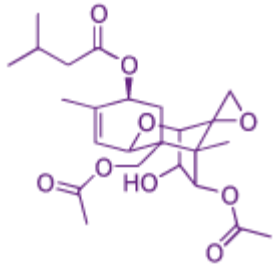
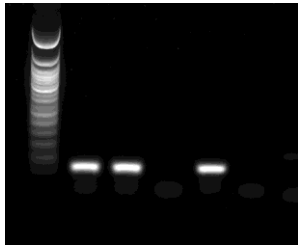
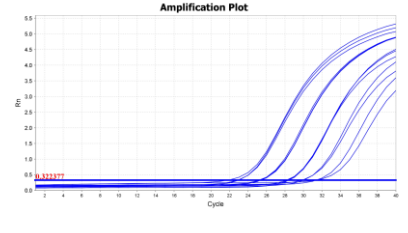
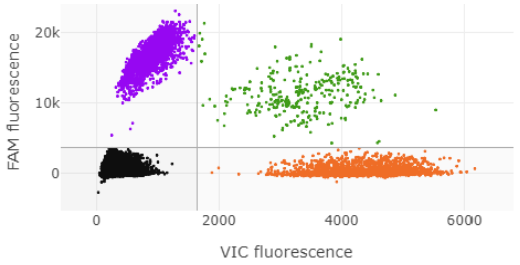
PCR diagnostics of Trichothecene producing *Fusarium* fungi

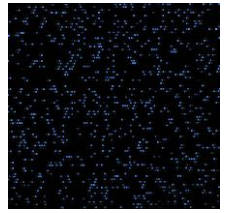
Natarajan Subramani
Post doc researcher (Mycotox-I)
UCD, Dublin











Importance of Trichothecenes

Trichothecenes are a very large family of chemically related mycotoxins produced by various species of F*usarium, Myrothecium, Stachybotrys, Trichothecium, Trichoderma* and *Cephalosporium*

Cereals contaminated with trichothecenes lead to serious economic losses and leads to mycotoxicosis in humans and animals

Fusarium mould is a major plant pathogen and produces a number of trichothecene mycotoxins including deoxynivalenol (or vomitoxin), nivalenol, diacetoxyscirpenol, and T-2 toxin, HT-2 toxin

Identification of *Fusarium* species is a critical point in predicting the potential mycotoxigenic risk of the isolates

(Moss, 1991; Niessen and Vogel, 1998)

Trichothecenes producing *Fusarium* species

Species	Mycotoxins
F. graminearum ***F.aveneceum*** ***F.culmorum*** ***F.langsethiae***	**Type A Trichothecenes** • T-2 toxin • HT-2 toxin
F.poae ***F.equiseti*** ***F.crookwellense*** ***F.acuminatum*** ***F.sporotrichioides*** ***F.sambucinum***	**Type B Trichothecenes** • Nivalenol • Deoxynivalenol • Fusarenon-X

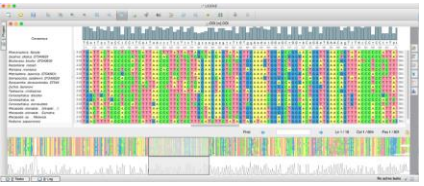
(Streit *et al.*, 2012; EFSA, 2017)

Problems in the accurate identification of *Fusarium* species

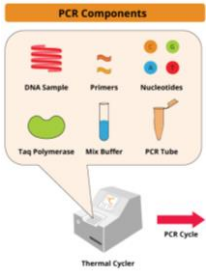
- Cultural and morphological characters are highly variable depending on the media and cultural conditions
- Degeneration of the cultures and production of mutants
- Conventional identification based on morphological characters is not enough to identify at the species level
- Time consuming
- Requires expertise in taxonomy

PCR is an effective, rapid, and Sensitive approach

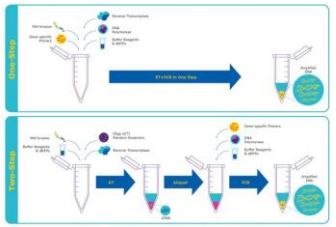
1. Primer design



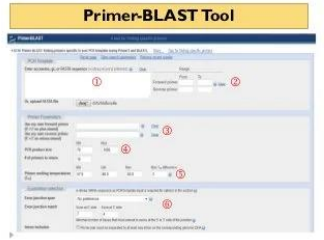
2. Primer alignment



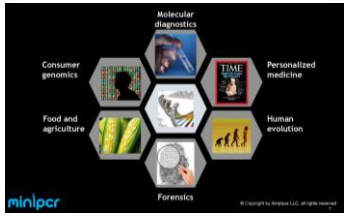
3. A first PCR program



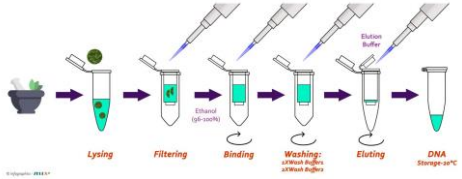
4. PCR optimization



5. Primer specificity



6. Applicability of the PCR



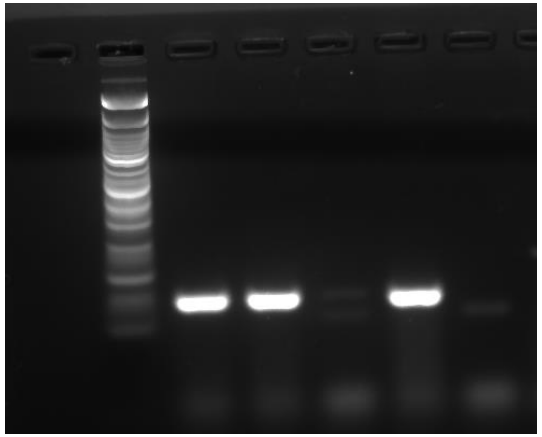
7. DNA extraction

8. Ready - to - use PCR

Source: Konietzny and Greiner, 2003

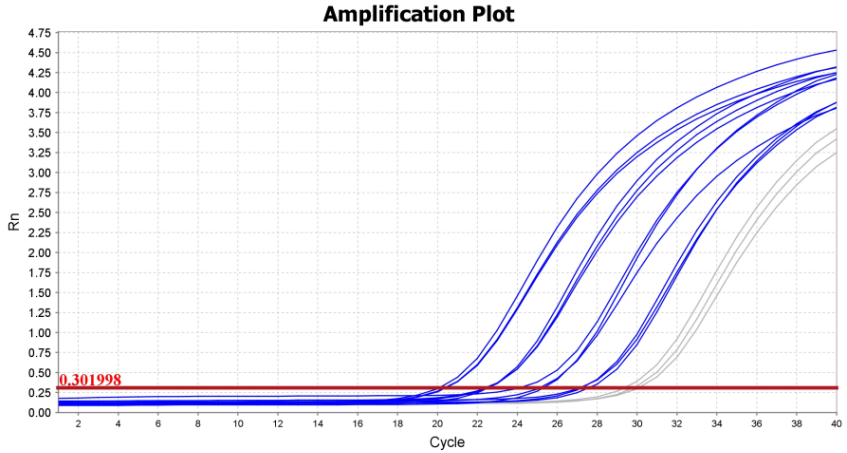
PCR methods used for the *Fusarium* diagnostics

