

# Development of a qualitative and quantitative qPCR assay to detect teliospores of *Tilletia controversa* in wheat seed samples

Institute for Crop Science and Plant Breeding  
Seed Testing and Seed Research

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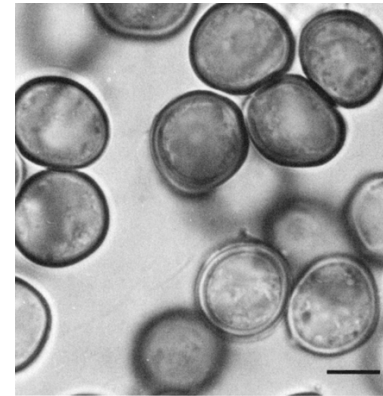
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Institute for Epidemiology and Pathogen Diagnostics, Germany

<sup>3</sup>Technical University of Munich, TUM School of Life Sciences,  
Chair of Technical Microbiology, Germany

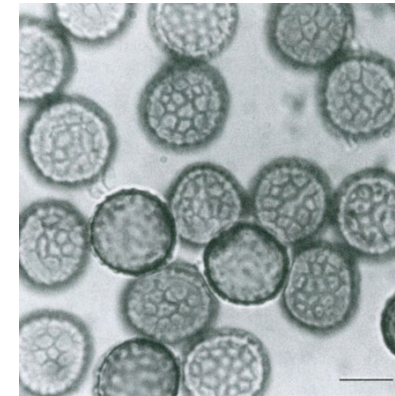
# Differentiation of *Tilletia* spp.

## Relevance of differentiation

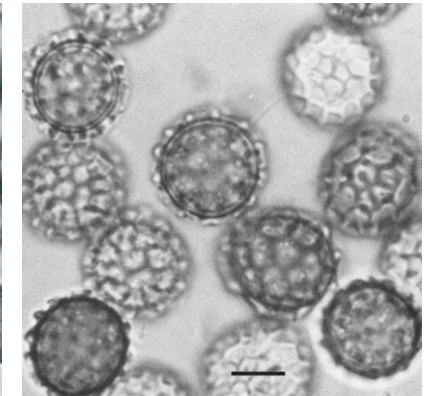
- (Chemical) seed treatment
- Choice of wheat variety (susceptibility)
- International seed trading (quarantine regulations)



*Tilletia laevis*



*Tilletia caries*

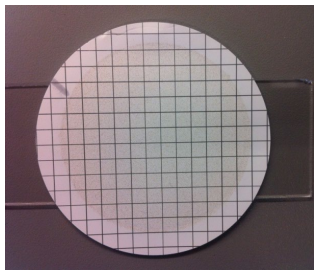


*Tilletia controversa*

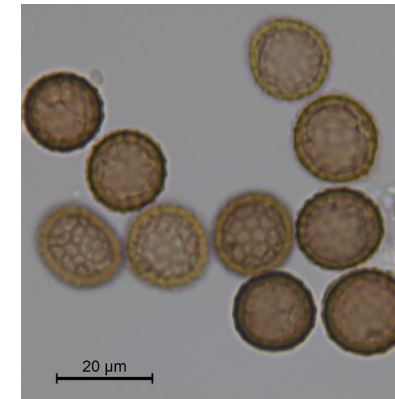
Bar = 10 µm. © Vánky, 2012: Smut Fungi of the World. APS

## Current method

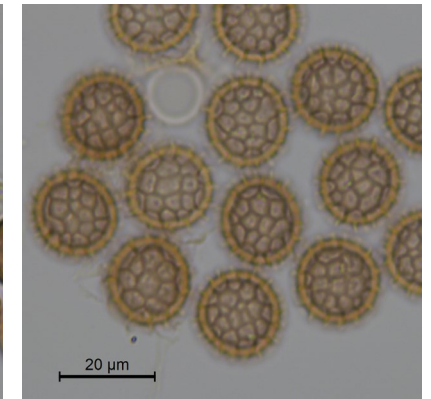
‘Optimized filtration method’ (2016) according to ISTA Working Sheet No 53 (1984), slightly modified



- Needs well educated and regularly trained professionals
- Microscopic spore counting is exhausting and time consuming



