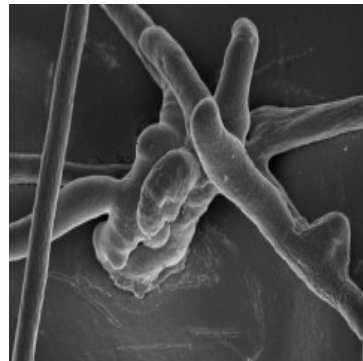
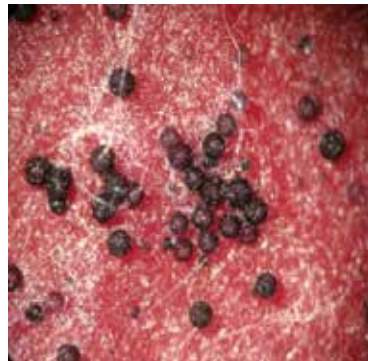
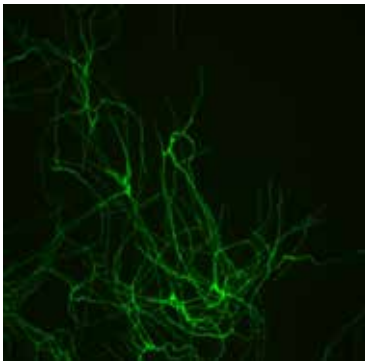


Intraspecies interaction of *Fusarium graminearum* contributes to reduced toxin production and virulence

Gopal Subramaniam
Sean Walkowiak



Research Focus



- § Regulation of DON Biosynthesis
- § Virulence/PAMPs
Plant responses
- § Mitigation Strategies



**Intraspecies interaction of *Fusarium
graminearum* contributes to reduced toxin
production and virulence**

A comparative genomics/proteomics approach

Fusarium Head Blight in Wheat, Barley...



Ear rot of maize

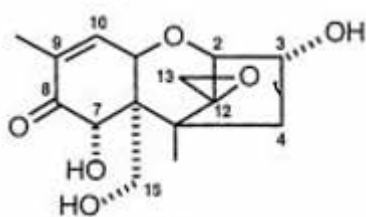
- § Reduced grain yield and quality
- § Mycotoxin deposition

Deoxynivalenol (DON)

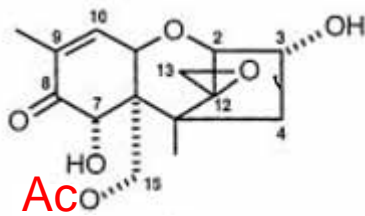
- **food and feed safety issues**
- **potential export barrier**



