

Cereal grain mycoflora on single seeds

Comparison of metabarcoding and VideometerLab for pathogen detection on cereal grain

Mogens Nicolaisen, Merete H Olesen, Birte Boelt, Johannes Ravn Jørgensen



Søren Knudsen 

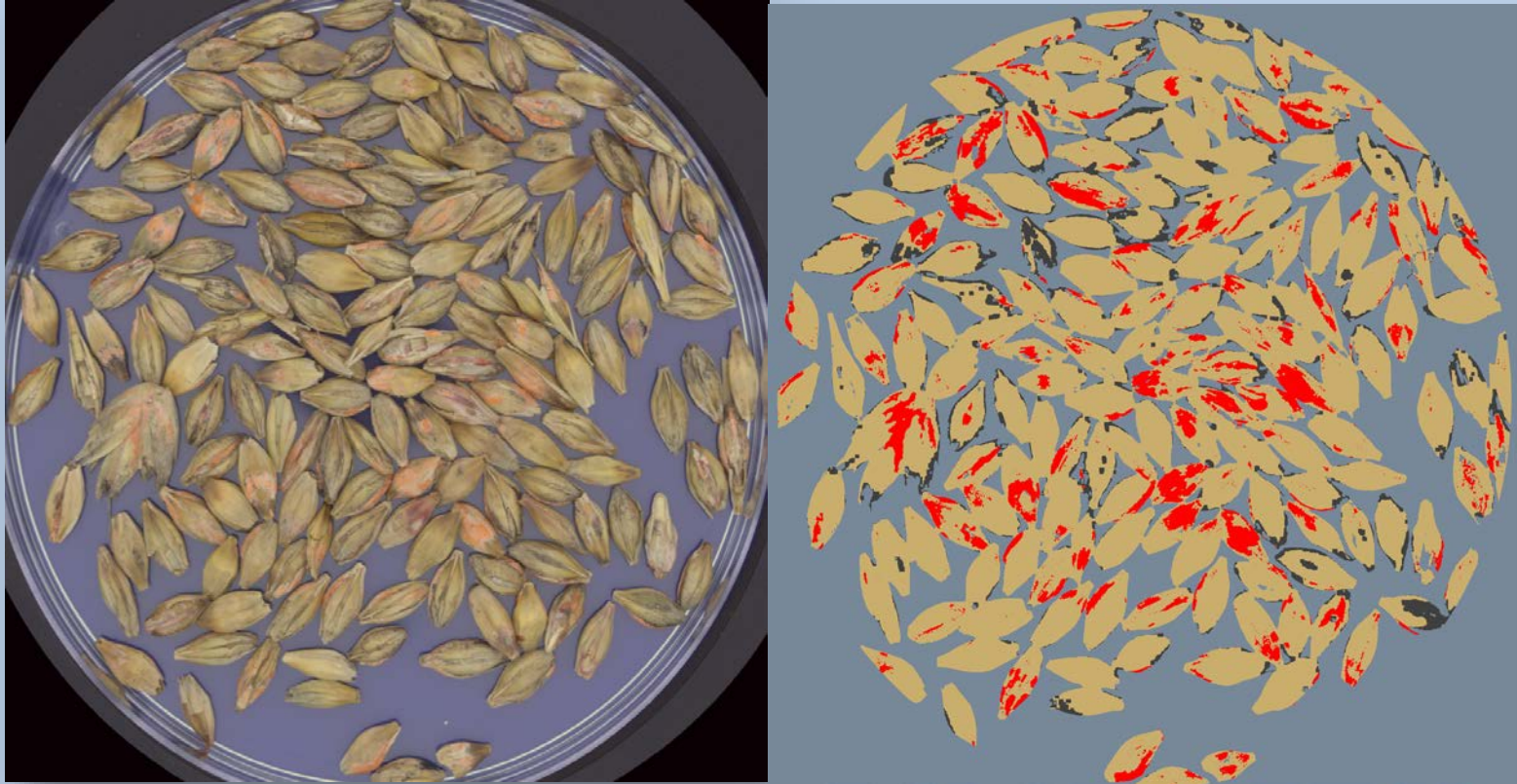
Kim Jørgensen
Dorte Rennebjerg



Jens Michael Carstensen



Videometer image

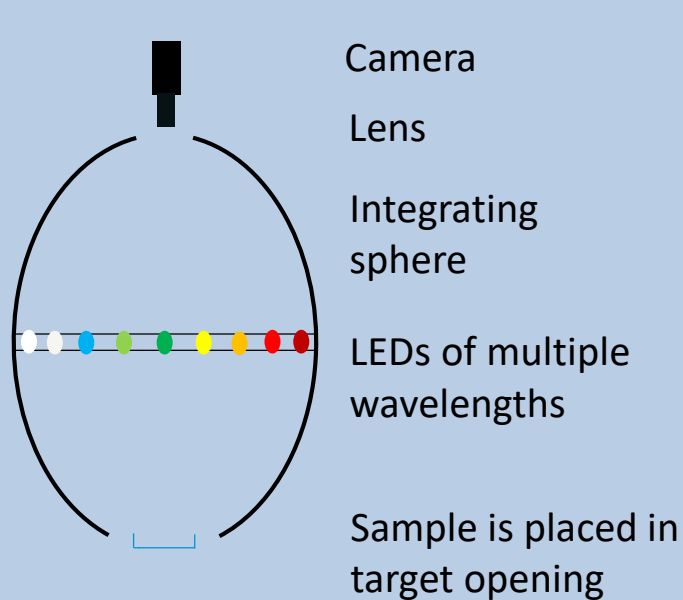


Red color = *Fusarium*

Gray color = grey and black moulds

Brown = barley without moulds

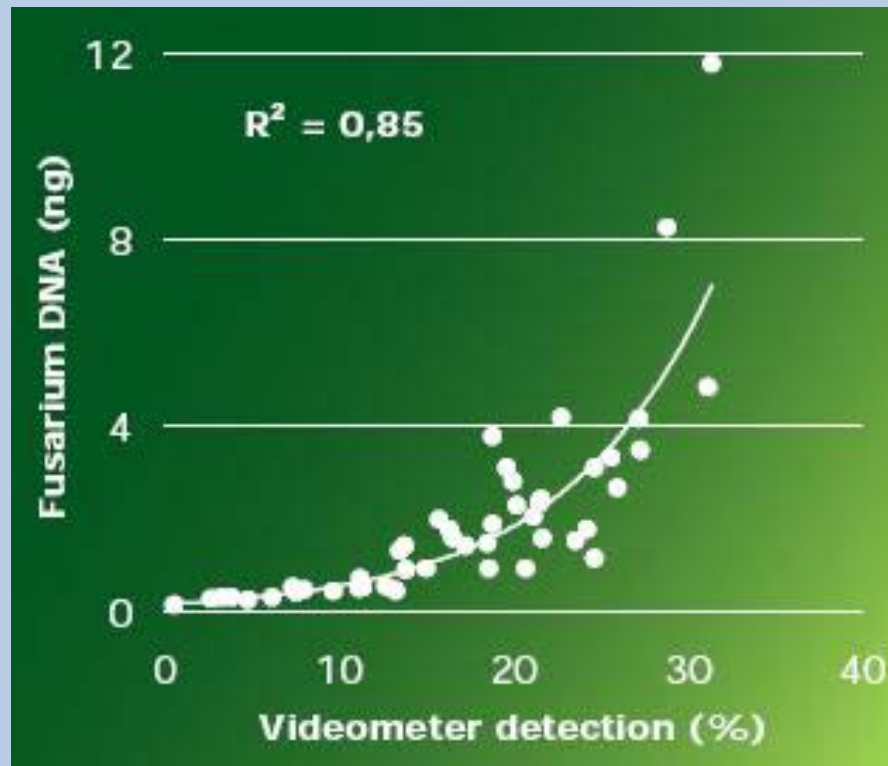
Videometer Spectral Imaging



- LEDs: Stable, durable, large selection, rapidly developing technology