Conventional PCR



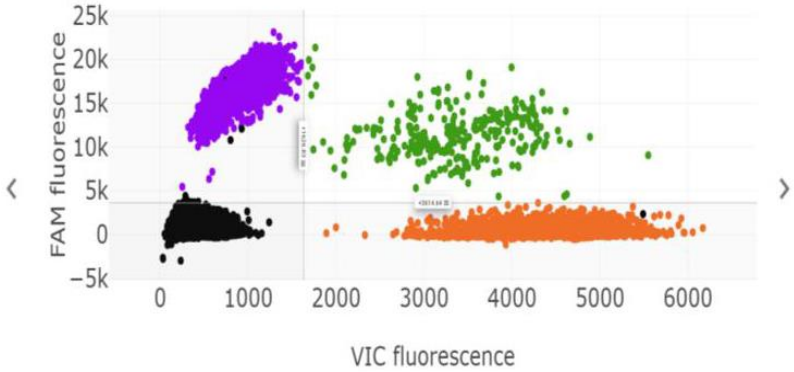
- End point
- Semi-quantitative

Quantitative PCR (qPCR)



- Real time analysis
- Relative and Absolute quantification

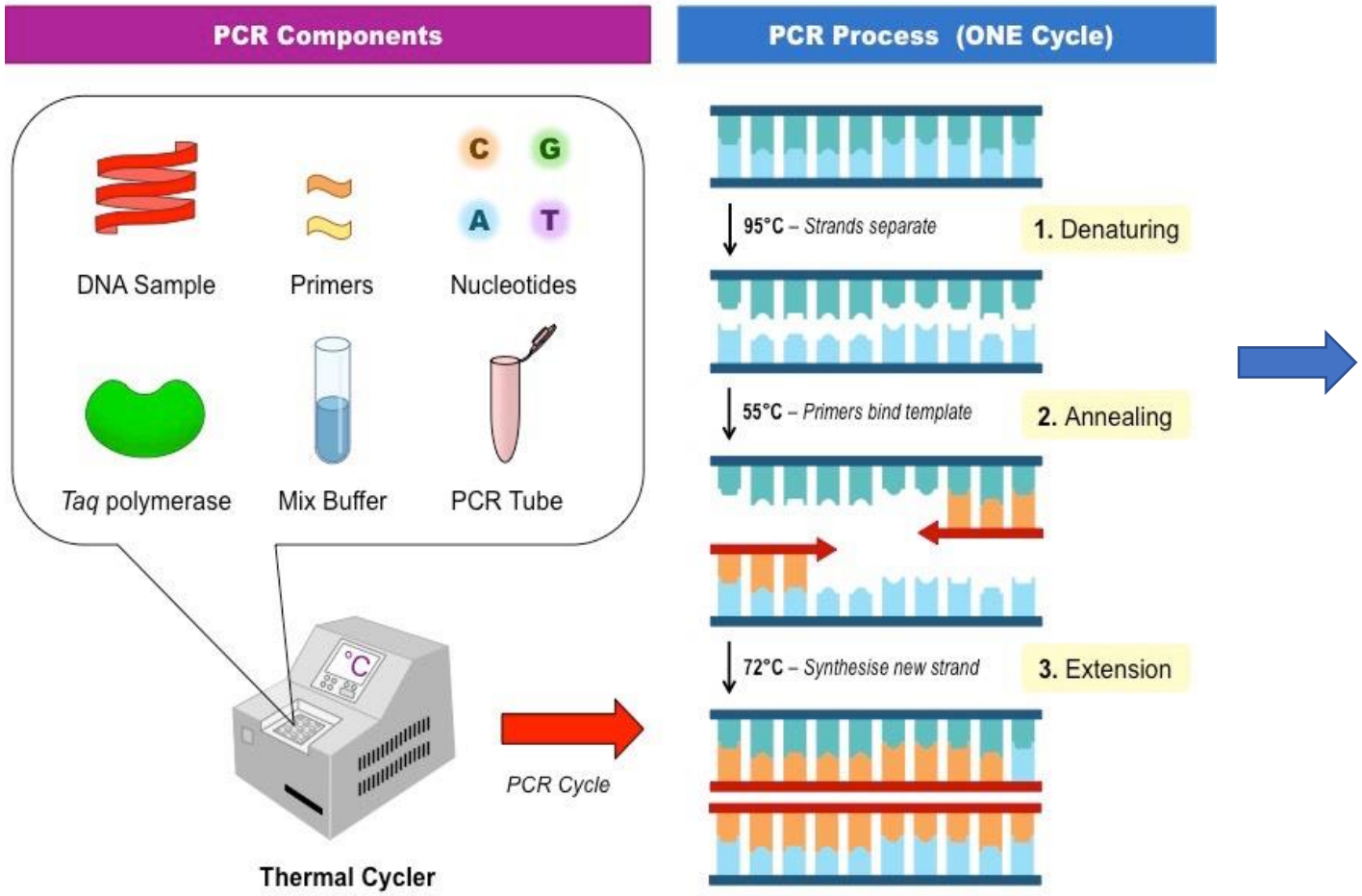
Digital Droplet PCR

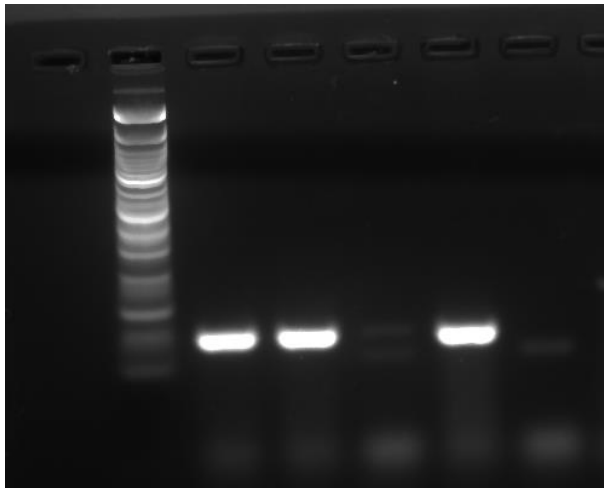


- End point
- Absolute quantification

1. End point (Conventional) PCR

- The primers target sequences on DNA and amplification follows the usual steps of denaturation, annealing and elongation





Visualization through agarose gel electrophoresis

Supporting Findings- 01

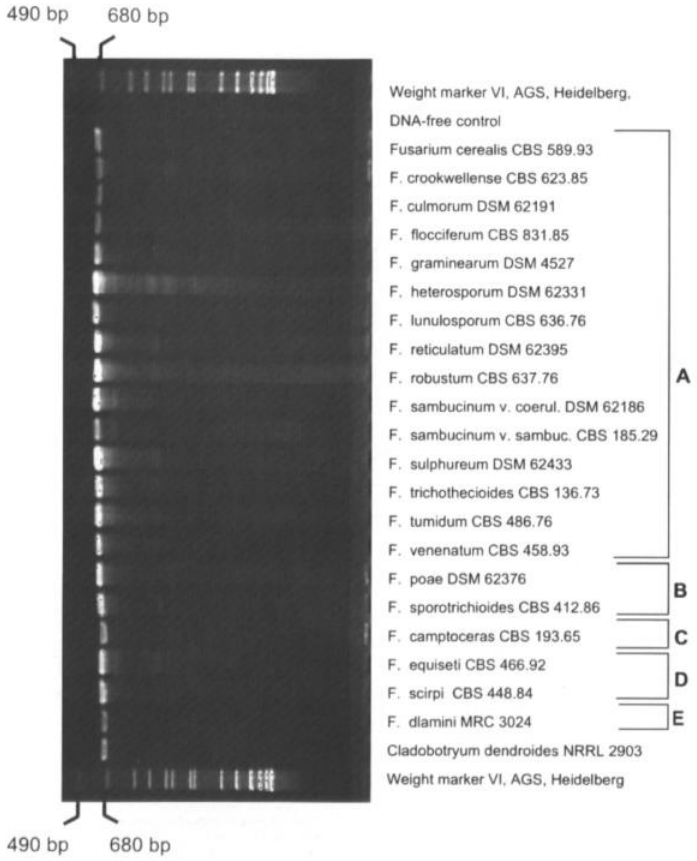
System. Appl. Microbiol. 21, 618–631 (1998)
© Gustav Fischer Verlag
SYSTEMATIC AND APPLIED MICROBIOLOGY