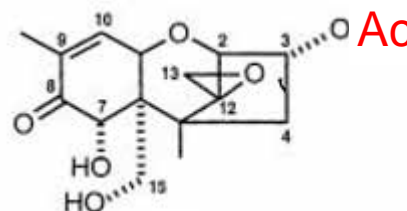
Head blight



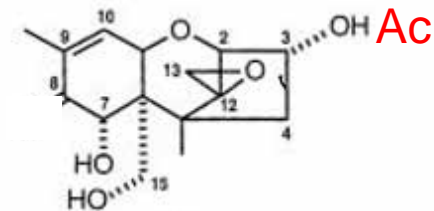
DON



15-ADON

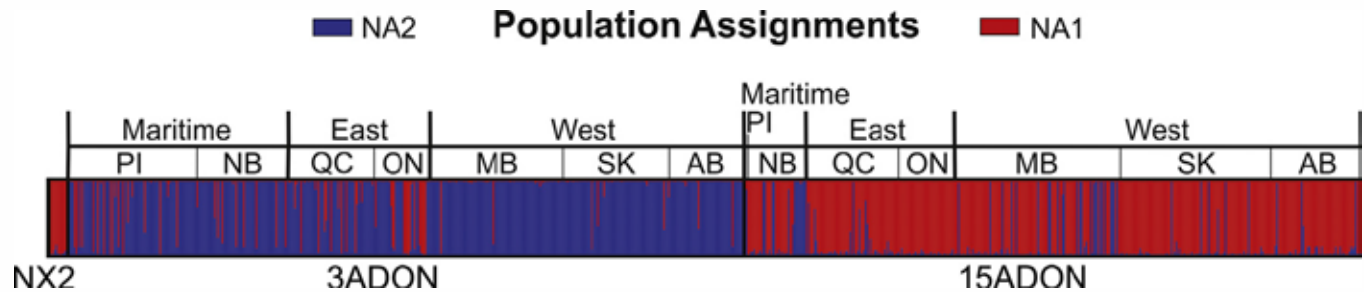


3-ADON

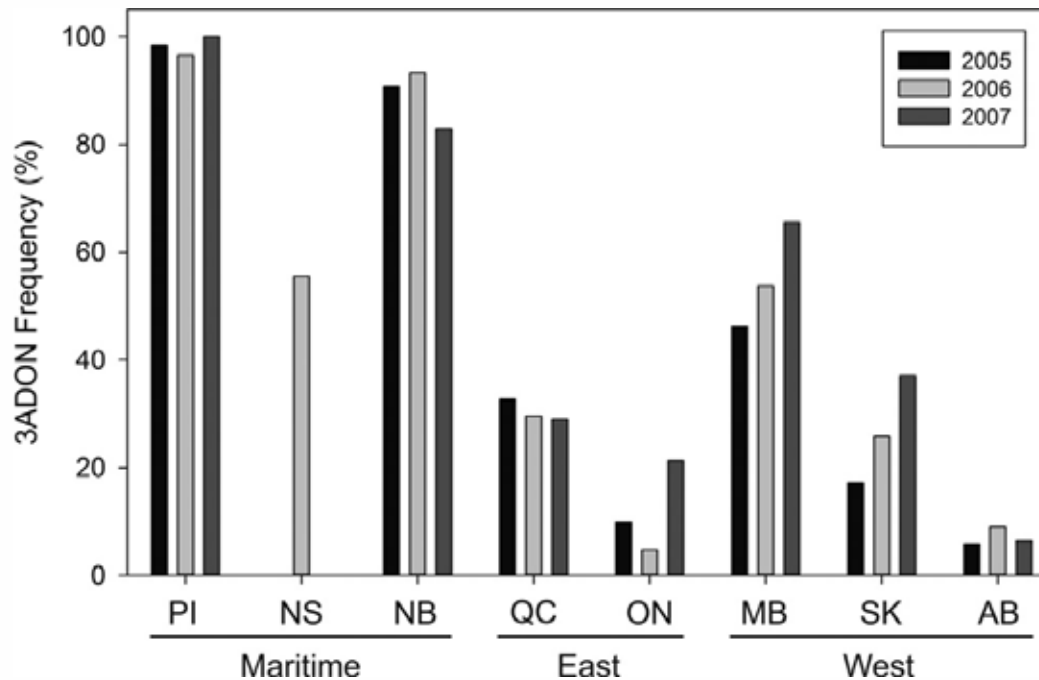


NX2

Distribution of *F. graminearum* across Canada

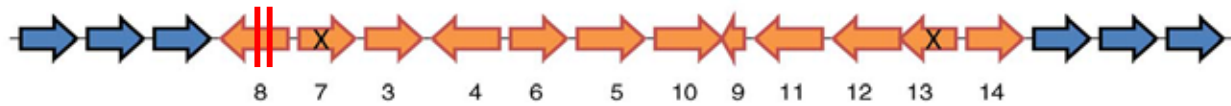


(Kelly et al., 2015)