*Tilletia caries*



*Tilletia controversa*

# Cooperation project

With support from



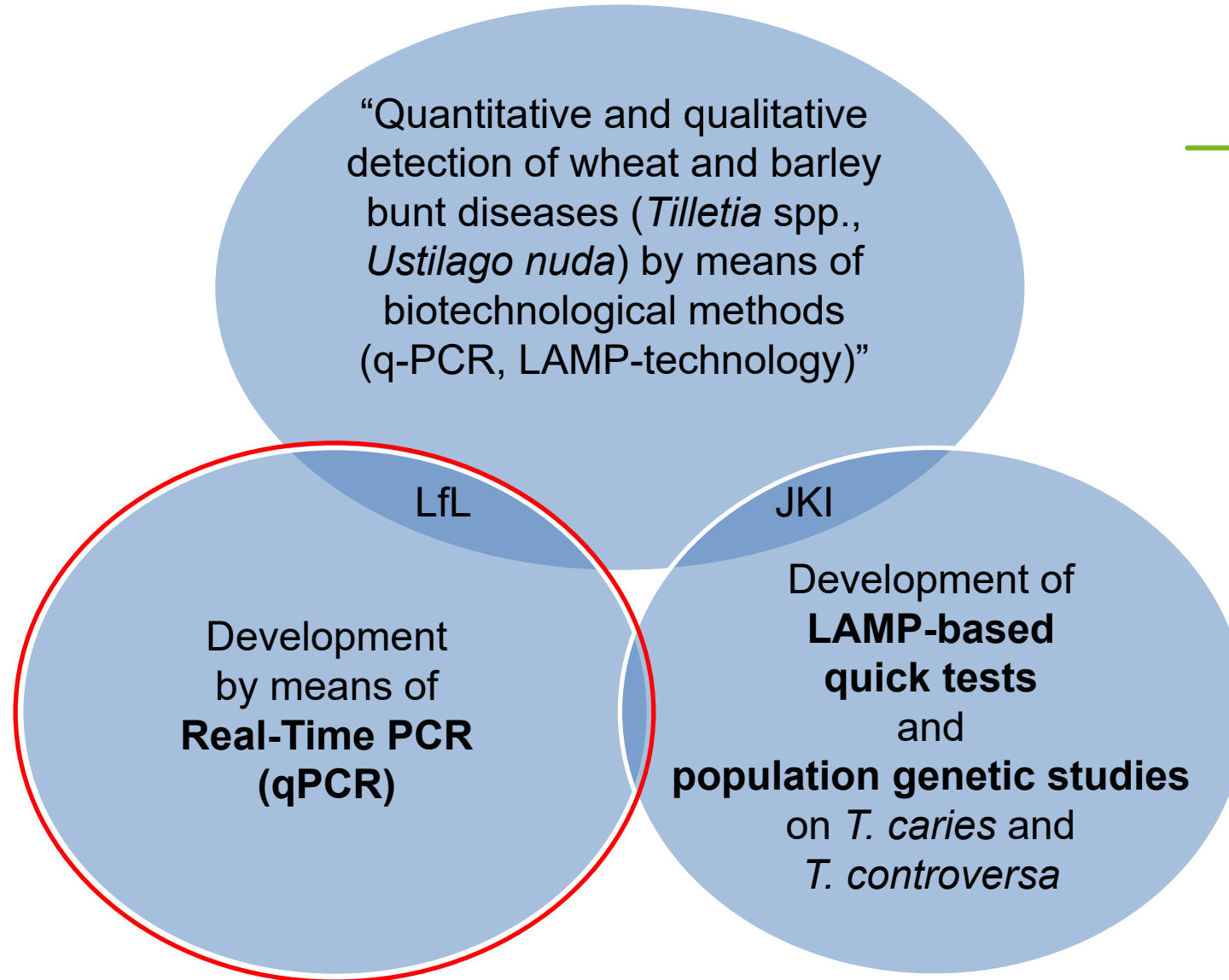
Federal Ministry  
of Food  
and Agriculture

by decision of the  
German Bundestag

## BÖLN

Bundesprogramm Ökologischer Landbau  
und andere Formen nachhaltiger  
Landwirtschaft

(federal program for organic  
and sustainable farming)