Group Specific PCR-Detection of Potential Trichothecene-Producing Fusarium-Species in Pure Cultures and Cereal Samples

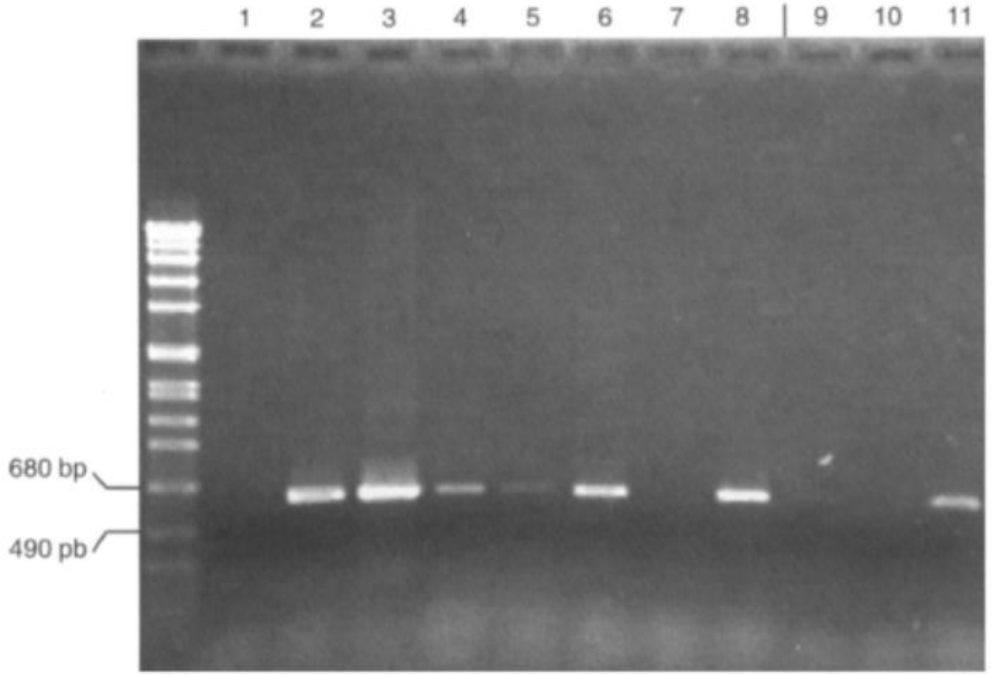
M. LUDWIG NIESSEN, and RUDI F. VOGEL

Lehrstuhl für Technische Mikrobiologie, Technische Universität München, Freising-Weihenstephan, Germany

- A PCR based assay which analyses *Fusarium* species potentially producing trichothecenes was developed using a pair of primers derived from the DNA-sequence of the trichodiene synthase gene(tri5)



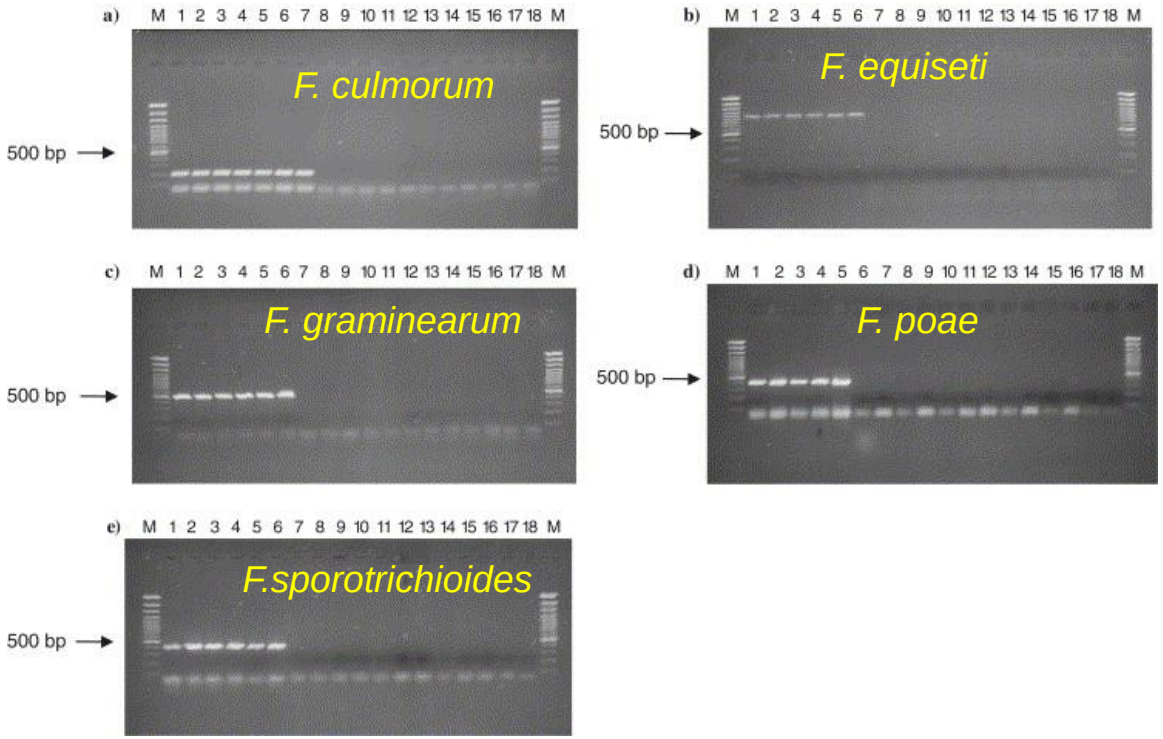
Amplification of a 658 bp fragment from DNA isolated from 21 species and varieties of Fusarium and Cladobotryum dendroides NRRL 2903 using primer pair Tox5-1rrox5-2



Tox5 (tri5) PCR with DNA extracts obtained from wheat malt and barley malt

Supporting Findings- 02

- Specific PCR assays developed for the detection of *Fusarium graminearum, F. culmorum, F. poae, F.sporotrichioides and F. equiseti*
- The specific primers were designed based on IGS sequences (Intergenic Spacer of rDNA)



ELSEVIER

Systematic and Applied Microbiology

Volume 28, Issue 6, 1 August 2005, Pages 562-568

PCR detection assays for the trichothecene-producing species *Fusarium graminearum, Fusarium culmorum, Fusarium poae, Fusarium equiseti* and *Fusarium sporotrichioides*

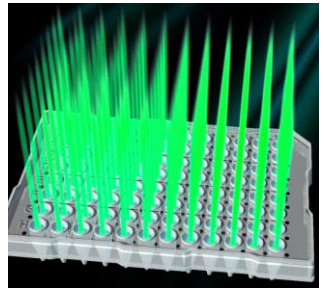
Miguel Jurado [a], Covadonga Vázquez [b], Belén Patiño [b], M. Teresa González-Jaén [a]

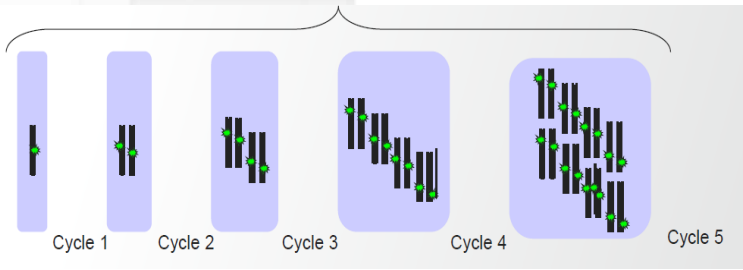
Table 3. Occurrence of *F. graminearum* (FG), *F. culmorum* (FC), *F. equiseti* (FE), *F. sporotrichioides* (FSP) and *F. poae* in samples of wheat