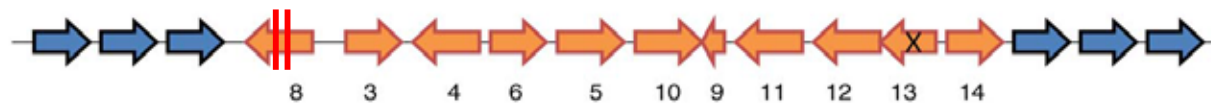


Genetics of Chemotypes

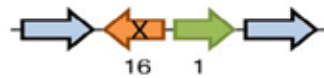
F. graminearum 15ADON



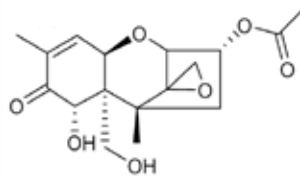
F. graminearum 3ADON



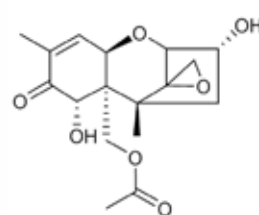
Current Opinion in Plant Biology



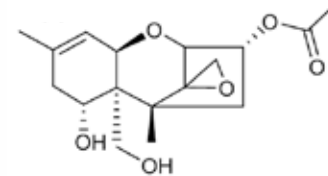
Rep & Kistler, 2010



3-ADON

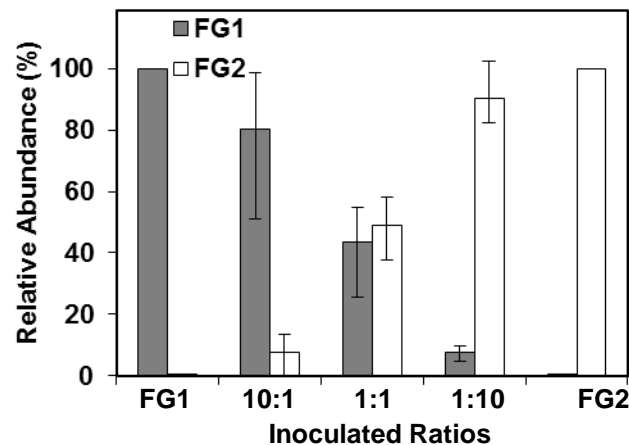
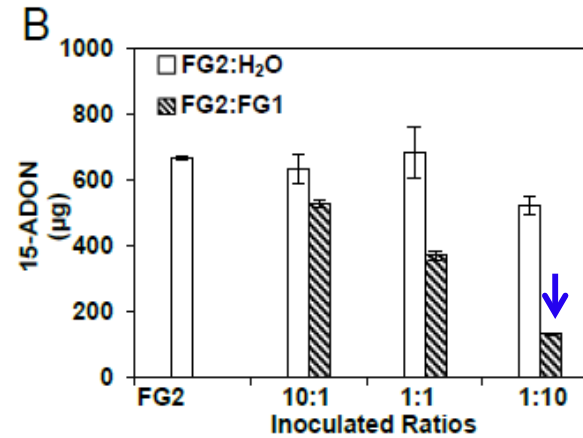
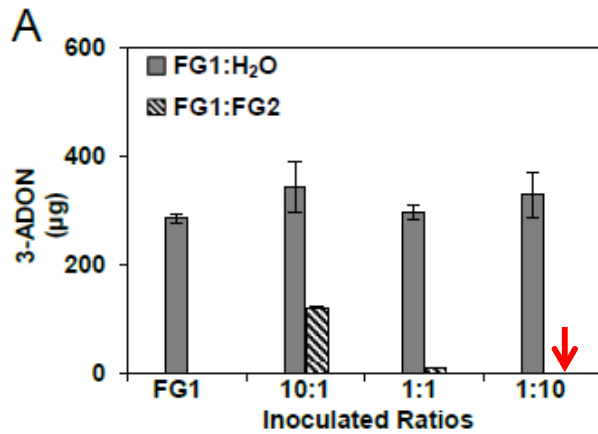