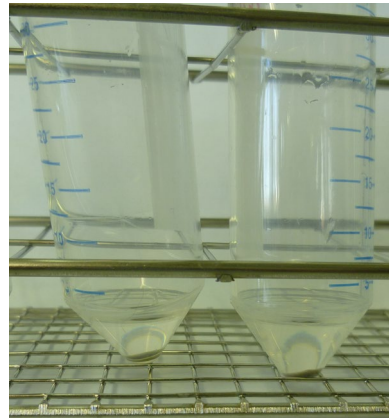
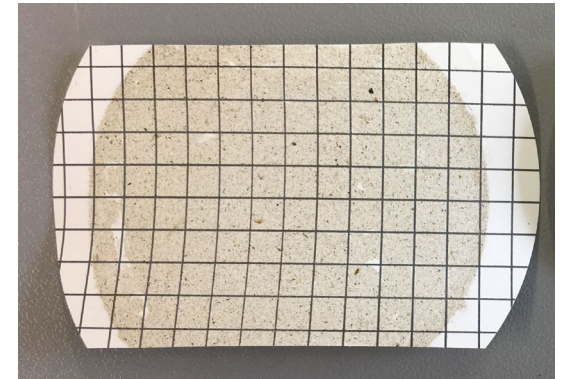


# Sample preparation

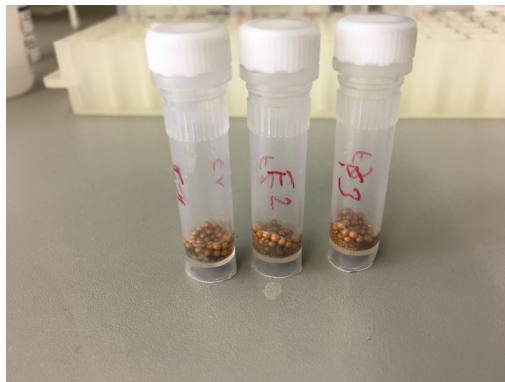
Workflow – detection of *Tilletia* spp. in wheat samples



Filtration method  
as reference



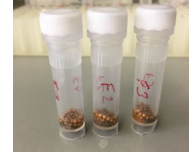
qPCR method





# qPCR assay to detect *T. controversa*

Sample preparation



DNA isolation

all teliospores considered

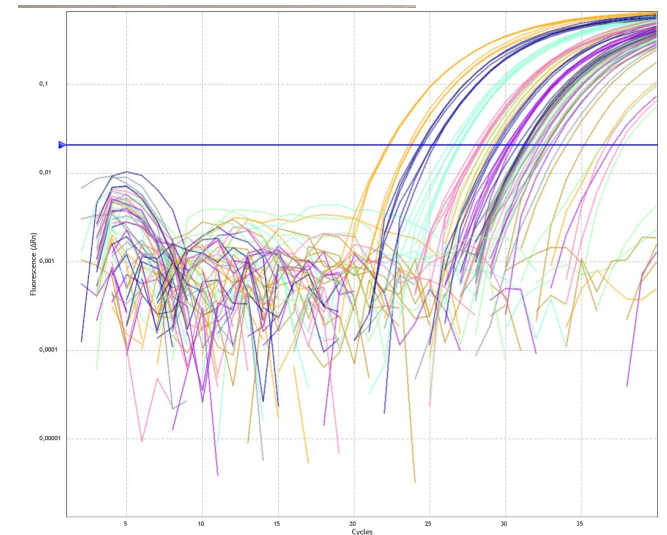
Species-specific  
primer and probe

Program  
(temperatures,  
cycle numbers)

