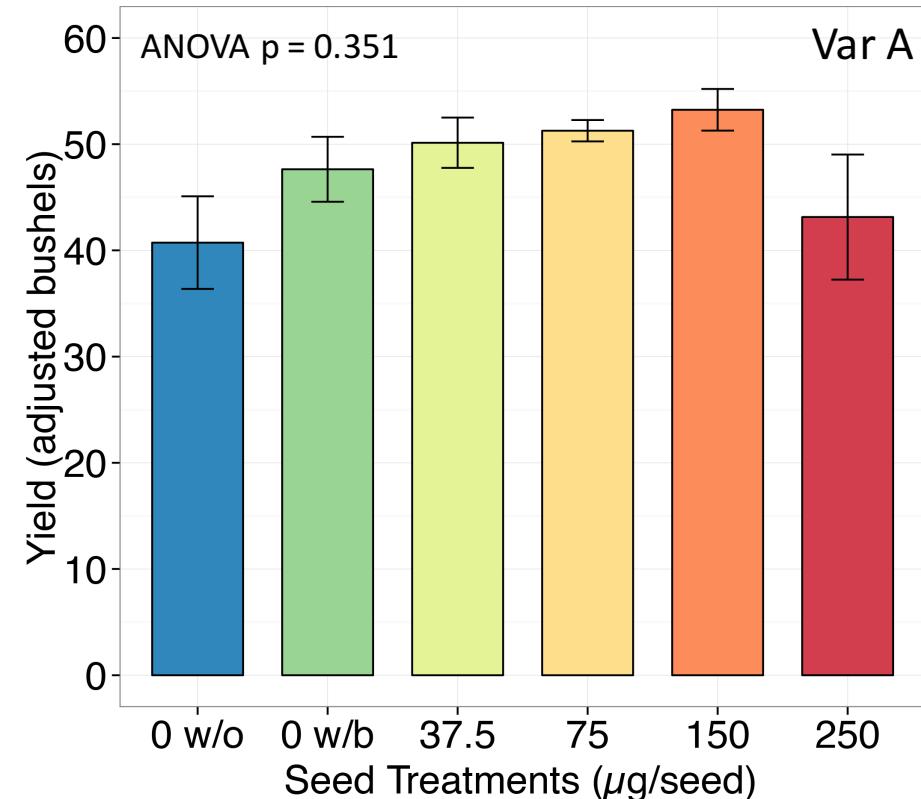


At R3 stage, seed treatment with fluopyram showed significant reduction of *F. virguliforme* root colonization



Tukey HSD Mean separation significant level $\alpha = 0.05$

Yield responses to seed treatments



Soybean yield was favored by seed treatment, but it was not significant between fluopyram treatments.

Tukey HSD Mean separation significant level $\alpha = 0.05$