15-ADON



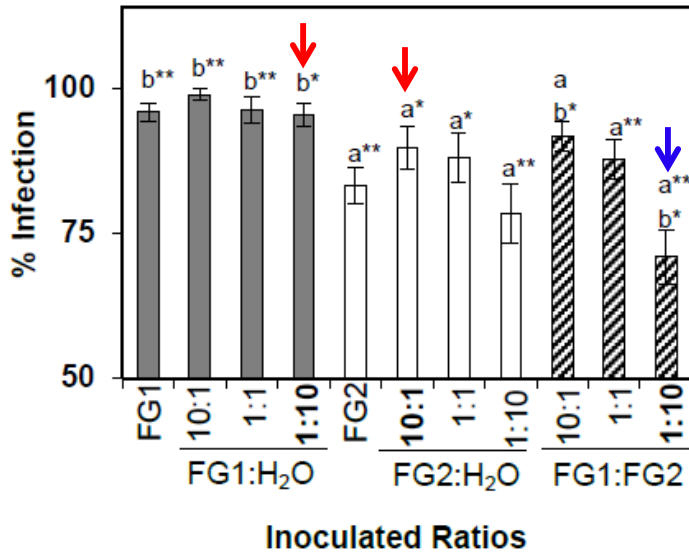
NX-2

3-ADON (FG1) & 15-ADON (FG2) Interfere each other *in Vitro*

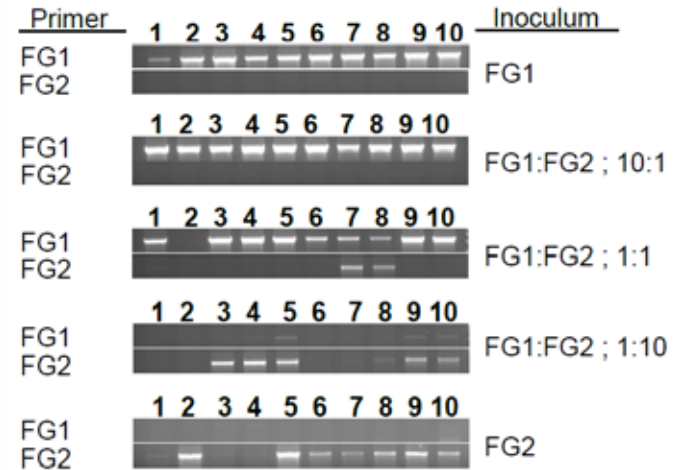


No displacement

3-ADON (FG1) & 15-ADON (FG2) Interfere during Infection



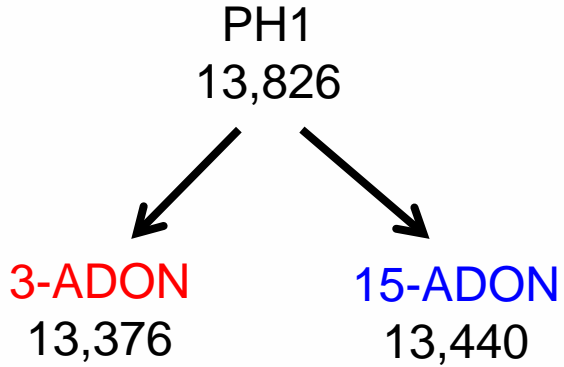