qPCR

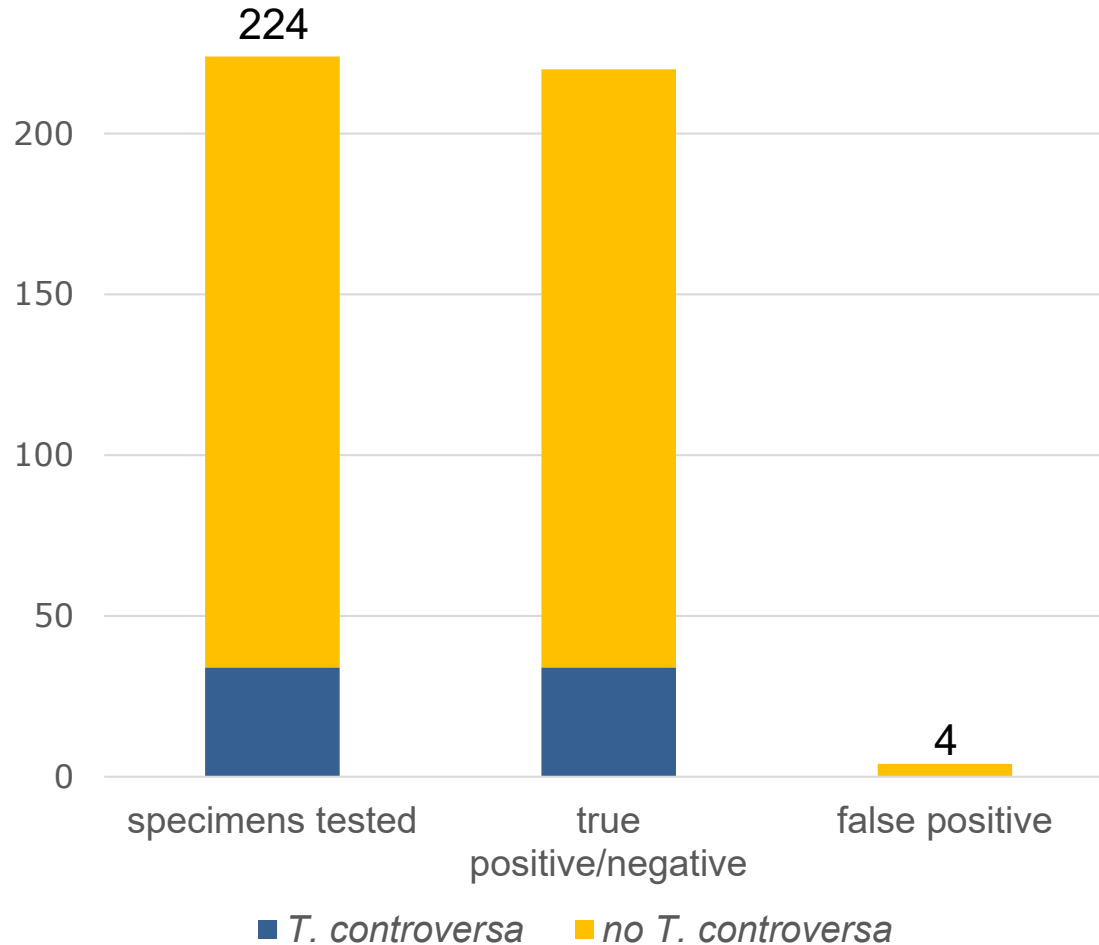
Master Mix  
(concentration of  
primer, probe,  
template)

Quantification standards  
(cloning, define copy number)



# Specificity and sensitivity

## Qualitative test



Accuracy = 98.21 %

## Quantification standards

- cloning
- MPN PCR

Dilution	10 <sup>-7</sup>	10 <sup>-8</sup>	10 <sup>-9</sup>	10 <sup>-10</sup>	10 <sup>-11</sup>
positive PCR-reaction / triplicate	3	3	1	1	0

