Novel methods for detection of pathogens on seeds

Mogens Nicolaisen

Annemarie Fejer Justesen

Aarhus University, Dept. Agroecology, Flakkebjerg

AARHUS
UNIVERSITY

Two new initiatives with elements of pathogen detection

- Testa
- SpectraSeed

  - development of robust and standardized detection methods
  - testing of 'generic' methods that detect 'all' pathogens

Seed health: development of seed treatment methods, evidence for seed transmission and assessment of seed health

Budget: 3 m€

- Seed transmission

- Sampling strategies

- Detection/diagnosis

- Disinfection methods

- Validation of methods

- FERA, UK
- PLANT RESEARCH INTERNATIONAL, NL
- INRA, F
- UNIVERSITA DEGLI STUDI DI TORINO , I
- UNIVERSITY OF PRETORIA, SA
- SASA, UK
- AARHUS UNIVERSITET, DK
- GEVES, F
- NIAB, UK
- NAK TUINBOUW, NL
- UNIVERSITA DEGLI STUDI DI MODENA E REGGIO EMILIA
- VIDEOMETER, DK
- EPPO

AARHUS
UNIVERSITY

- Brassica:
  - *Xanthomonas campestris pv. campestris*
  - *Phoma lingam*
  - *Fusarium oxysporum (raphani, conglutinans, matthioli)*
- Legumes
  - *Xanthomonas axonopodis pv. phaseoli*
  - *Aphelecoides*
  - *Ditylenchus dispaci*
- Barley, wheat, rice
  - *Fusarium spp*
  - *Tilletia spp*
- Maize
  - *Pantoea stewartii*
- Tomato
  - *Pepino mosaic virus*
  - *Pospiviroids*
  - *Clavibacter michiganensis subsp. michiganensis*
  - *Pseudomonas syringae pv. tomato*
  - *Xanthomonas spp.*
- Cucurbits
  - *Viruses: CGMMV etc*
  - *Acidovorax avenae subsp. citrulli*

What is needed?

- Lots of PCR assays already exist for almost all pathogens
  - Different protocol for each assay
  - Not easy to transmit protocol from one lab to another
  - Ease of use is not prioritized

Diagnosis/detection

– Make existing protocols more robust and standardized

- Sampling methodology (WP2)
  - Representative samples
- Sample extraction (DNA)
  - extraction methods that are automatable
- Test conditions
  - PCR SYBR/TaqMan
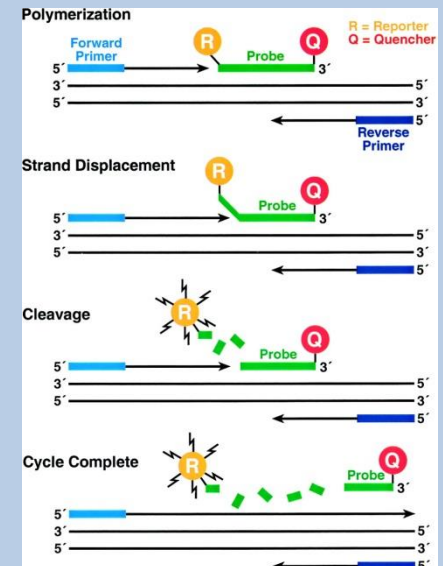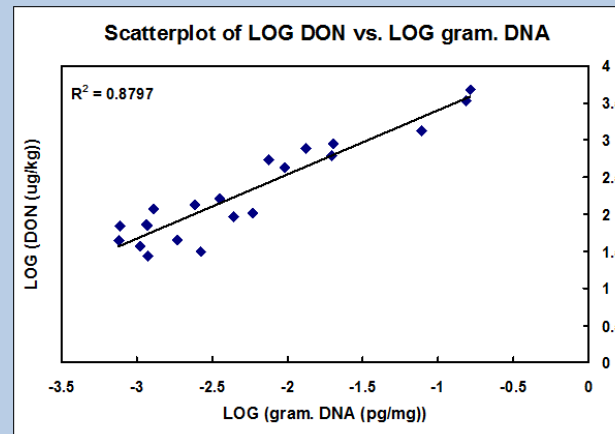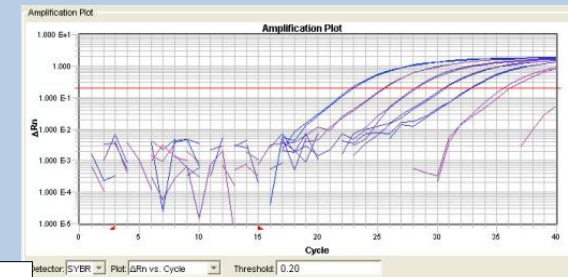
Species specific Real-Time PCR assays