- Up to 20 different high-resolution bands acquired sequentially in 0.5-1.5 seconds depending on camera
- May be combined with emission filters, backlight, and darkfield illuminant

Validation by Carlsberg Research Center:

Comparison between VideometerLab® measurements and the level of Fusarium DNA quantified by real-time PCR on batches of seed

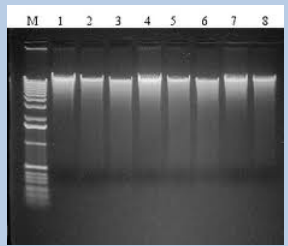


Excellent correlation with Fusarium DNA level
($R^2=0,85$)

Metabarcoding workflow

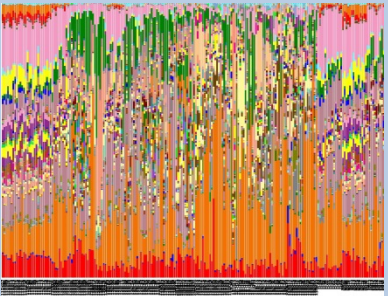
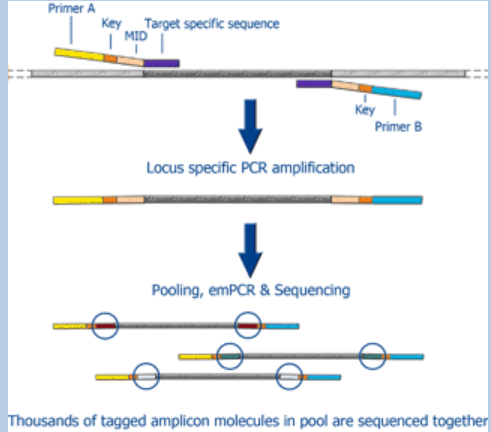


DNA extraction

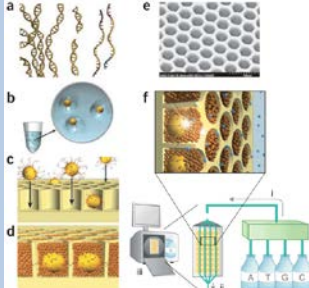


Fusarium qPCR

PCR amplification of ITS1
(ITS1-F/58A2R)