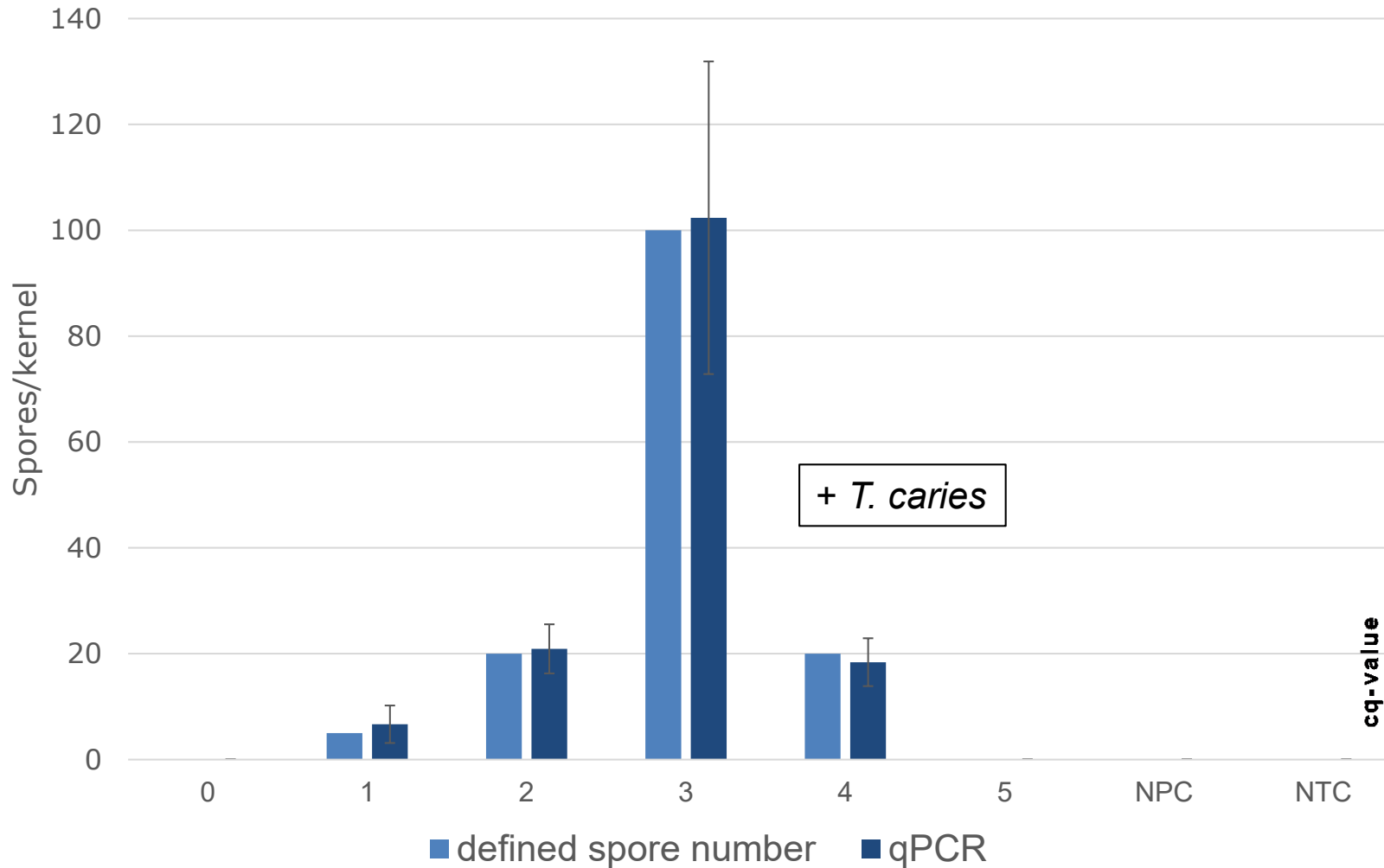
MPN Index	most probable number in 1 µl stock solution	
	copies [billion]	spores [billion]
0.74	24.66	12.33

Standard	initiale quantity	
	spores	spores per kernel
S1	1233000	2880.84
S2	411000	960.28
S3	137000	320.09
S4	45667	106.70
S5	15222	35.57
S6	5074	11.86
S7	1691	3.95
S8	564	1.32