Sample	FG	FC	FE	FSP	FP
U6	–	–	+	–	–
L1	+	+	+	–	–
L3	+	–	+	–	–
E1	–	+	–	–	–
BE1	–	–	+	–	–
BO1	+	–	–	–	–
N1	+	+	+	–	–

2. Quantitative PCR

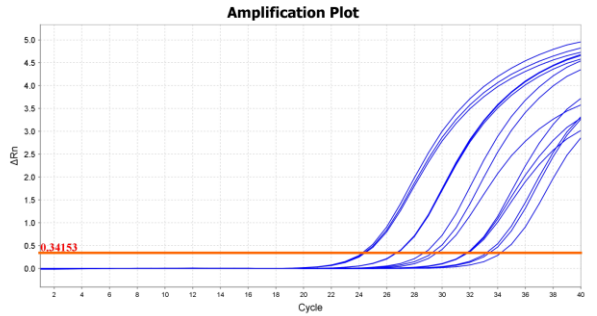
- It allows the detection of PCR products as they accumulate in real time during the PCR amplification process
- RT-PCR systems rely upon the detection and quantification of fluorescent reporter
- The signal of which increases in direct proportion to the amount of PCR product in a reaction
- **Absolute quantification:** Serially dilute some reference material to generate a set of standards and test these standards alongside unknowns on each plate of Real-Time PCR

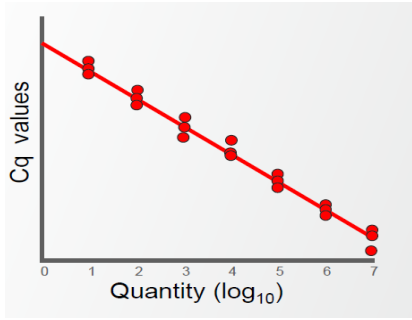




Advantages

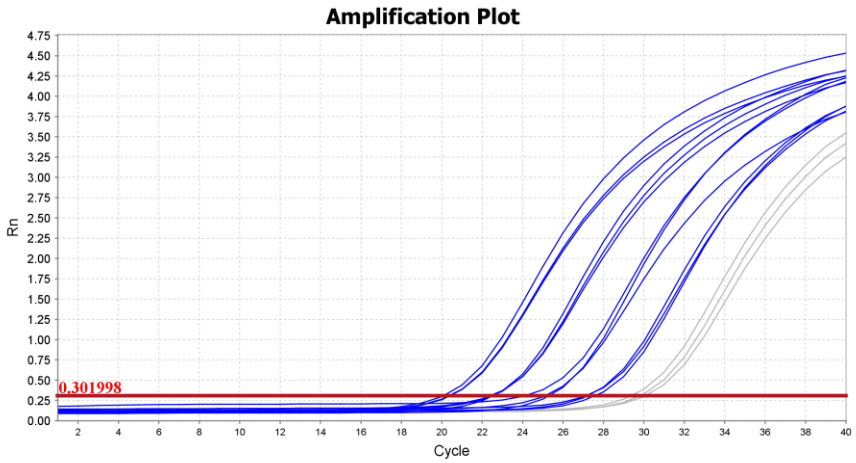
- ✓ Post-PCR processing is not required
- ✓ Quantitative
- ✓ Specificity and sensitivity
- ✓ Specific amplification can be confirmed by melt curve analysis



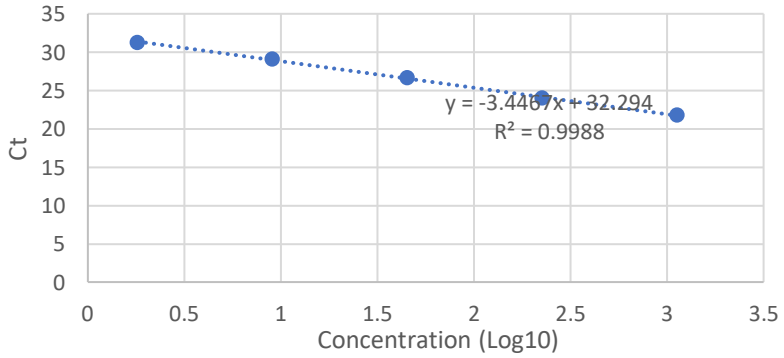


Validation of Trichothecene genes (TRI5) using qPCR

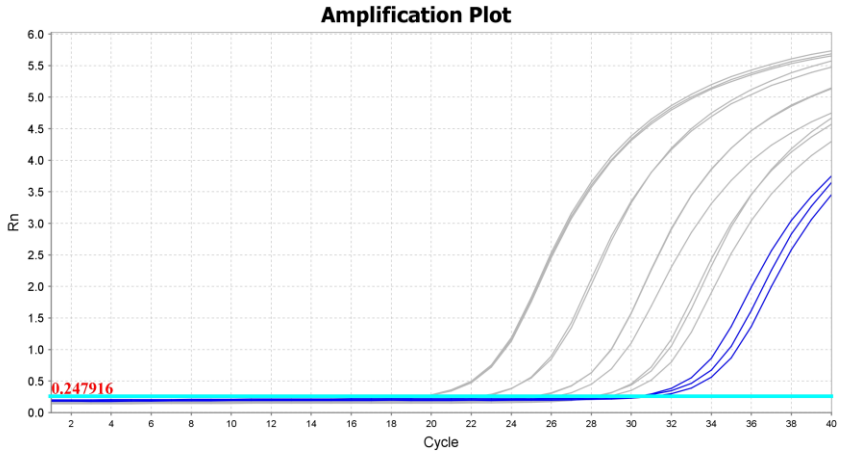
Fusarium langsethiae (F1201059)



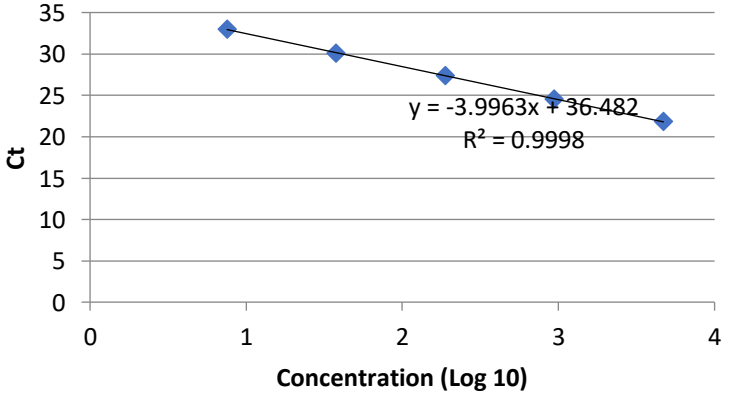
Standard curve (5 fold dilution)



Fusarium culmorum HUCU3



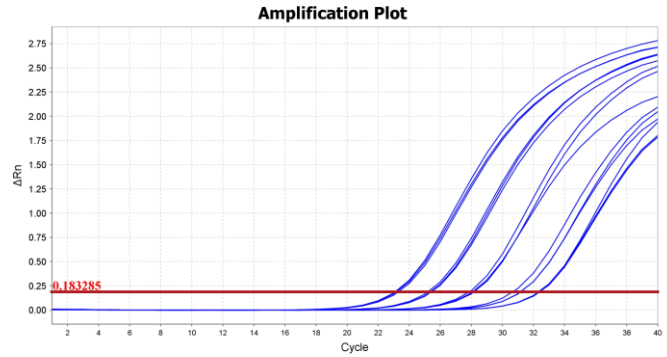
Standard curve (5 fold dilution)