Virulence reduced during competition



Chemotypes Co-habit

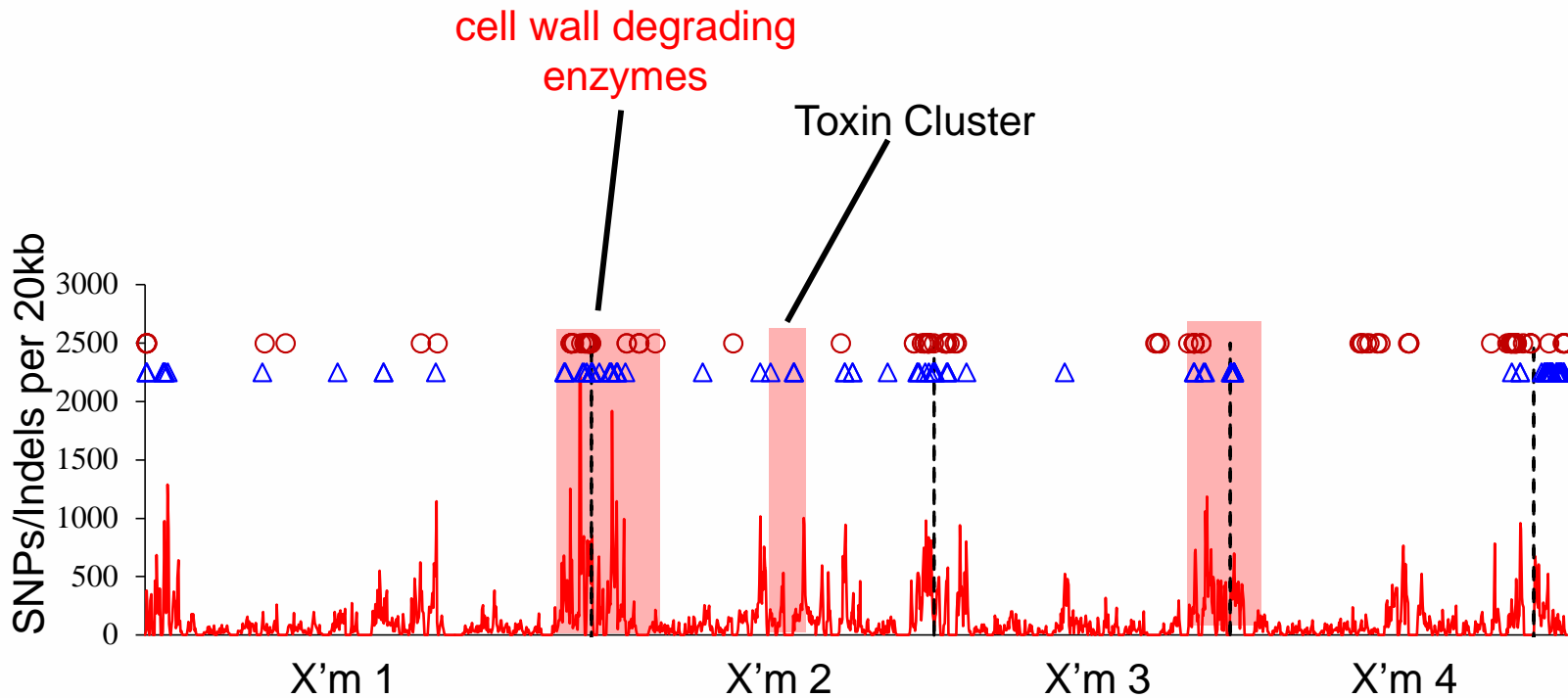
Genome comparison between 3-ADON and 15-ADON

	PH-1	3-ADON	15-ADON
Method	Sanger	Illumina GAI	Illumina GAI
Genome Size (Mb)	36.6	36.6	36.5
Coverage (fold)	10x	144x	86x
Aligned bases (%)	97	97	98
GC %	48	48	48