LOD

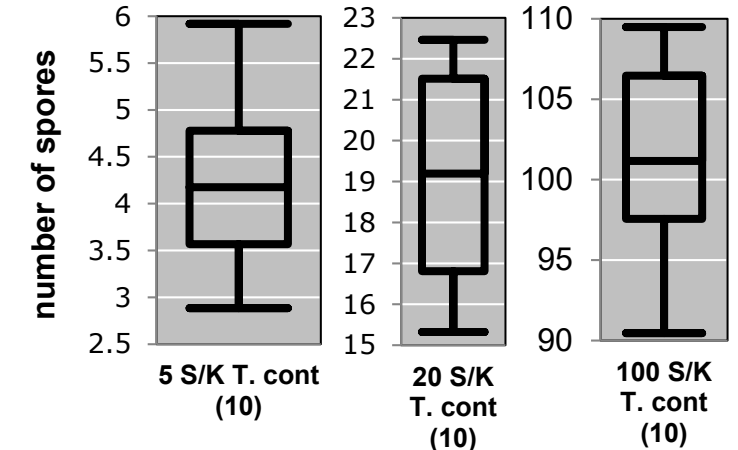
# Artificially infested wheat seeds

*T. controversa*

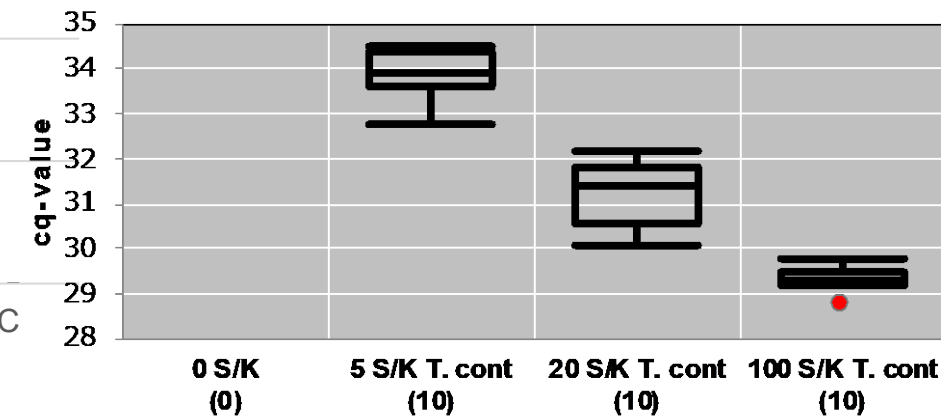


Homogeneity test

Filtration method

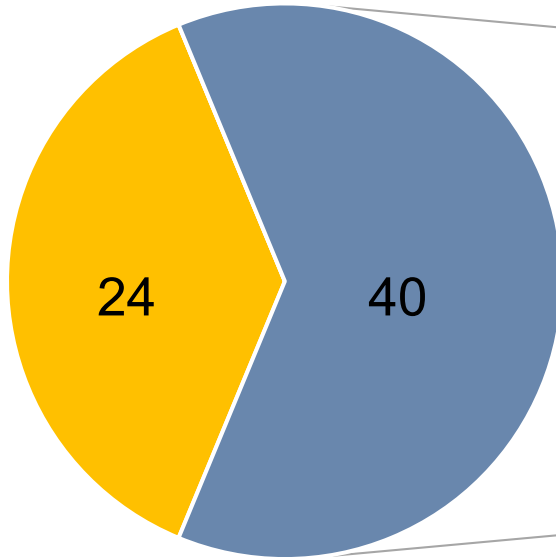


qPCR method (probe)