Amplicon 454 sequencing



~200.000 sequences



Data analysis

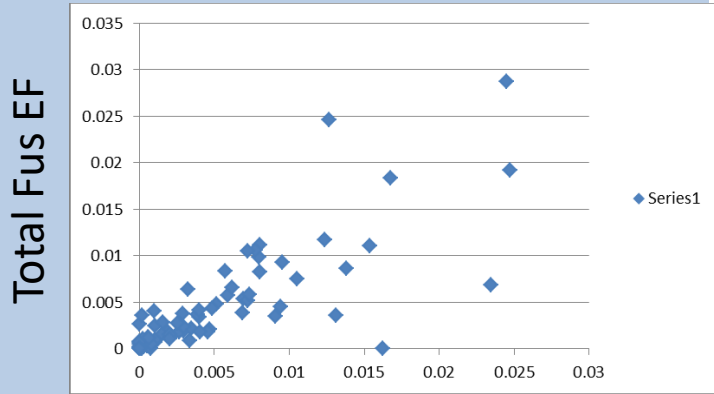
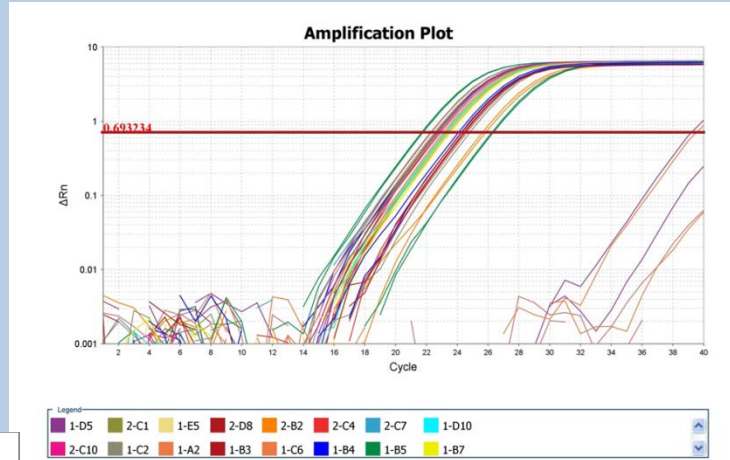


Metabarcoded samples

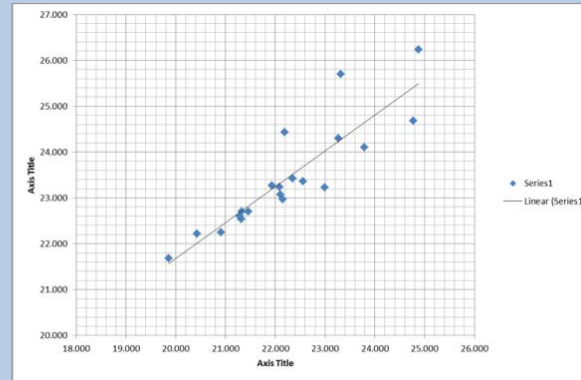


Fusarium qPCR

11 assays for individual *Fusarium* species, one generic



Summarized



EF



Real-time PCR for quantification of eleven individual *Fusarium* species in cereals

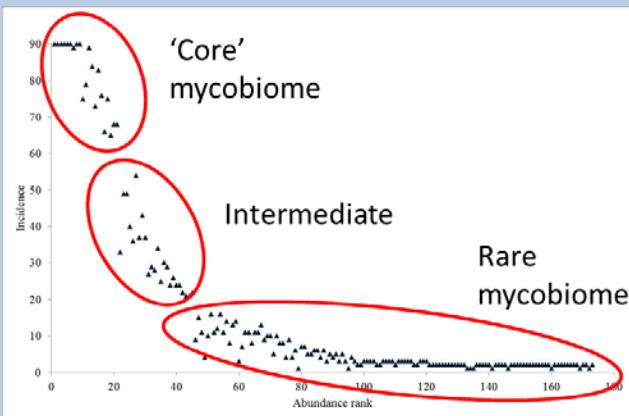
Mogens Nicolaisen ^{a,*}, Skaidrė Supronienė ^b, Linda Kærsgaard Nielsen ^a, Irene Lazzaro ^a, Niels Henrik Spliid ^a, Annemarie Fejer Justesen ^a

^a Aarhus University, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Denmark
^b Lithuanian Institute of Agriculture, Lithuania