Genome alignment ~97%; Gene content ~ 97%

Gene features in 3-ADON and 15-ADON genomes

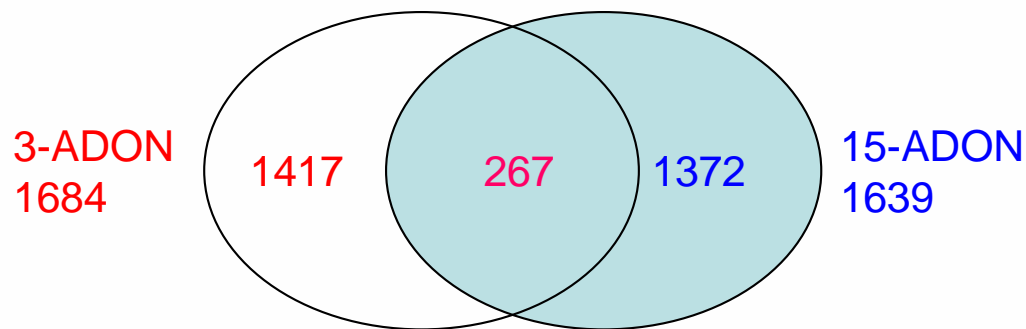


- Poorly conserved genes in 3- ADON
- △ Poorly conserved genes in 15- ADON

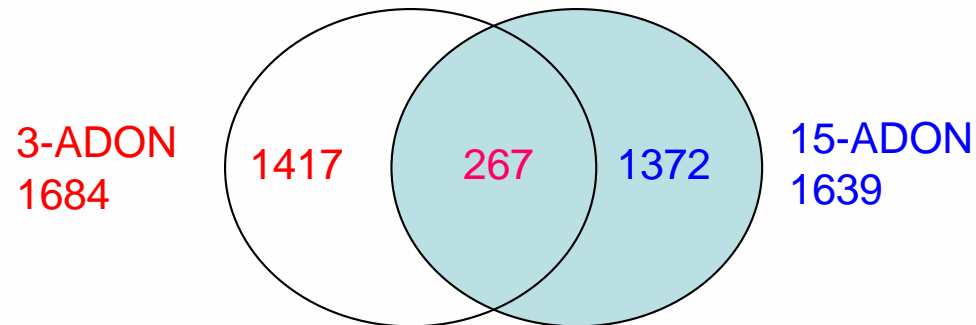
Differential Expression Between Strains

Experimental Condition	Reference Genome	
	FG1	FG2
FG1 vs FG2	1684	1639
FG1:FG2 ; 10:1 ^a	67	76
FG1:FG2 ; 1:1 ^a	56	58
FG1:FG2 ; 1:10 ^a	64	55

FG1: 3-ADON
FG2: 15-ADON



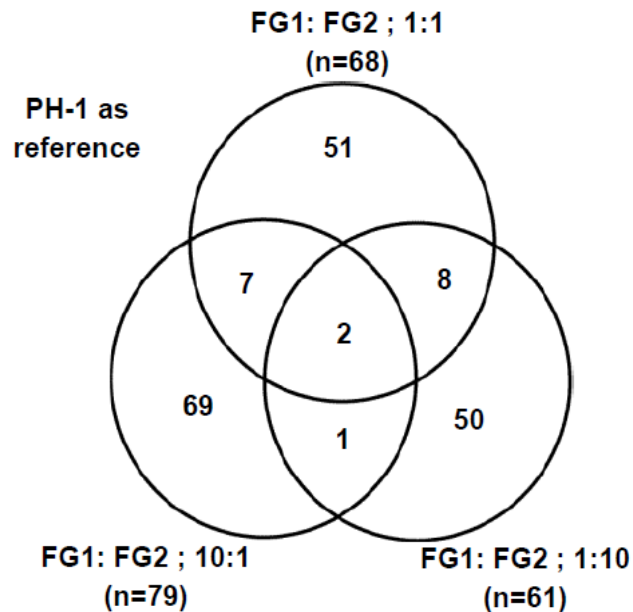
Functional categories of genes between two strains



- 3-ADON dominated by metabolic genes (aspartate, pyruvate and aromatic families)
exception: Phenylalanine metabolism)
- 15-ADON dominated cell-differentiation (eg: Hyphae formation); Electron transport

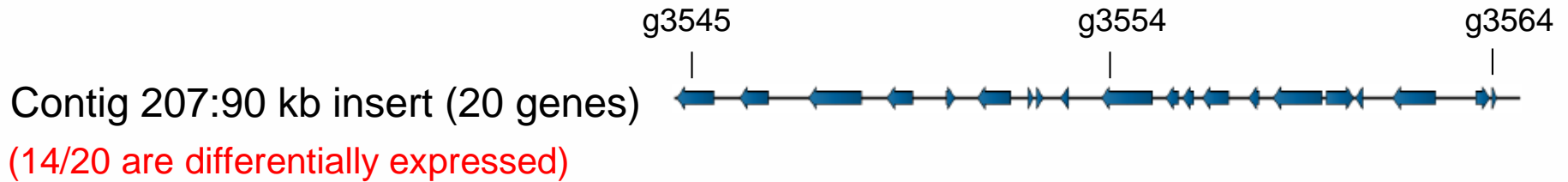
Differential Expression During Competition