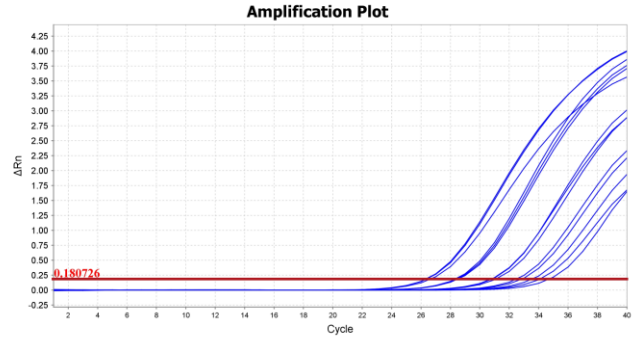
(Source: Mycotox-I)

Validation of Trichothecene genes (TRI6) using qPCR

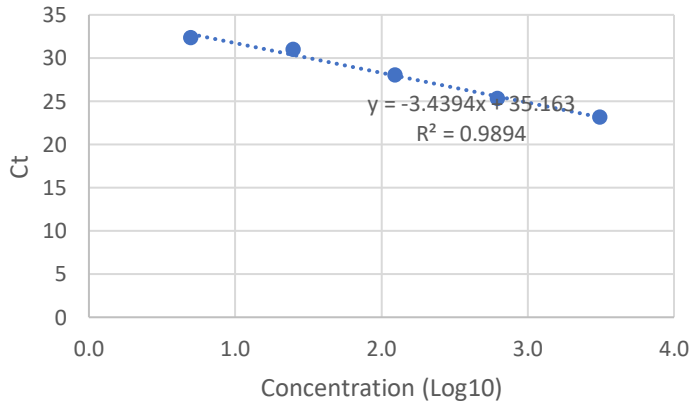
Fusarium langsethiae (F1201059)



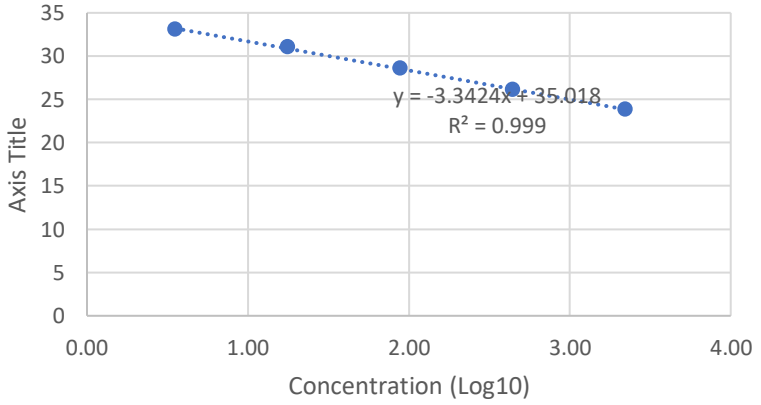
Fusarium sporotrichoides



Standard curve (5 fold dilution)



Standard curve (5 fold dilution)

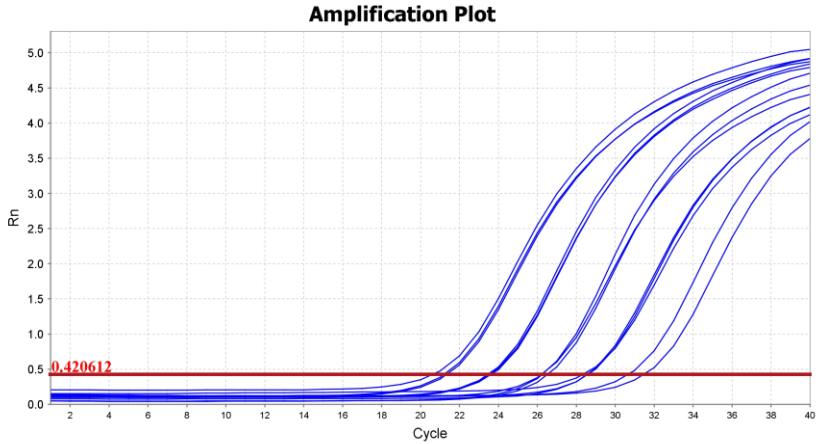


(Source: Mycotox-I)

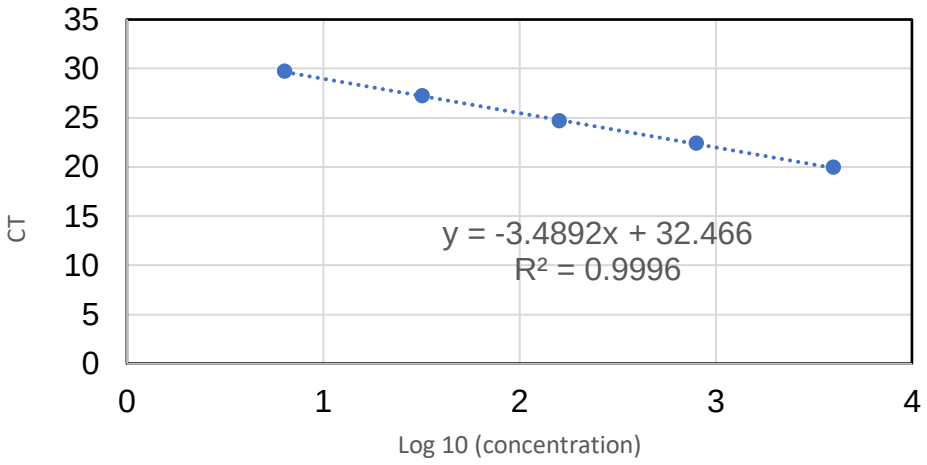
Validation of Species specific genes using qPCR

(Elongation factor 1 alpha gene)

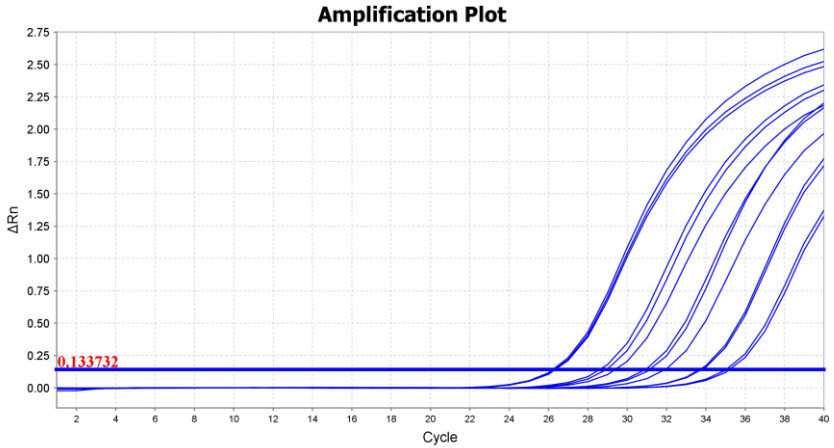
Fusarium langsethiae (F1201059)