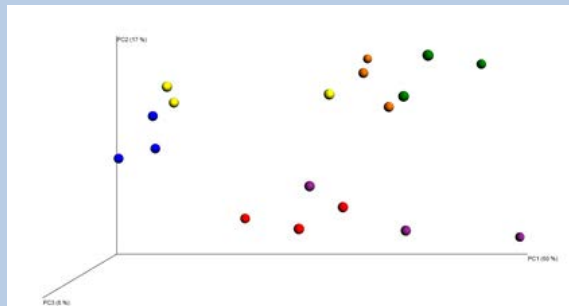
Assay	Fragmearum	Fculmorum	Fpoae	Flangsethiae	Fsporotrichoides	Fquiseti	Ftrincinctum	Favenaceum	Fverticilloides	Fsubglutinans	Fproliferaum
Water											
<i>F. oxysporum</i> 8329											
<i>F. oxysporum</i> 9514											
<i>F. flocciferum</i> 8932											
<i>F. flocciferum</i> 1434											
<i>F. sambucinum</i> 2524											
<i>F. sambucinum</i> 1731											
<i>F. pseudogram.</i> R-05291											
<i>F. pseudogram.</i> 1547											
<i>F. cerealis</i> CBS195.80			21.0	19.9							
<i>F. cerealis</i> CBS589.93											
<i>F. venenatum</i> 1337											
<i>F. venenatum</i> 1338											
<i>F. proliferatum</i> B8376B											22.9
<i>F. proliferatum</i> A8376A											21.1
<i>F. subglutinans</i> 8567										23.5	
<i>F. subglutinans</i> 8005										23.5	
<i>F. verticilloides</i> 8000											
<i>F. verticilloides</i> 8002										20.1	
<i>F. avenaceum</i> 8500										22.8	
<i>F. avenaceum</i> 9605										23.3	
<i>F. trincinctum</i> 2952											
<i>F. trincinctum</i> 8424										25.2	
<i>F. equiseti</i> 8752										20.4	
<i>F. sporotrichoides</i> 2680					23.8						
<i>F. sporotrichoides</i> 1926					23.8						
<i>F. langsetiae</i> 9955											
<i>F. langsetiae</i> 8051								23.0			
<i>F. poae</i> 8452										23.2	
<i>F. poae</i> 1766										22.1	
<i>F. culmorum</i> 9560										22.7	
<i>F. culmorum</i> 8984										19.6	
<i>F. fragmearum</i> 1955										22.7	
<i>F. fragmearum</i> 9319										21.4	

Metabarcoding

Wheat seed lots from ~200 conventional farms at harvest



	Sequences	BLAST Id	Disease in wheat
OTU1	48161	<i>F. graminearum</i> group (Fgr)	Fusarium head blight
OTU2	45980	<i>Lewia infectoria</i>	Black head mold
OTU3	36072	<i>Cladosporium herbarum</i>	Black head mold
OTU4	22066	<i>Didymella exitialis</i>	Ascochyta leaf scorch
OTU5	16215	<i>Fusarium avenaceum</i> (Fav)	Fusarium head blight
OTU6	9959	<i>Epicoccum nigrum</i>	Black head mold
OTU7	9327	<i>Microdochium nivale</i>	Fusarium head blight
OTU8	2607	<i>Alternaria alternata</i>	Black head mold
OTU9	2241	<i>Cladosporium cladosporioides</i>	Black head mold
OTU10	2139	<i>Pyrenophora tritici-repentis</i>	Tan spot
OTU11	1870	<i>Fusarium poae</i> group (Fpo)	Fusarium head blight
OTU12	1822	<i>Mycosphaerella graminicola</i>	Septoria tritici blotch
OTU13	1421	<i>Botrytis cineria</i>	Gray mold
OTU14	1404	<i>Phaeosphaeria nodorum</i>	Stagonospora nodorum blotch
OTU15	1209	<i>Cryptococcus victoriae</i>	non pathogenic
OTU16	745	<i>Sporobolomyces</i> sp	Black head mold
OTU17	493	<i>Stemphyllium</i>	Black head mold
OTU18	374	<i>Cryptococcus tephrensii</i>	non pathogenic
OTU19	327	<i>Alternaria</i> sp.	Black head mold
OTU20	312	<i>Phaeosphaeria avenaria</i> f. sp. tr	Stagonospora nodorum blotch
OTU21	289	<i>Dioszegia hungarica</i>	non pathogenic