Experimental Condition	Reference Genome	
	FG1	FG2
FG1 vs FG2	1684	1639
FG1:FG2 ; 10:1 ^a	67	76
FG1:FG2 ; 1:1 ^a	56	58
FG1:FG2 ; 1:10 ^a	64	55



Functional categories: ALL in metabolism

Interesting Features of 3-ADON Strain



Gene	Predicted protein function
1 g3545	ankyrin repeat
2 g3546	Hypothetical protein
3 g3547	ATP-dependent DNA helicase
4 g3548	telomere-associated helicase
5 g3549	hypothetical protein
6 g3550	ANK superfamily
7 g3551	predicted protein
8 g3552	hypothetical protein
9 g3553	hypothetical protein
10 g3554	Unnamed protein product

Gene	Predicted protein function
11 g3555	hypothetical protein
12 g3556	hypothetical protein
13 g3557	hypothetical protein
14 g3558	hypothetical protein
15 g3559	hypothetical protein FOMG_17501
16 g3560	peptidase s8 family protein
17 g3561	major facilitator superfamily mfs-1
18 g3562	DDE superfamily endonuclease
19 g3563	predicted protein
20 g3564	uncharacterized proteinh

Genes of Contig 207 present in other 3-ADON Chemotypes

Strain	Chemotype	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	% Similarity	Origin																				
241165 (reference)	3																					100	MB																			
S3BN-10-008	3																					100	SK																			
TG2010_19/TG04_05	3																					100	MB																			
TG2010_30/TG04_16	3																															84	MB									
LH-03A	3																																					84	NB			
S6A-10-003	3																																						79	SK		
S9B-10-001	3																																						79	SK		
LH-14	3																																							63	QC	
A6-10-001	3																																								58	AB
LH-04A	NX																																								53	NS
TG2010_50/TG04_36	3																																								42	MB
A5-10-005	3																																								42	AB
S7A-10-011	3																																							37	SK	
A1-10-043	3																																							37	AB	
A6-10-002	3																																							37	AB	
A7-10-005	3																																						37	AB		
LH-17	3																																							37	NB	
A1-10-008	3																																							26	AB	
LH-16	3																																							26	NB	
LH-31	3																																							26	NB	
A2-10-008	3																																							21	AB	
A4-10-009	NX																																							21	AB	
S4A-10-003	3																																						16	SK		
S4B-10-003	3																																					16	SK			
TG2010_63/TG04_49	3																																						16	MB		
TG2010_69/TG04_55	3																																					5	MB			
LH-23	3																																					5	NS			

