



Fusarium graminearum mycotoxins associated with grain mold of maize and sorghum in South Africa.

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Initial aims of the study:



1. Determine the incidence and distribution of *F. graminearum* in selected maize (*and sorghum*) cultivars
2. Quantify DON and Zearalenone in the respective samples (ELISA)
3. Develop a weather x FGSC mycotoxin model (pending)

Refined aims of the study

3. Determine the specific host x species relationship
4. Determine the trichothecene chemotypes
5. Analysis of grain DON, NIV and Zearalenone using LC-MS



1. Determine the incidence and distribution of *F. graminearum* in selected maize (*and sorghum*) cultivars

- 308 maize samples over period 2006/07 – 2008/09
- 6 cultivars
- 27 localities
- Isolates on Nash-Snyder Medium
- Rt-PCR {*F. g* MGB primers and probe – Waalwijk *et al.* (2004)}

Quantitative detection (q-RtPCR) of *F. graminearum* in maize at various localities: 2006/07-2008/09



Locality	2007/8	2008/9
Bethlehem	68.90	440.60
Cedara 1st planting	99.47	145.88
Cedara 2nd planting	213.12	468.93
Hartebeesfontein	165.14	39.90
Jim Fouche	106.55	66.00
Leeudoringstad	819.75	66.00
Potchefstroom 1st planting	43.20	176.33
Potchefstroom 2nd planting	52.35	191.38
Potchefstroom 3rd planting	-	214.68
Vaalharts 1st planting	18.47	-
Vaalharts 2nd planting	56.00	39.90



Weather management?





Quantitative detection (q-RtPCR) of *F. graminearum* in maize cultivars: 2006/07-2008/09

Cultivar	FgC (pg)
LS 8521B	249.56
DKC80-10	269.93
PAN 6611	297.78
DKC78-15B	404.11
DKC80-12B	494.63
CRN 3505	553.97



Deployment of resistance?



2. Quantify DON and Zearalenone in the respective samples



Veratox® DON and Zearalenone quantitative test kits (Neogen® Corporation, 620 Lasher Place, Lansing, MI 48912, USA).

DON and Zearalenone concentrations recorded with ELISA at 27 localities: 2006/07-2008/09



LOCALITY	[DON ppm]	[ZEA ppb]
Bothaville	<0.250	132.35
Ottosdal	<0.250	147.58
Nampo	<0.250	151.79
Rushof	<0.250	158.93
Hartebeesfontein	<0.250	160.12
Viljoenskroon	<0.250	160.80
Wesselsbron	<0.250	168.05
Bainsvlei	<0.250	180.46
Ventersdorp	<0.250	201.40
Koster	<0.250	220.74
Delareyville	<0.250	237.09
Leeudoringstad	<0.250	256.50
Marquard	<0.250	316.23

DON and Zearalenone concentrations recorded with ELISA at 27 localities: 2006/07-2008/09 (cont)



LOCALITY	[DON ppm]	[ZEA ppb]
Danielsrus	<0.250	439.09
Jim Fouche	<0.250	485.16
Frankfort	<0.250	544.56
Tweeling	<0.250	664.60
Bloekomspruit	<0.250	684.88
Bethlehem	<0.250	770.76
Vaalharts	<0.250	796.03
Robertsdrift	<0.250	856.41
Nooitgedacht	<0.250	864.97
Cedara	<0.250	918.96
Warden	<0.250	1151.09
Wonderfontein	<0.250	1434.74
Tweebuffelsfontein	<0.250	1585.30
Potchefstroom	<0.250	1844.84

DON and Zearalenone concentrations recorded with ELISA in 6 cultivars: 2006/07-2008/09



Cultivar	[DON ppm]	[ZEA ppb]
DKC 80-12B	<0.250	591.61
DKC 78-15B	<0.250	626.76
PAN 6611	<0.250	681.72
CRN 3505	<0.250	683.88
LS-8521B	<0.250	738.28
DKC 80-10	<0.250	752.65