- **F. graminearum**
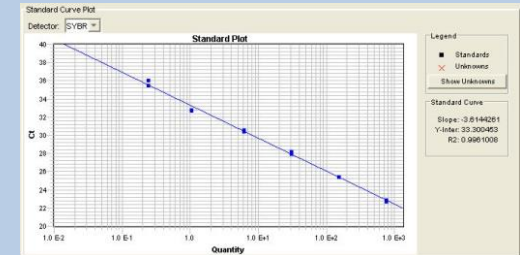- **F. culmorum**
- **F. avenaceum**
- **F. tricinctum**
- **F. poae**
- **F. langsethiae**
- **F. equiseti**
- **F. sporotrichioides**
- **F. proliferatum**
- **F. verticilloides**

› **Microdochium nivale**
  › Detects both M. nivale var. nivale and M. nivale var. majus

› **Tilletia caries, T. indica**
  › Assays already published

Scatterplot of LOG DON vs. LOG gram. DNA

$R^2 = 0.8797$

Test improvements

- DNA extraction
  - Try several methods
    - Test for i) ease of use ii) yield iii) reproducibility
    - MoBio Seed kit
    - LGC Genomics
    - CTAB

- TaqMan assays
  - Use same kits and conditions for all assays
  - Test assays in partner laboratories

AARHUS
UNIVERSITY

Testing of 'generic' methods that detect 'all' pathogens

"Next generation sequencing can be used to identify a huge number of individuals in a sample"

Diagnostic tool or research tool?

- Culture based methods
  - 100-1000 isolates

- Next generation sequencing
  - 100.000 to 1.000.000 individuals identified

AARHUS
UNIVERSITY

Workflow

Sample

DNA extraction
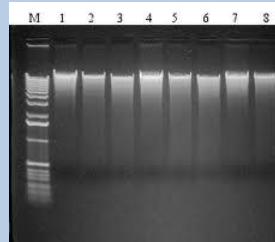
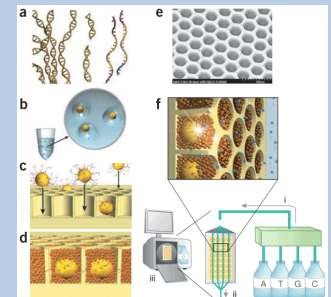PCR amplification
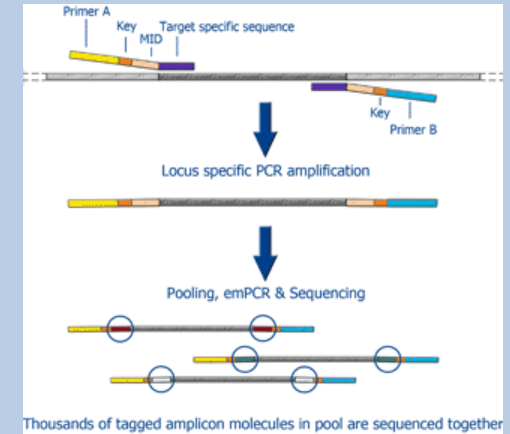
Identification of species (BLAST)

Clustering

~300.000 sequences

NGS sequencing

AARHUS
UNIVERSITY

Wheat grain fungal communities

- Wheat grain samples from 2004, 2005, 2006
- 30 samples from each year from a previous *Fusarium* survey

Year

Previous crop

Tillage

Wheat cultivar (including Fusarium 'resistance')

Fusarium biomass (Q-PCR data)