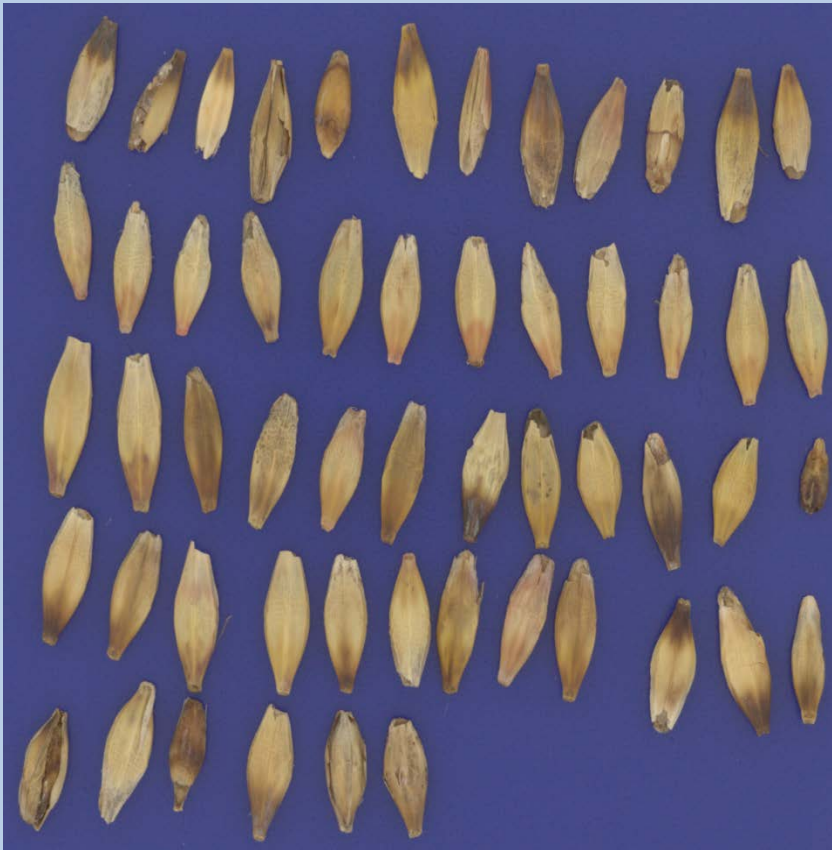


Cultivar effect

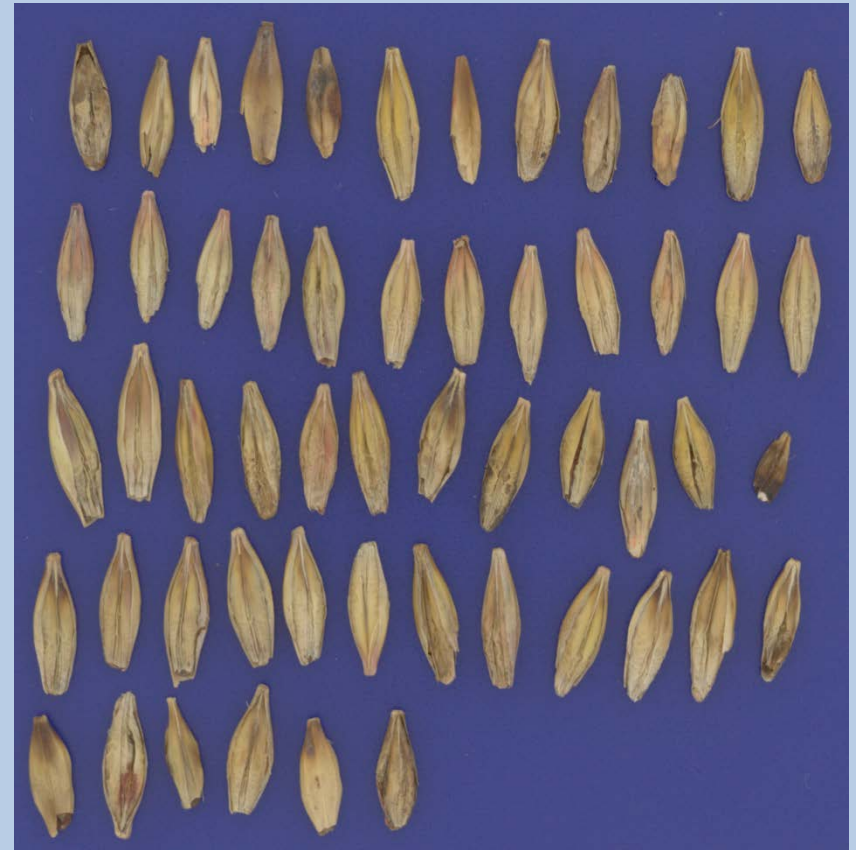


Malting barley seeds with visible signs of fungal infection

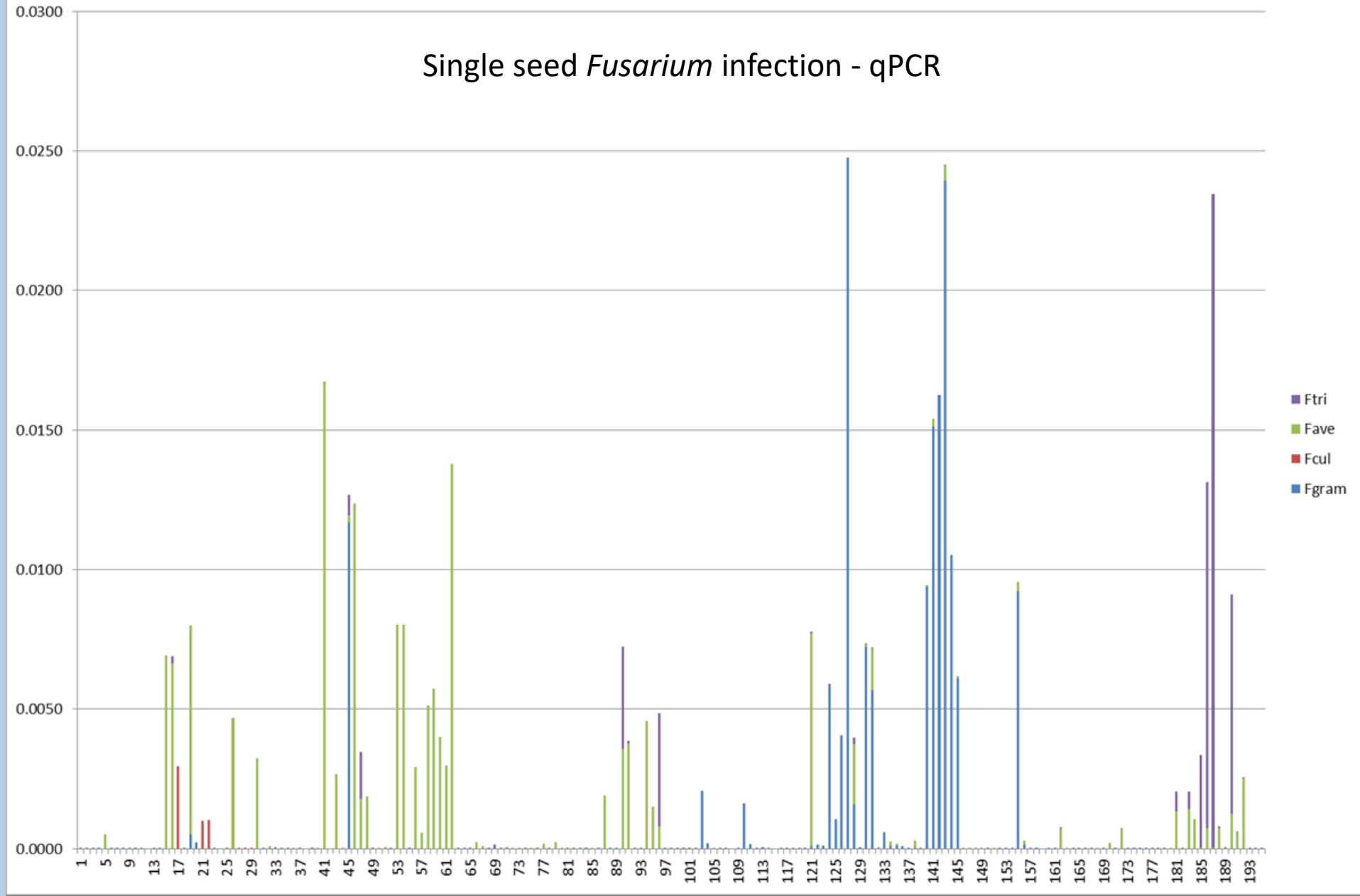
Dorsal



Ventral



Single seed *Fusarium* infection - qPCR

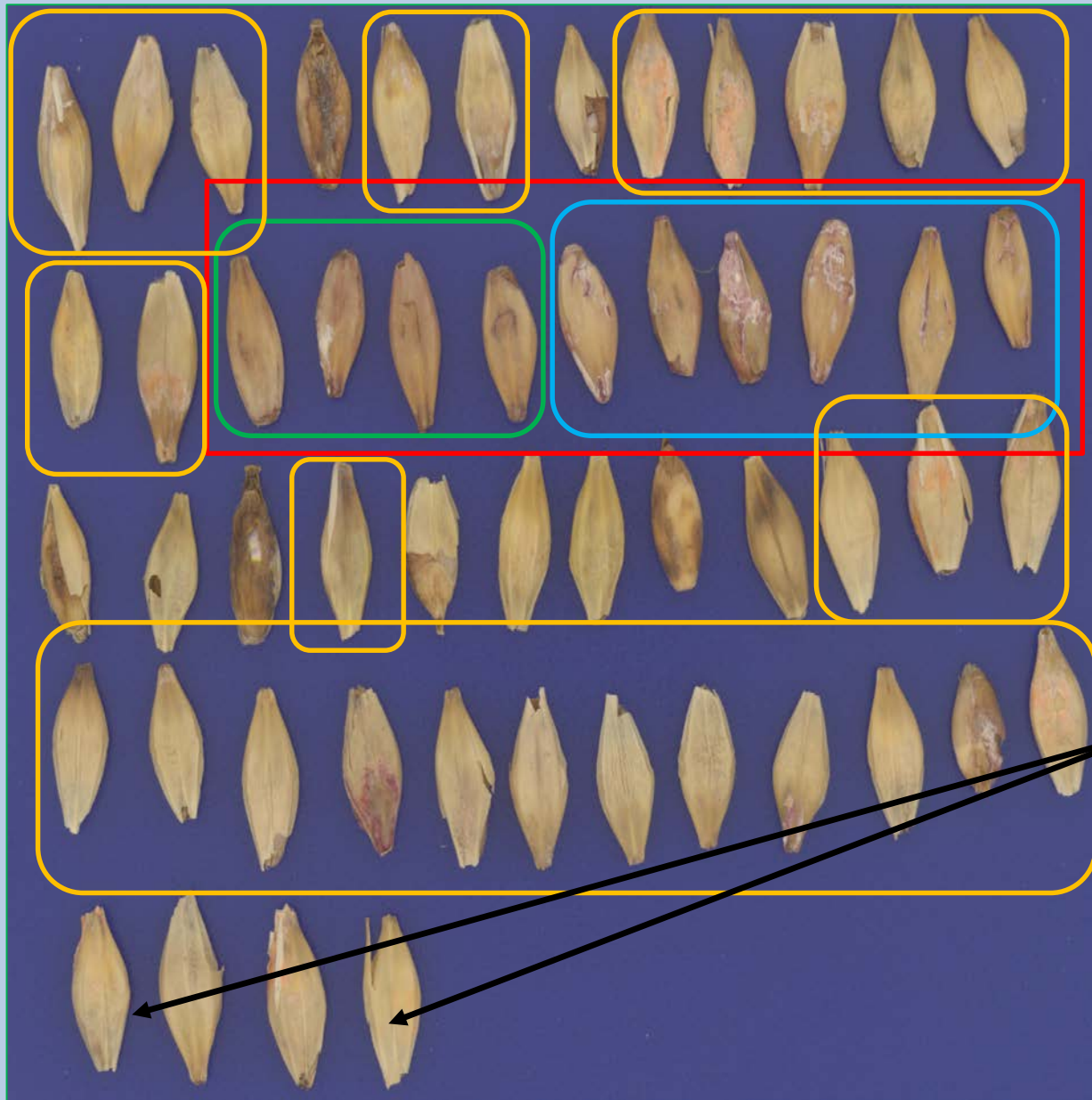


Sequences from entire dataset

ID	% abundance
F. avenaceum	38.814835
Pyrenophora teres	13.932855
F. poae	7.868754
Cladosporium	6.3350191
F. graminearum	5.6560397
Lewia infectoria	5.2410193
Didymella exitalis	4.0651601
Epicoccum nigrum	2.7365175
Microdochium bolleyi	2.5928317
Bipolaris eleusines	1.6260415
Alternaria alternata	0.8952025
Fungal(?)	0.8139792
Phaeosphaeria nodorum	0.6314249
Sporisorium	0.4012329
Shiraia bambusicola	0.3250396
Verticillium dahliae	0.3247018