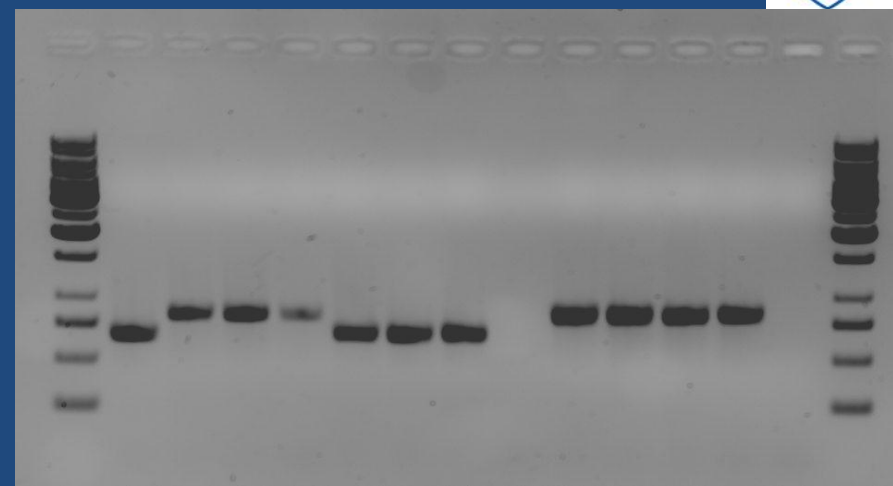
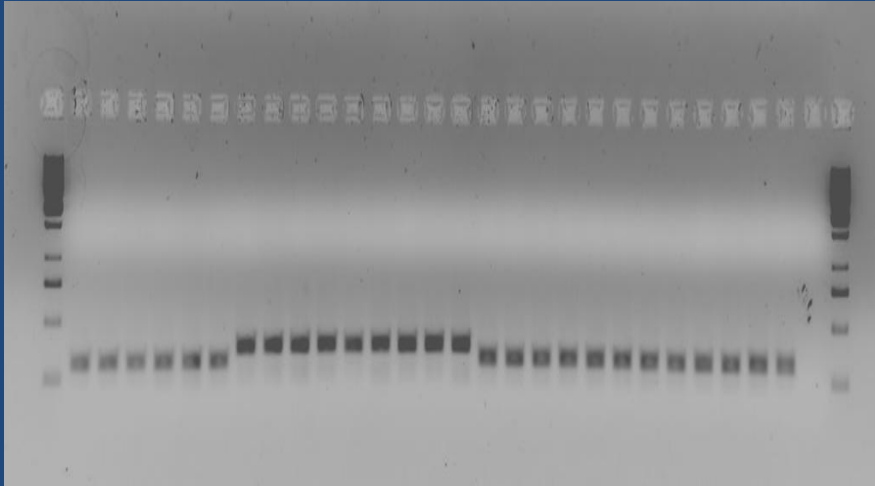
Note: no significant relationship between grain colonization by FGSC and toxin concentration

4. Determine the specific host x species relationship



- Sequence data generated from the TEF-1 α gene –
 - *F. boothii* only lineage in maize
 - *F. meridionale*, *F. acaciae-mearnsii* and *F. cortaderiae* in sorghum

4. Determine the trichothecene chemotypes



PCR products for trichothecene mycotoxin chemotypes using (a) ToxP1/ToxP2 primers to amplify a 300 and 360 bp fragments for DON and NIV respectively and (b) using *Tri12* primers which amplify 15-ADON (670 bp) and NIV (840bp) fragments.