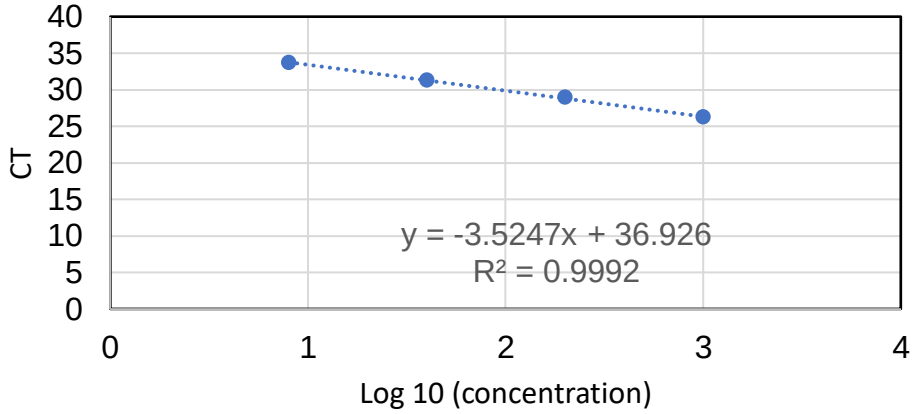
Standard curve (5 fold dilution)



Fusarium poae 202189



Standard curve (5 fold dilution)



(Source: Mycotox-I)

Supporting Findings- 01

- Species-specific qPCR analysis was utilized to quantify the DNA of *Fusarium culmorum* and *F. poae*

Plant Pathology (1999) 48, 209–217

Fusarium ear blight of wheat: the use of quantitative PCR and visual disease assessment in studies of disease control

F. M. Doohan[a]*†, D. W. Parry[b] and P. Nicholson[a]

[a]*John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH; and* [b]*Horticultural Research International, East Malling, West Malling, Kent ME19 6BJ; UK*

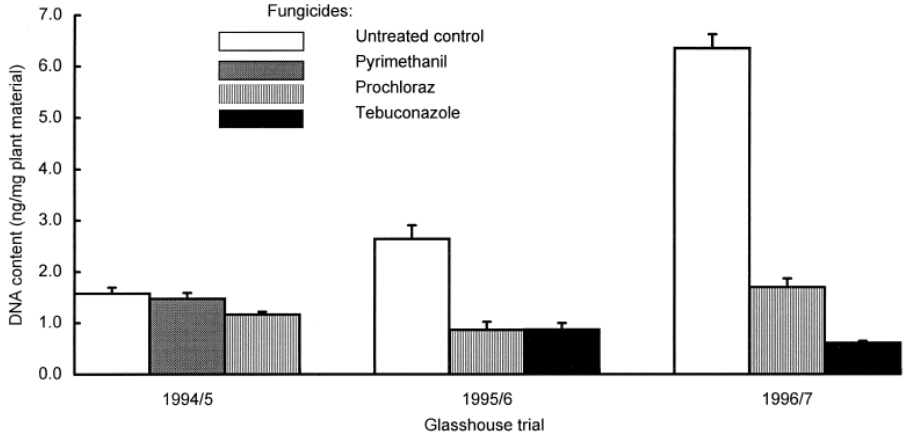


Figure 1 Analysis of the efficacy of fungicides against *Fusarium culmorum* ear blight of wheat (cv. Avalon) in the 1994–5, 1995–6 and 1996–7 glasshouse trials. Disease based on (a) visual disease assessment at GS 80 and (b) quantitative PCR analysis. Bars indicate standard error of the means.

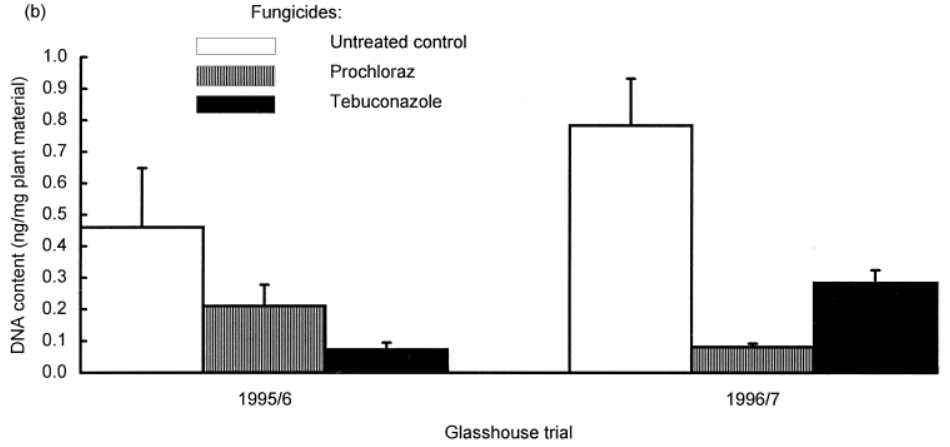
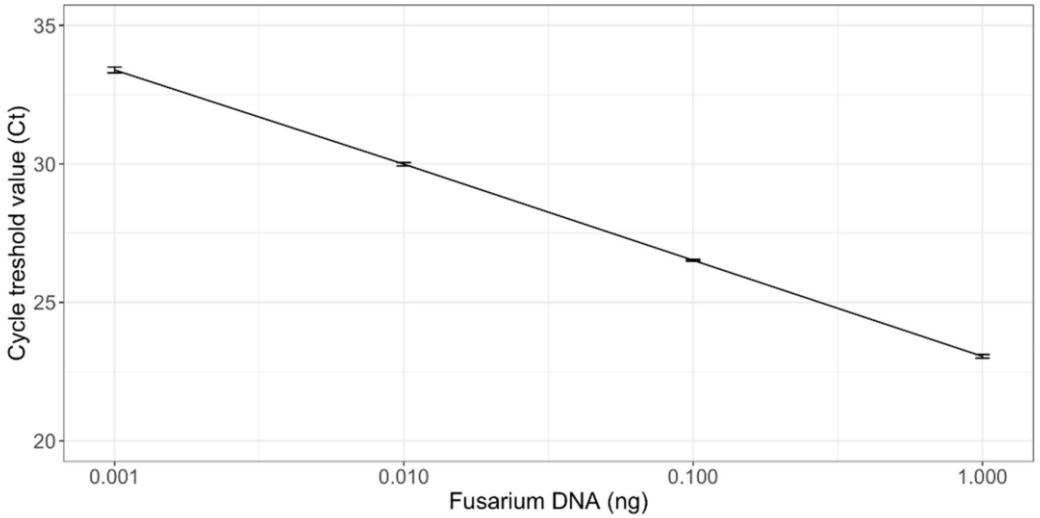


Figure 2 Analysis of the efficacy of fungicides against *Fusarium poae* ear blight of wheat (cv. Avalon) in the 1995–6 and 1996–7 glasshouse trials. Disease assessment based on (a) visual disease assessment at GS 80 and (b) quantitative PCR analysis. Bars indicate standard error of the means.

Supporting Findings- 02

- TaqMan-based qPCR method (FusE) targeting the Fusarium-specific elongation factor region (EF1*a*) was developed for the detection and quantification of *Fusarium* spp.