Toxins

AARHUS UNIVERSITY

Raw data from sequencing

| Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Total			
89	2409	826	38	36	348	48161	Cluster 1	DQ459835.1	Fusarium culmorum grp
872	376	368	1262	981	1660	45980	Cluster 2	GU584953.1	Lewia infectoria strain I45
515	534	211	418	644	587	36072	Cluster 3	HQ540688.1	Davidiellaceae sp. PIMO_9
59	40	87	76	164	25	22066	Cluster 4	EU167564.1	Didymella exitialis strain C
13	71	250	275	22	139	16215	Cluster 5	JF340258.1	Gibberella avenacea strai
55	38	70	250	156	180	9959	Cluster 6	JF440590.1	Epicoccum nigrum 18S ribo
76	20	46	40	20	4	9327	Cluster 7	AM502260.1	Microdochium nivale 18S r
2	4	10	23	82	76	2607	Cluster 8	FJ025231.1	Fungal endophyte sp. B16-
21	23	15	68	28	119	2241	Cluster 9	JF440587.1	Cladosporium tenuissimur
11	24	48	3	10	418	2139	Cluster 10	FJ907535.1	Pyrenophora tritici-repent
7	11	0	0	10	26	1870	Cluster 11	FR732053.1	Fusarium sp. IMM16 genon
12	0	13	8	21	1	1822	Cluster 12	AF181696.1	AF181696 Septoria passeri
15	8	6	20	3	17	1421	Cluster 13	HQ171053.1	Botryotinia fuckeliana isol
64	4	5	12	2	2	1404	Cluster 14	AF181710.1	AF181710 Phaeosphaeria r
24	27	11	4	47	68	1209	Cluster 15	FR717837.1	Cryptococcus victoriae gen
9	17	4	0	7	41	745	Cluster 16	FJ210642.1	Sporobolomyces sp. JJP-20
2	3	1	0	11	6	493	Cluster 17	HQ533146.1	Stemphylium sp. CNU0940
2	7	5	1	11	12	374	Cluster 18	AM160648.1	Cryptococcus sp. HB 1222
2	1	0	4	5	7	327	Cluster 19	HQ166323.1	Fungal sp. FL-2010d isolate