3-ADON chemotype not detected

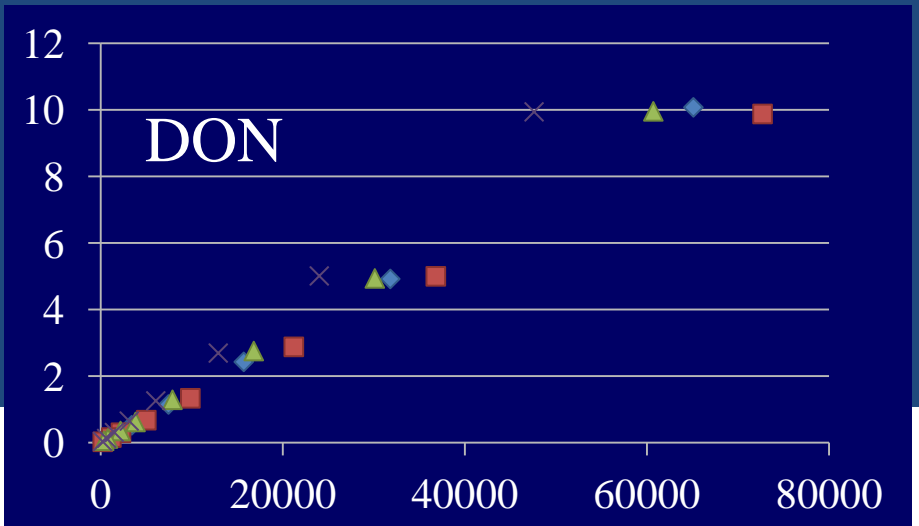
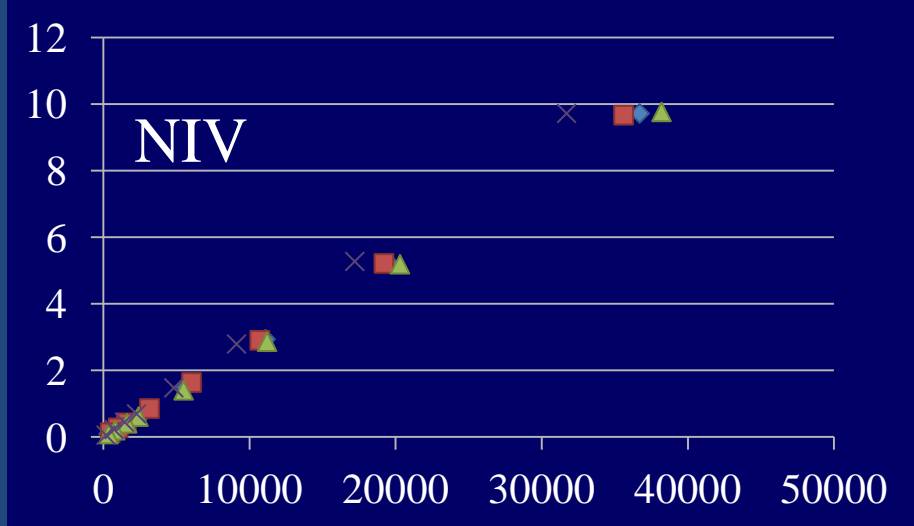
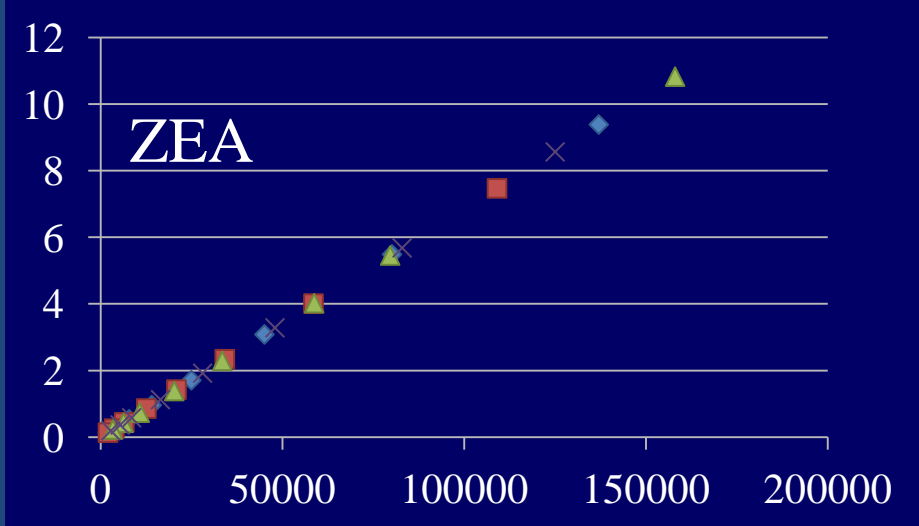
DON and 15-ADON chemotype only chemotype detected in maize