- ✓ Standard curve of the FusE qPCR assay showing Fusarium DNA concentration (ng) against the cycle threshold (Ct) values of a single qPCR run.
- ✓ The Fusarium DNA concentration range was 0.001 to 1 ng per reaction

toxins

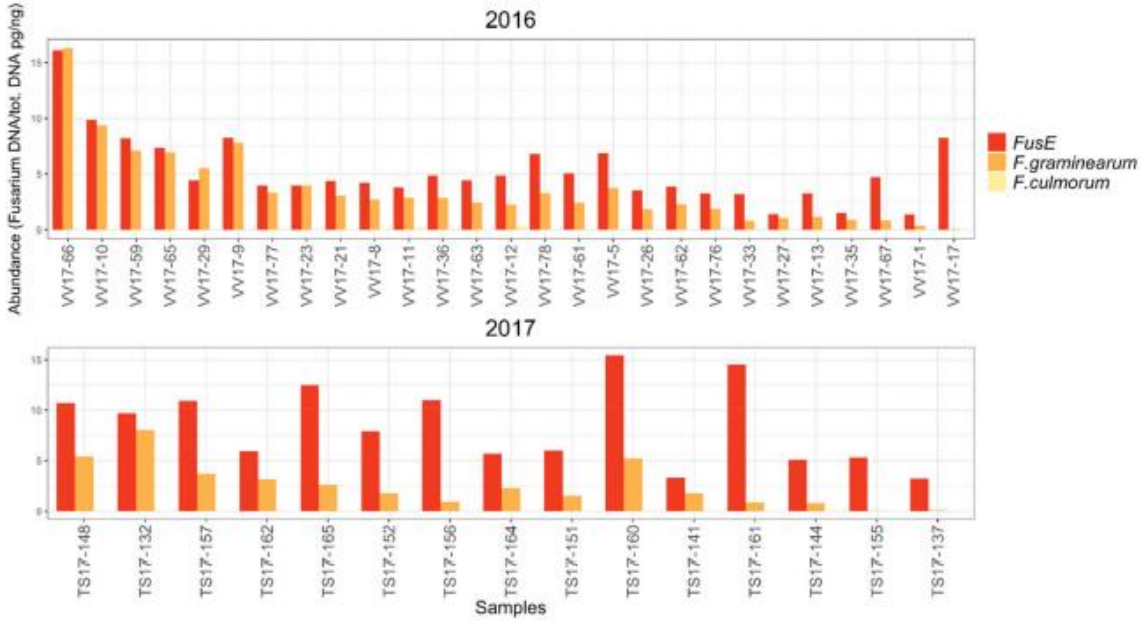
MDPI

Article

Taqman qPCR Quantification and *Fusarium* Community Analysis to Evaluate Toxigenic Fungi in Cereals

Elina Sohlberg [1,*], Vertti Virkajärvi [1], Päivi Parikka [2], Sari Rämö [2], Arja Laitila [1] and Tuija Sarlin [1]

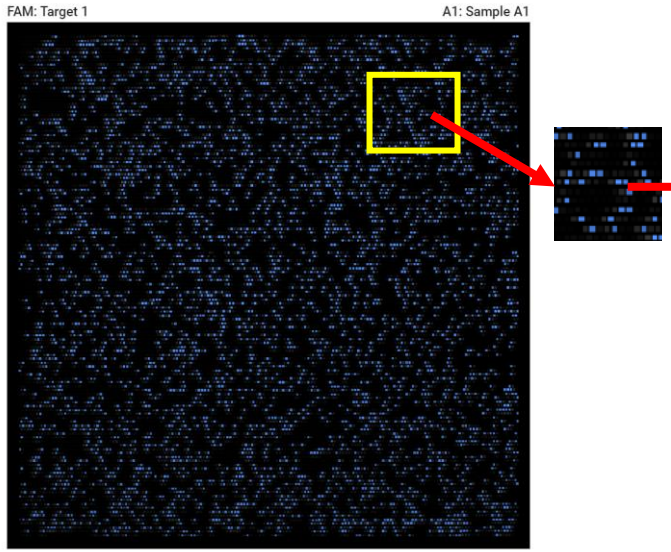
[1] VTT Technical Research Centre of Finland Ltd., FI-02044 Espoo, Finland; vertti@no-office.co (V.V.); Arja.Laitila@outlook.com (A.L.); tuija.sarlin@vtt.fi (T.S.)
[2] Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland; ext.paivi.parikka@luke.fi (P.P.); sari.ramo@luke.fi (S.R.)
* Correspondence: elina.sohlberg@vtt.fi

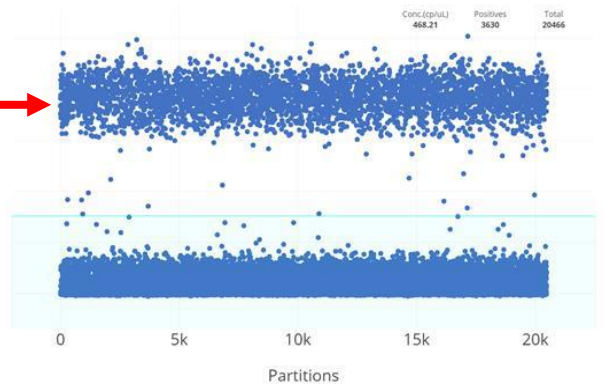


Amount of *Fusarium* spp. DNA, *F. graminearum* DNA, and *F. culmorum* DNA in the oat samples

3. Digital Droplet PCR

- Digital PCR is a method of quantifying nucleic acid targets without standard curves by dividing the bulk reaction into thousands of smaller, independent reaction
- Microchambers with a target molecule are easily distinguished from microchambers with no target molecule.
- The number of fluorescent microchambers are counted and used to determine the absolute quantity of the target





High-fluorescence positive reactions

Low-fluorescence negative reactions

Advantages

- Digital PCR provides an absolute quantification of target(s)Improved accuracy and precision over qPCR
- No standard curve required (not a relative quantification)
- More reproducible from lab to lab
- Resistant to inhibitors
- Diluting target simultaneously dilutes inhibitors
- Ideally suited for targets at lower concentrations
- Provides higher statistical power to quantify low concentration samples

Weaknesses

- Limited dynamic range compared to qPCR
- qPCR generally considered as cheaper, faster, and higher throughput (although this is gradually changing)

Source: Thermo Fischer scientific

Supporting Findings- 01

- ✓ Primers targeting TRI 5 gene (specific to *F. graminearum*) was targeted in this study
- ✓ Antagonist bioassays were performed on detached spikelet at 20 °C with a photoperiod and analyzed after 8 days of co-culture
- ✓ Main graphic presents quantity of *F. graminearum* biomarkers in amount of copy.µL−1 in presence of different BCAs treatments. Myco:Mycostop® , Xeda: Xedavir® , Poly: Polyversum®
- ✓ A focus was realized on *F. graminearum*–Xeda interaction in blue circle: quantity of *F. graminearum* biomarkers copy and Xeda biomarker