Incidence
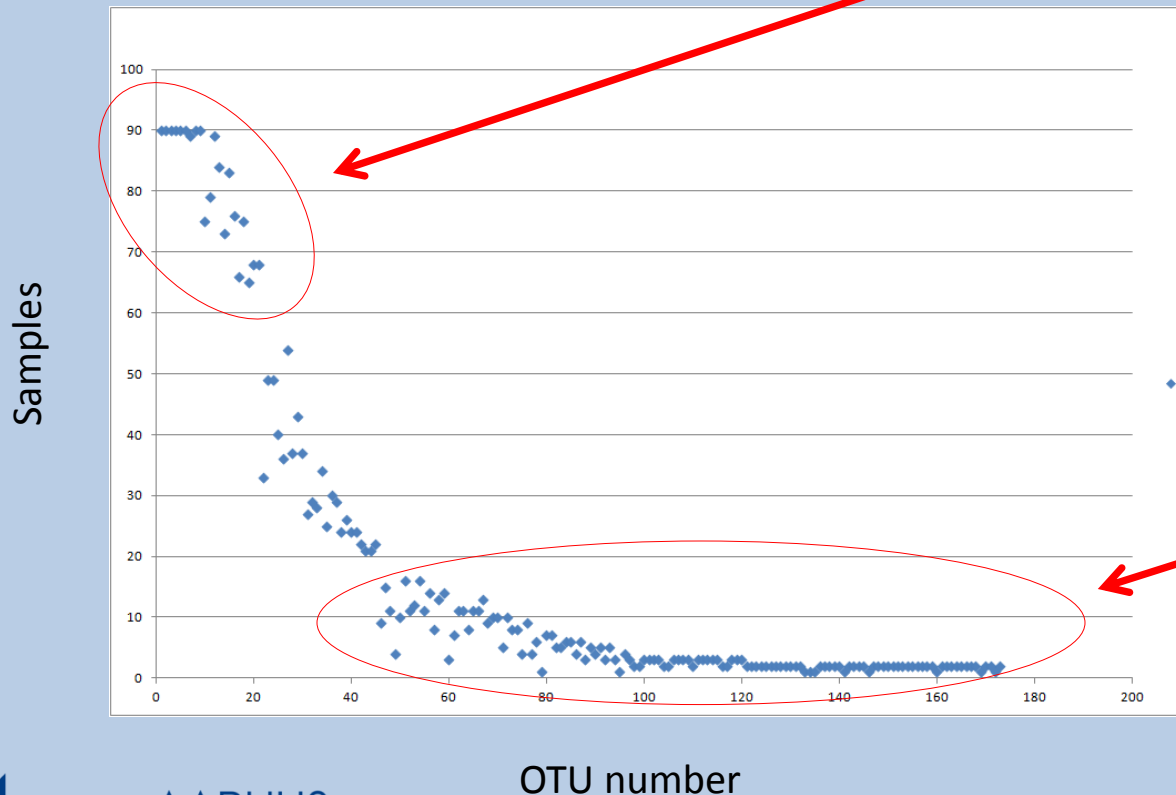
Core species

Fusarium culmorum 'group'
Lewia infectoria (Alternaria)
Cladosporium sp
Didymella exitialis (Ascochyta, leaf scorch)
Gibberella avenacea
Epicoccum nigrum
Microdochium nivale
Alternaria alternata
Cladosporium cladosporioides
Pyrenophora tritici-repentis (tan spot, DTR)
Fusarium poae
Mycosphaerella graminicola (Septoria tritici blotch)
Sclerotinia sclerotiorum/Botryotinia fuckeliana
Phaeosphaeria nodorum (Septoria)
Cryptococcus sp
Sporobolomyces sp
Stemphylium/Pleospora sp (teleomorph)
Cryptococcus sp
Lewia infectoria
Phaeosphaeria sp
Dioszegia hungarica

Rare biosphere

OTU number

AARHUS UNIVERSITY

Fungal 'interactions'

Cluster	Best identification
1	Fusarium culmorum 'group'
2	Lewia infectoria (Alternaria)
3	Cladosporium sp
4	Didymella exitialis (Ascochyta, leaf scorch)
5	Gibberella avenacea
6	Epicoccum nigrum
7	Microdochium nivale
8	Alternaria alternata
9	Cladosporium cladosporioides
10	Pyrenophora tritici-repentis (tan spot, DTR)
11	Fusarium poae
12	Mycosphaerella graminicola (Septoria tritici blotch)
13	Botryotinia fuckeliana
14	Phaeosphaeria nodorum (Septoria)
15	Cryptococcus sp
16	Sporobolomyces sp
17	Stemphylium/Pleospora sp (teleomorph)
18	Cryptococcus sp
19	Lewia infectoria
20	Phaeosphaeria sp
21	Dioszegia hungarica
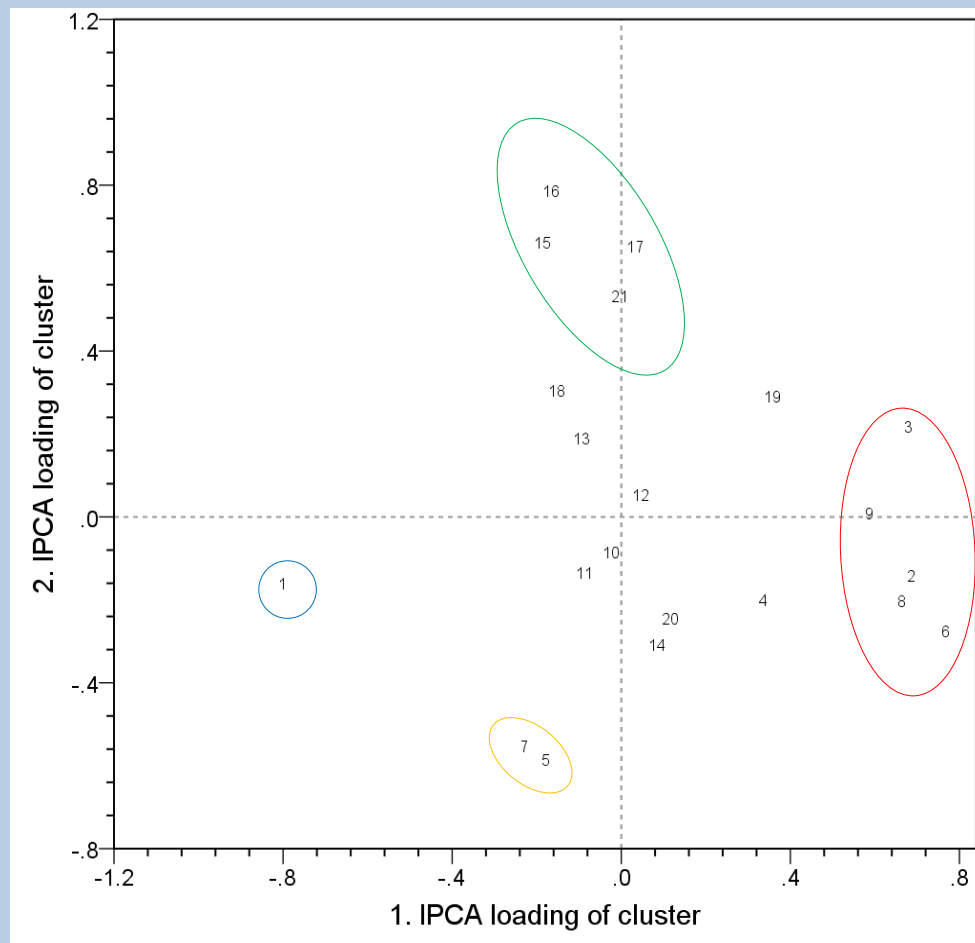
Agronomic factors and fungal communities