NIV chemotype only chemotype in sorghum

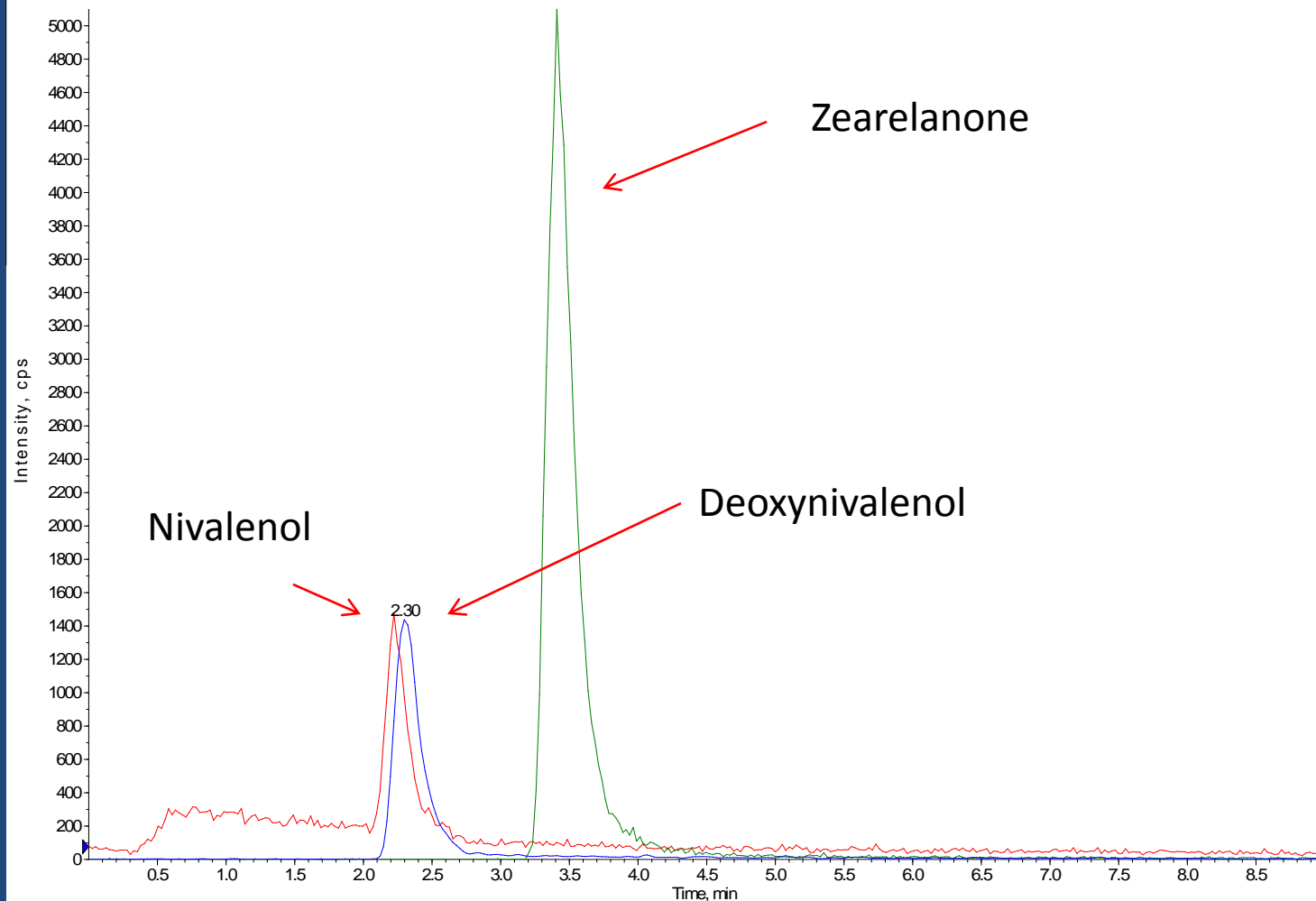
5. Analysis of grain DON, NIV and Zearalenone using LC-MS



Repeatability of evaluation method



14/10
17/10
18/10
21/10



Retention times: Nivalenol 2.22 min
Deoxynivalenol 2.30 min
Zearelanone 3.41 min

DON, NIV and Zearalenone concentrations recorded with LC-MS at 27 localities: 2006/07-2008/09