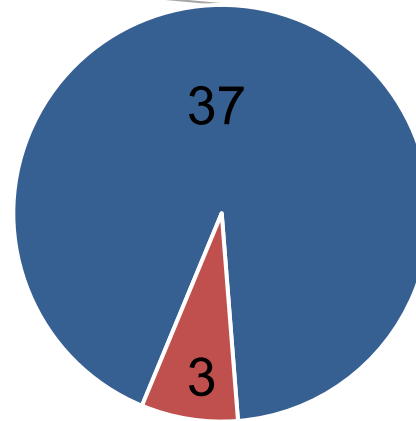


# Naturally infested wheat seeds (N=64)

filtration method



qPCR method

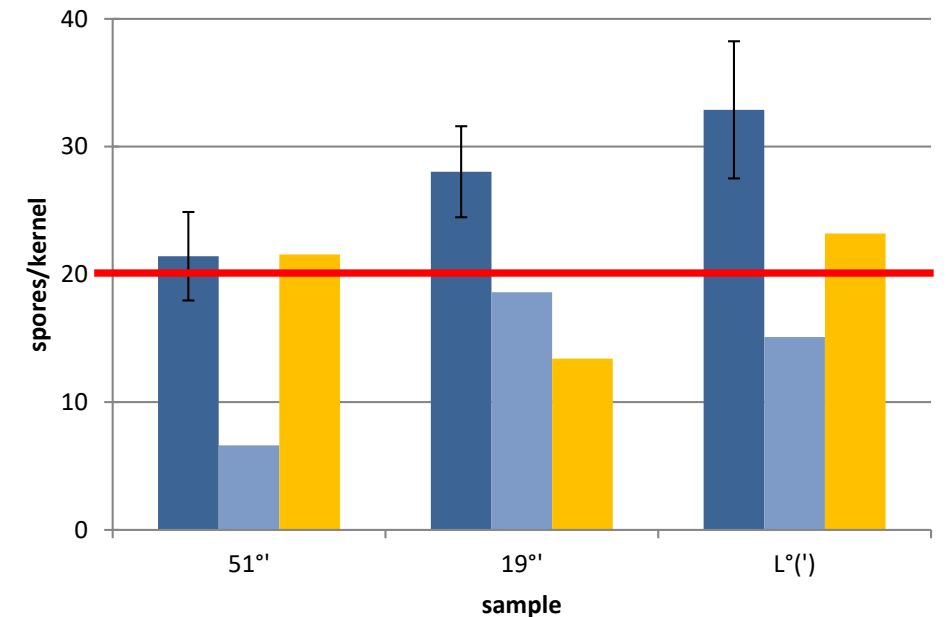


< detection limit

- common bunt or not infested
- dwarf bunt qPCR true positive
- dwarf bunt qPCR false negative

95.31 % of the samples above or below the threshold (Germany 20 s/k)  
 ✓ consistently determined with both methods

3 samples inconsistent

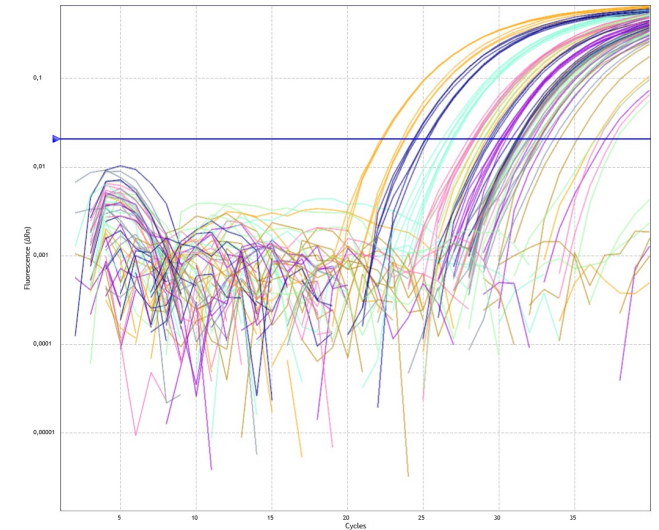
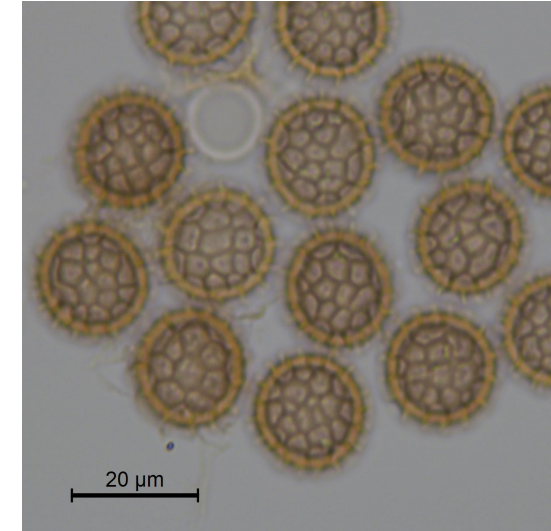


- *T. controversa* (qPCR)
- *T. controversa* (filtration)
- *T. caries* (filtration)



# Summary and Conclusion

- Good quality of DNA and sufficient yield
    - LOD around 1 s/k
  - Species-specific primer and probe to detect *T. controversa*
    - No cross-reaction with *T. caries*, *T. laevis*, *T. indica*, ...
  - Consistent determination with qPCR assay and filtration method
    - German thresholds
- Thus, the developed qPCR assay is a promising tool to be used in seed testing laboratories in future



# Acknowledgement



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Berta Killermann



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Ludwig Niessen

With support from



Federal Ministry  
of Food  
and Agriculture

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by decision of the  
German Bundestag