OTU	Factor or factor combination				Mean amount of DNA	95% CI lower bound	95% CI upper bound	Mean comparison (Bonferroni-corrected) [a]
					back-transformed			
Mean across OTU 1 – 21	Year	2003	Tillage	normal	16.1	12.5	20.7	a
				reduced	30.1	16.4	54.7	a
		2004		normal	23.1	18.0	29.5	a
				reduced	25.5	14.0	45.7	a
		2005		normal	25.7	19.3	33.9	a
				reduced	12.4	6.1	24.2	a
	Previous crop	Barley	Tillage	normal	24.0	16.9	33.9	b
				reduced	4.8	0.8	17.7	a
		Corn		normal	27.4	19.0	39.4	a
				reduced	45.1	22.3	90.1	a
		Rape		normal	18.2	12.5	26.1	-
				reduced [b]				-
		Wheat		normal	19.8	15.3	25.6	a
				reduced	55.9	40.5	77.2	b
Fusarium culmorum	Year			2003	453.0	268.9	762.6	ab
				2004	541.9	321.8	911.3	b
				2005	188.0	111.5	317.0	a
Lewia infectoria	Fusarium susceptibility			medium	301.2	197.5	458.9	a
				high	503.2	339.4	746.0	b
	Year	2003	Tillage	normal	122.7	81.7	183.9	a
				reduced	252.4	93.6	677.6	a
		2004		normal	629.2	420.6	941.9	a
				reduced	713.8	271.9	1871.4	a
		2005		normal	974.5	616.7	1541.3	a
				reduced	380.5	129.6	1113.3	a
	Previous crop	barley	Tillage	normal				
				reduced				
		corn		normal	457.5	251.6	830.3	a
				reduced	1375.1	433.8	4353.7	a
		rape		normal	358.2	198.5	645.1	-
				reduced [b]				-
		wheat		normal	444.9	294.0	672.8	a
				reduced	1578.7	923.3	2699.0	b

Conclusions

- NGS reveals a high diversity of fungi in wheat

- Fungal 'interactions' and the influence of environmental factors can be investigated at high level of resolution

- Potential for diagnostic tool?

Thank you!

- Annemarie F. Justesen
- Mogens Nicolaisen
- Jiaoyu Wang
- Kamilla Knorr
- Hans Pinnschmidt
- Lise Nistrup Jørgensen
- Lotte Olesen