Pyrenophora teres	F. poae	F. avenaceum	Epicoccum nigrum	Didymella exitalis	Cladosporium	Lewia infectoria	Bipolaris eleusines	F. graminearum	Microdochium bolleyi	Alternaria alternata	Phaeosphaeria nodorum	Verticillium dahliae	Shiraia bambusicola	Fungal	Sporisorium	Others
0.3	0.1	8.0	4.6	0.1	1.0	0.2	0.1	13.1	55.6	1.3	1.6	0.0	0.0	0.0	0.0	1.6
0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
0.0	0.0	1.8	0.2	0.1	1.2	0.1	0.0	0.7	0.0	0.4	0.3	0.1	0.1	0.0	0.0	3.2
37.4	0.1	0.7	0.8	0.3	1.3	0.6	0.0	22.8	0.1	1.1	3.4	33.7	0.0	0.0	0.0	7.9
0.0	0.0	0.3	4.9	0.1	3.2	12.8	0.8	0.3	0.0	0.9	47.7	0.0	22.4	0.0	0.0	6.4
0.0	0.1	7.9	2.7	0.7	2.0	15.0	63.0	1.0	0.1	0.8	3.1	0.1	0.0	0.0	0.0	3.6
0.2	0.1	5.8	3.4	0.1	0.4	0.4	0.1	60.3	24.6	1.4	0.1	0.2	0.2	0.0	0.0	2.7
0.2	0.1	1.0	0.3	0.1	3.3	25.7	0.0	1.8	35.8	0.3	0.4	8.1	0.0	0.0	0.0	22.8
15.7	0.0	0.2	0.6	7.7	17.2	1.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	6.3
38.8	0.2	0.2	1.1	25.8	12.3	1.2	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	20.2
16.6	0.4	0.2	0.3	5.9	24.6	31.4	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	20.6
50.7	0.0	0.0	2.4	30.5	5.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.4
80.8	0.1	0.0	0.3	1.1	17.4	1.3	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	14.8
25.4	0.0	22.1	0.1	38.1	1.7	4.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	8.5
15.3	0.0	0.0	0.6	2.0	5.4	0.3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	6.3
28.3	0.0	0.6	0.5	5.1	6.9	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.3
55.3	1.1	0.2	1.6	10.4	14.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8
22.6	0.0	0.0	0.7	8.8	2.9	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5
59.3	0.0	0.1	0.2	12.2	11.8	0.4	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.8
45.8	0.0	1.1	0.4	16.7	19.8	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.5
16.3	0.0	0.1	3.1	0.8	4.9	47.7	0.0	0.0	0.8	0.0	0.0	0.1	0.0	0.0	0.0	26.0
17.1	0.7	0.3	0.8	1.3	5.0	7.8	0.0	0.1	0.0	0.1	0.0	2.1	0.0	0.0	0.0	10.4
73.3	0.0	0.2	0.5	1.1	3.0	2.1	0.0	0.1	0.0	0.1	0.0	3.7	0.0	0.0	0.0	16.0
2.0	0.5	5.0	0.8	3.1	5.1	51.8	0.0	0.4	0.7	0.1	0.4	0.0	0.0	0.0	0.0	30.4
26.1	0.3	0.2	0.3	0.4	1.7	1.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	69.5
73.9	0.1	0.1	0.4	6.8	4.3	1.5	0.0	0.1	0.0	0.2	1.6	0.0	0.0	0.0	0.0	19.3
0.1	75.4	0.4	7.8	0.1	0.7	7.1	0.0	0.1	0.1	0.1	24.0	0.0	0.0	0.0	0.0	2.9
0.0	78.1	0.1	0.6	0.1	8.5	1.6	0.0	0.0	0.0	0.2	29.0	0.0	0.0	0.0	0.0	7.9
0.6	0.4	38.7	10.7	0.2	20.4	9.5	0.0	0.2	0.2	0.7	2.9	1.9	0.0	0.0	0.0	16.7
0.5	0.5	0.0	0.4	0.8	0.7	0.7	0.0	0.0	0.1	0.7	0.0	0.0	0.0	0.0	0.0	0.8
0.4	0.5	0.7	15.4	2.5	28.6	7.9	0.0	0.1	0.0	22.8	0.1	0.0	0.0	0.0	0.0	24.7
0.0	97.1	0.1	0.4	0.0	0.2	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	1.9
62.9	0.4	0.0	3.5	5.1	8.8	1.4	0.0	0.2	0.0	0.1	0.1	0.0	0.0	0.0	0.0	17.4
78.7	0.2	0.0	0.1	1.2	3.9	0.5	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	16.2
88.0	0.1	0.1	0.8	1.0	7.1	0.6	0.0	0.1	0.0	0.1	0.5	0.0	0.0	0.0	0.0	7.5
28.9	0.3	0.2	1.5	16.4	14.6	37.9	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	22.1
3.7	0.4	0.1	70.2	1.9	7.2	0.9	0.0	0.1	0.0	0.4	0.0	0.0	0.0	0.0	0.0	14.9
22.1	0.1	0.6	1.4	130	28.1	1.3	0.0	0.6	0.0	25.0	0.8	0.0	0.0	0.0	0.0	29.5
85.9	0.1	0.3	1.1	0.5	1.2	3.1	0.0	0.0	0.2	0.4	0.3	0.0	0.0	0.0	0.0	5.7
43.0	0.1	1.5	2.1	1.4	1.8	0.0	0.0	0.1	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.3
72.2	0.2	1.3	0.2	6.8	8.2	2.8	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.9
0.5	57.9	0.3	1.0	12.9	10.8	2.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	13.1
0.1	88.4	16.6	1.7	3.5	2.1	0.2	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	7.8