toxins

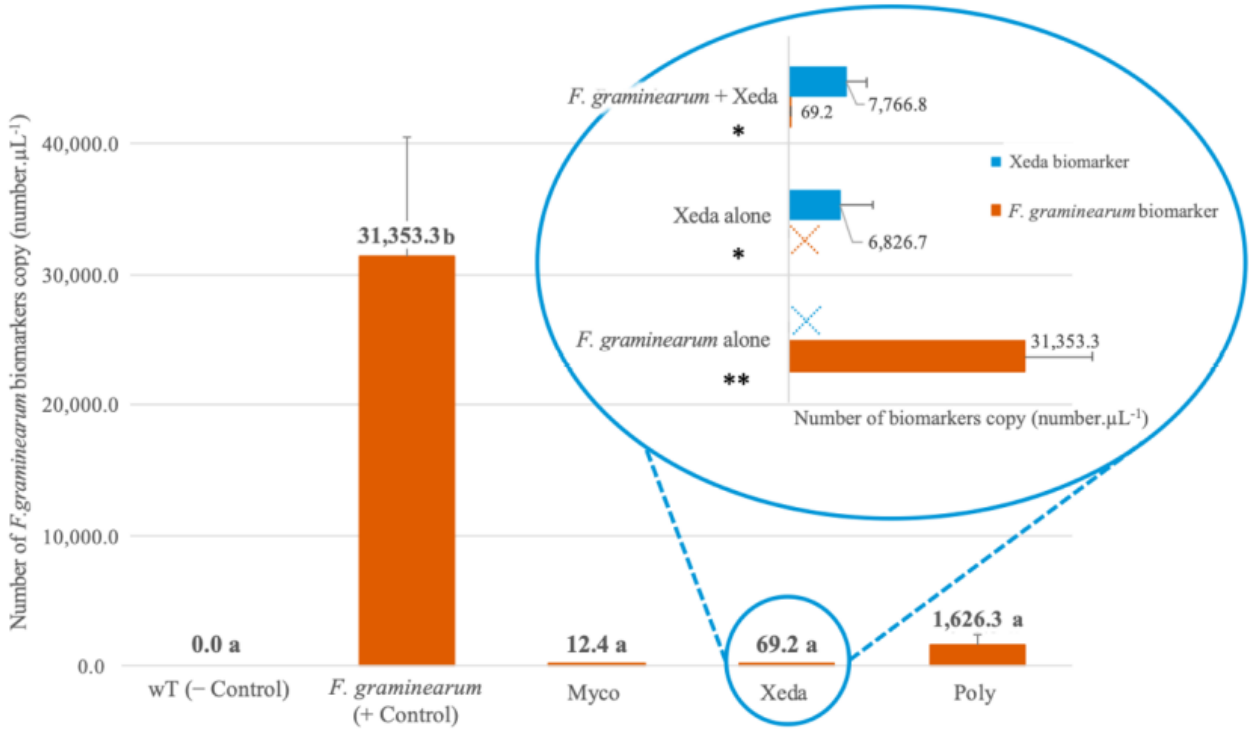
MDPI

Article

Biocontrol Agents Reduce Progression and Mycotoxin Production of *Fusarium graminearum* in Spikelets and Straws of Wheat

Lucile Pellan [1,*], Cheikh Ahmeth Tidiane Dieye [1], Noël Durand [1,2], Angélique Fontana [1], Sabine Schorr-Galindo [1] and Caroline Strub [1,*]

[1] Qualisud, Univ Montpellier, Avignon Université, CIRAD, Institut Agro, IRD, Université de La Réunion, F-97490 Montpellier, France; cheikh.dieye@umontpellier.fr (C.A.T.D.); noel.durand@cirad.fr (N.D.); angelique.fontana@umontpellier.fr (A.F.); sabine.galindo@umontpellier.fr (S.S.-G.)
[2] CIRAD, UMR Qualisud, F-34398 Montpellier, France
* Correspondence: pellan.lucile@gmail.com (L.P.); caroline.strub@umontpellier.fr (C.S.); Tel.: +33-467-3212 (L.P.); +33-467-143-201 (C.S.)



Supporting Findings- 02

ELSEVIER
Journal of Microbiological Methods
Volume 190, November 2021, 106319

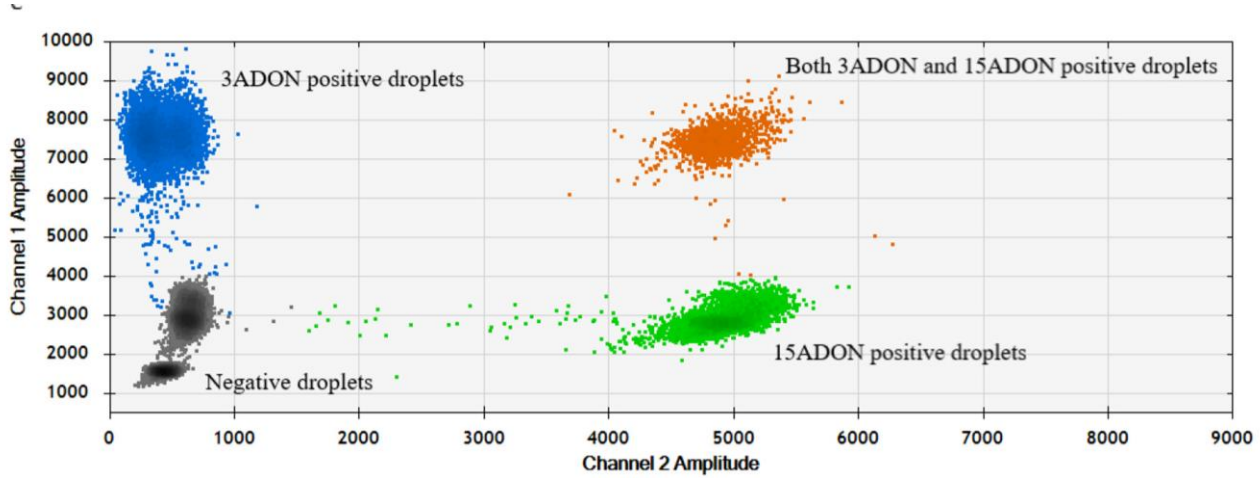
Simultaneous quantitation of 3ADON and 15ADON chemotypes of DON-producing *Fusarium* species in Chinese wheat based on duplex droplet digital PCR assay

Song Shan Wang, Hua Cui, Meng Ze Chen, Li Li, Yu Wu, Song Xue Wang

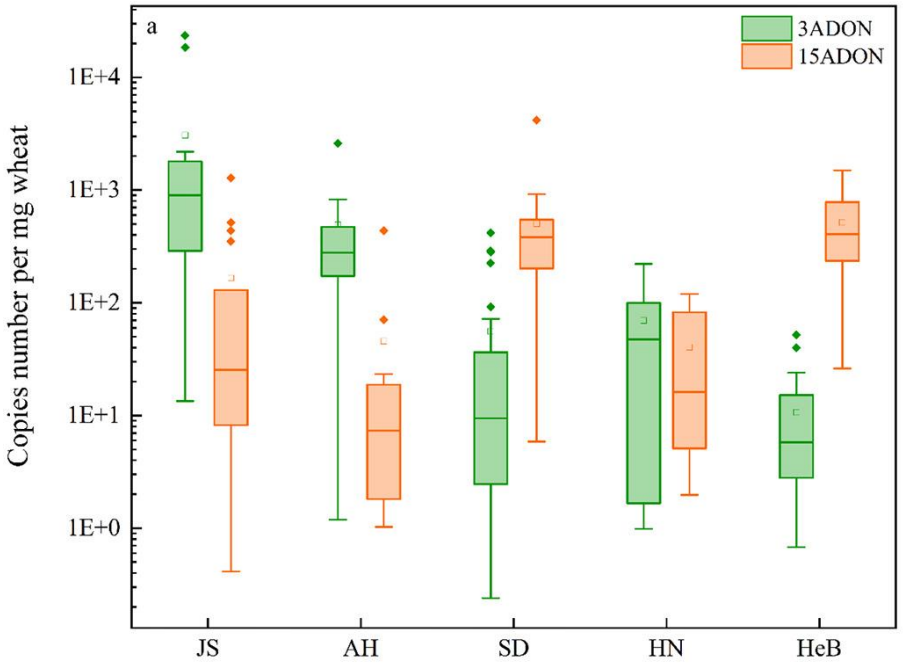
- In this study, a duplex droplet digital PCR (duplex ddPCR) assay was developed that allowed for the simultaneous quantitation of 3ADON and 15ADON chemotypes of DON-producing *Fusarium* species

Primers and probes

- 3ADON-F/3ADON-R (3-MGB-probe, 5′-6-FAM and MGB-3′) 15ADON-F/15ADON-R (15-MGB-probe, 5’-HEX and MGB-3′)



2D amplitudes showed 3ADON and 15ADON chemotypes. Channel 1 (Ch1) and Channel 2 (Ch2) indicate the FMA channel (3ADON chemotype) and HEX channel (15ADON chemotype), respectively



Box -plot showed concentration of 3ADON and 15ADON chemotypes in wheat samples. Samples were collected from Jiangsu (JS), Anhui (AH), Shandong (SD), Henan (HN) and Hebei (HeB) provinces of China

Thank You