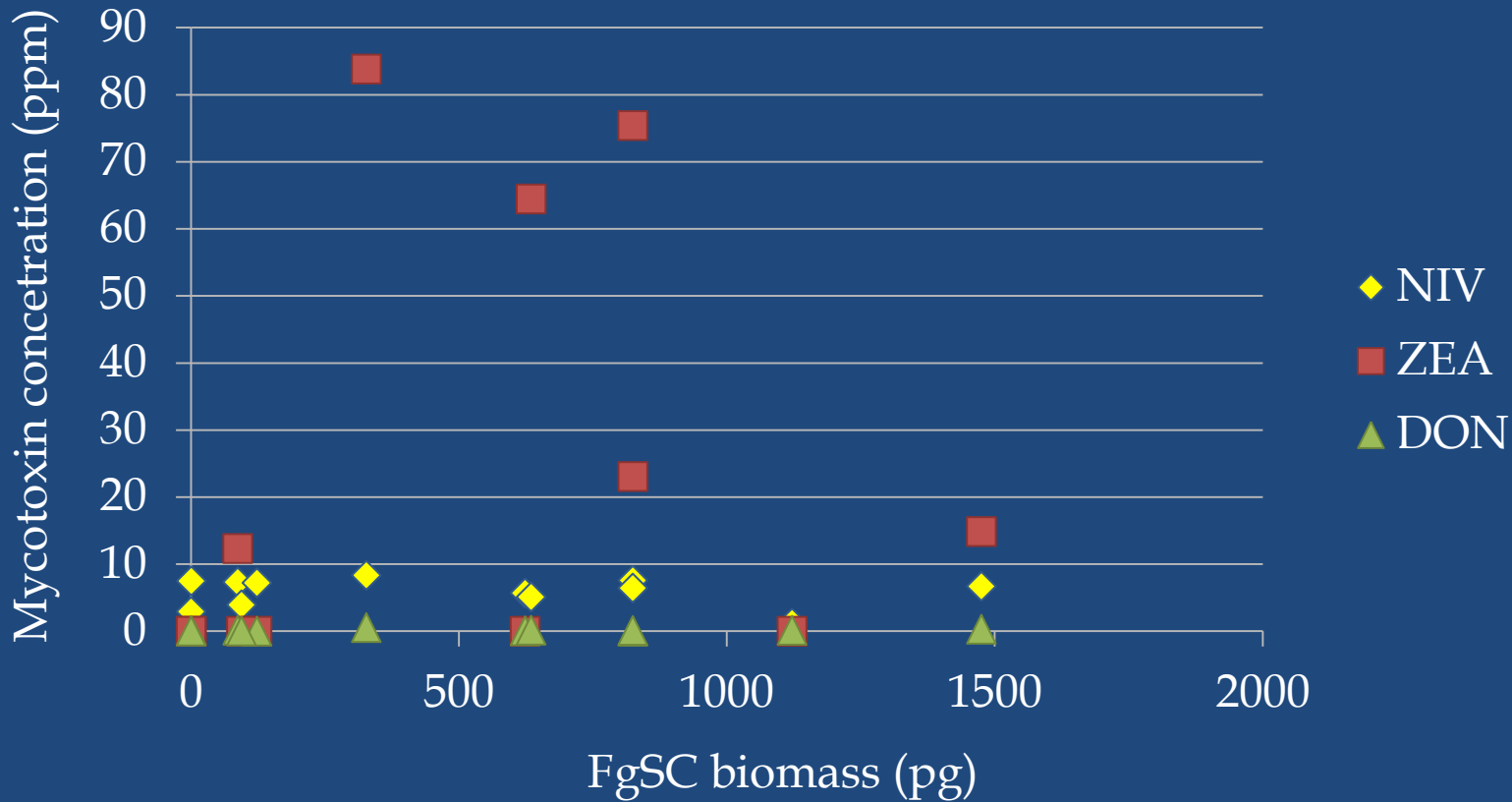


Locality	Fg DNA pg/mg	317 Zea ppm	371 NIV ppm	355 DON ppm
Bethlehem	87.00	12.34	7.33	0.15
Bloekomspruit	824.50	23.07	7.59	0.01
Bothaville	623.37	0.00	5.70	0.06
Cedara	634.63	64.46	5.11	0.16
Delmas	824.33	75.40	6.44	0.12
Jim Fouche	122.85	0.00	7.22	0.02
Leeudoringstad	94.00	0.00	3.94	0.04
Potchefstroom	327.00	83.83	8.35	0.55
Tweeling	1475.00	14.86	6.70	0.31
Vaalharts	0.43	0.00	7.50	0.09
Ventersdorp	0.00	0.00	2.94	0.03
Vrede	1121.67	0.00	1.22	0.15



DON and Zearalenone concentrations recorded with LC-MS in 6 cultivars: 2006/07-2008/09

Cultivar	Fg DNA pg/mg	317 Zea ppm	371 NIV ppm	355 DON ppm
PAN 6611	243.02	81.92	4.17	0.58
DKC80-12B	358.33	10.08	8.98	0.06
LS 8521B	426.04	18.38	5.27	0.07
CRN 3505	426.75	22.86	6.33	0.24
DKC78-15B	485.95	4.86	7.28	0.15
DKC80-10	542.21	20.36	3.28	0.11



No relationship between *F. graminearum* biomass and DON, NIV and Zearalenone concentrations recorded with LC-MS: 2006/07-2008/09



Conclusions

1. *F. boothii* is the only member of the FgSC isolated from maize grain
2. The chemotype and toxins are not aligned
3. Cultivars differ in their susceptibility to colonization by FgSC
4. Colonization of grain by FgSC and toxin concentrations are not aligned
5. Weather plays a role in colonization and toxin production and requires elucidation



Thank You
Dankie

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