0.2	85.3	0.1	0.2	0.5	1.8	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	19.9
0.1	70.1	0.1	0.5	0.7	1.7	1.6	0.0	0.0	0.1	0.5	0.0	0.0	0.0	0.0	0.0	24.5
0.1	0.0	0.0	0.0	0.8	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1
0.0	67.0	14.9	8.1	0.1	0.5	1.1	0.0	0.1	0.0	0.3	0.0	0.0	0.0	0.0	0.0	7.9
0.0	0.0	95.1	0.0	0.0	0.5	0.3	0.0	0.1	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.1
0.0	0.0	34.4	0.0	0.0	0.0	0.0	0.1	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	90.4	0.0	0.1	0.1	0.0	0.0	0.1	3.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1
0.0	0.1	4.3	0.5	0.4	1.4	1.6	0.0	4.5	54.1	0.0	0.0	0.0	0.1	0.0	0.0	2.6
0.0	0.0	93.9	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	84.8	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
0.0	0.2	4.9	0.9	4.8	3.8	4.8	0.0	32.6	40.8	0.0	0.0	0.0	0.0	0.0	0.0	7.2
0.0	0.0	90.4	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4
0.0	0.1	89.1	0.0	0.2	0.1	0.1	0.0	0.3	0.4	0.0	0.0	0.0	0.0	0.2	0.0	0.7
0.0	0.0	95.1	0.0	0.1	0.0	0.1	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1
0.0	0.0	99.7	0.0	0.1	0.0	0.5	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2
0.0	0.0	90.0	0.0	0.0	0.0	0.0	0.0	0.1	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.1
0.0	0.0	90.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
0.0	0.0	90.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	99.2	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
0.0	0.0	84.8	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1
0.0	0.0	1.6	1.2	18.3	44.3	9.5	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.1
0.0	0.6	1.0	0.0	69.3	11.3	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.1	11.9	5.4
0.0	0.2	0.1	0.0	0.0	2.4	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9
0.0	0.1	91.4	0.0	0.0	0.0	0.0	0.0	1.6	5.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	46.8	0.1	0.1	0.0	16.4	22.9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.7	12.9
0.0	0.5	4.6	6.1	51.4	22.1	0.0	0.0	0.1	1.2	0.0	0.0	0.0	0.0	0.0	0.0	13.9
0.0	0.2	1.4	0.8	47.9	0.4	0.0	2.9	0.0	0.1	0.0	0.0	0.0	1.4	25.5	17.4	0.0
0.0	0.0	2.1	0.1	0.1	29.3	0.3	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.7
0.0	0.0	0.2	0.9	1.5	29.4	40.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.4
0.0	0.0	91.4	0.1	0.0	0.0	0.0	0.0	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	88.3	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5
0.0	0.0	97.4	0.0	0.0	0.0	0.0	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1
0.0	0.0	91.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.7
0.0	0.0	90.8	0.0	0.0	0.0	0.1	0.1	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	2.5
0.0	0.1	94.8	0.0	0.1	0.1	0.8	0.0	0.1	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.5
0.0	89.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
0.0	85.1	0.1	0.0	0.1	0.6	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.5

52 "red" kernels (dorsal)



Artificially
infected
malt

F. culmorum

F. avenaceum

F. avenaceum
/*tricinatum*

Pyrenospora
Lewia infectoria
Microdochium
Cladosporium
Fusarium poae

Conclusions

- **Metabarcoding** is suitable for as studying the microbial composition of seeds – our next aim is to study **gushing**
- **VideometerLab** detects *Fusarium* on single seeds – in agreement with qPCR and NGS
- Seeds generally infected by only **one species**
- ‘Black’ seeds are mostly *Pyrenophora teres*
- Videometer *Fusarium* model may be improved and extended to other species e.g. **Microdochium**



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