Genes of Contig 207 present in other 15-ADON Chemotypes

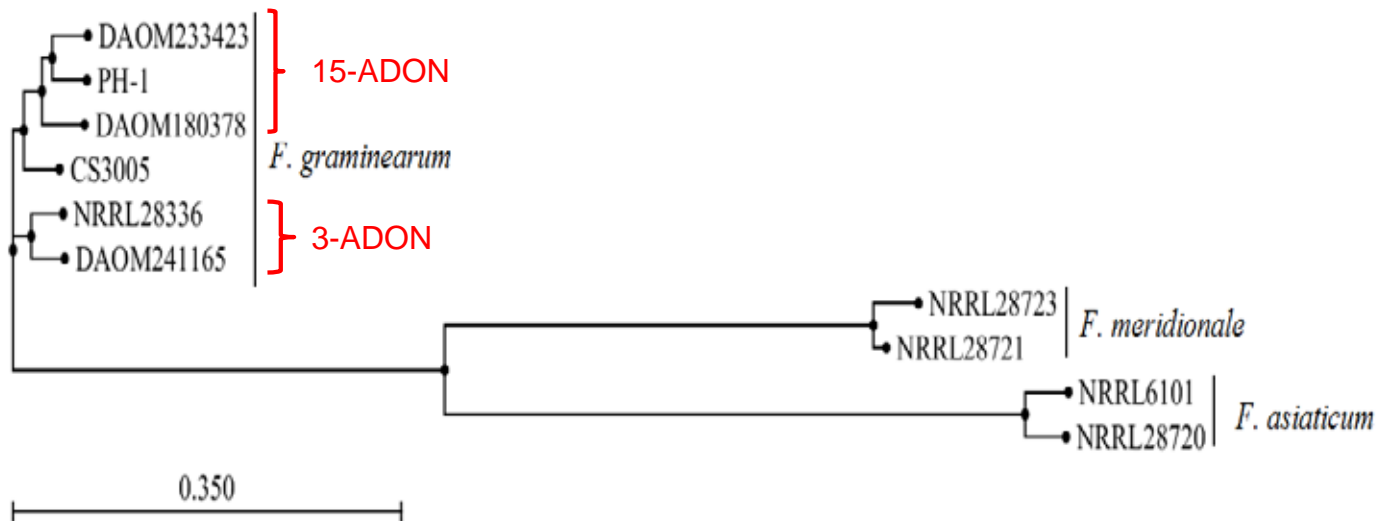
Strain	Chemotype	1	2	3	4	5	6	7	8	9	10	11	13	14	15	16	17	18	19	20	% Similarity	Origin	
241165 (reference)	3																					100	MB
TG2010_61/TG04_47	15																					68	MB
S4A-10-002	15																					63	SK
TG2010_31/TG04_17	15																					63	MB
TG2010_34/TG04_20	15																					63	MB
A7-10-004	15																					63	AB
S6B-10-009	15																					47	SK
233423	15																					32	KA
S6A-10-002	15																					21	SK
TG2010_70/TG04_56	15																					21	MB
A2-10-007	15																					21	AB
A4-10-003	15																					21	AB
LH-35	15																					21	ON
S4B-10-004	15																					16	SK
S7A-10-012	15																					16	SK
TG2010_44/TG04_30	15																					16	MB
A1-10-007	15																					16	AB
A1-10-042	15																					16	AB
A3-10-001	15																					16	AB
LH-07	15																					16	QC
LH-33	15																					16	ON
S3BN-10-007	15																					11	SK
S9B-10-002	15																					11	SK
TG2010_20/TG04_06	15																					11	MB
LH-19	15																					11	NB
LH-21	15																					11	NS
LH-13	15																					5	QC
LH-26	15																					5	NS

Fusarium Comparative Genomics

Isolate Accession	Species	Toxin Type	Isolate Information		Pathology on Wheat
			Host	Location	
PH-1; 31084	<i>F. graminearum</i>	DON, 15-ADON	Corn	Michigan	+++
180378	<i>F. graminearum</i>	DON, 15-ADON	Corn	Ontario	+++
29169	<i>F. graminearum</i>	DON, 15-ADON	Wheat	Kansas	+++
28336	<i>F. graminearum</i>	DON, 3-ADON	Wheat	Ohio	++
37440	<i>F. graminearum</i>	DON, 3-ADON	Wheat	Manitoba	+++
6101	<i>F. asiaticum</i>	DON, 3-ADON	Barley	Japan	-
28720	<i>F. asiaticum</i>	DON	Corn	Nepal	++
28723	<i>F. meridionale</i> / <i>F. asiaticum</i>	Niv	Corn	Nepal	+
28721	<i>F. meridionale</i>	Niv, 4-ANiv	Corn	Nepal	+

Goal: identify core (shared) genes of a species
+ its strain-specific (niche/host) genes

Fusarium Comparative Genomics



Phylogeny based on 90% identity (all genomes) and 700k SNPs/InDels

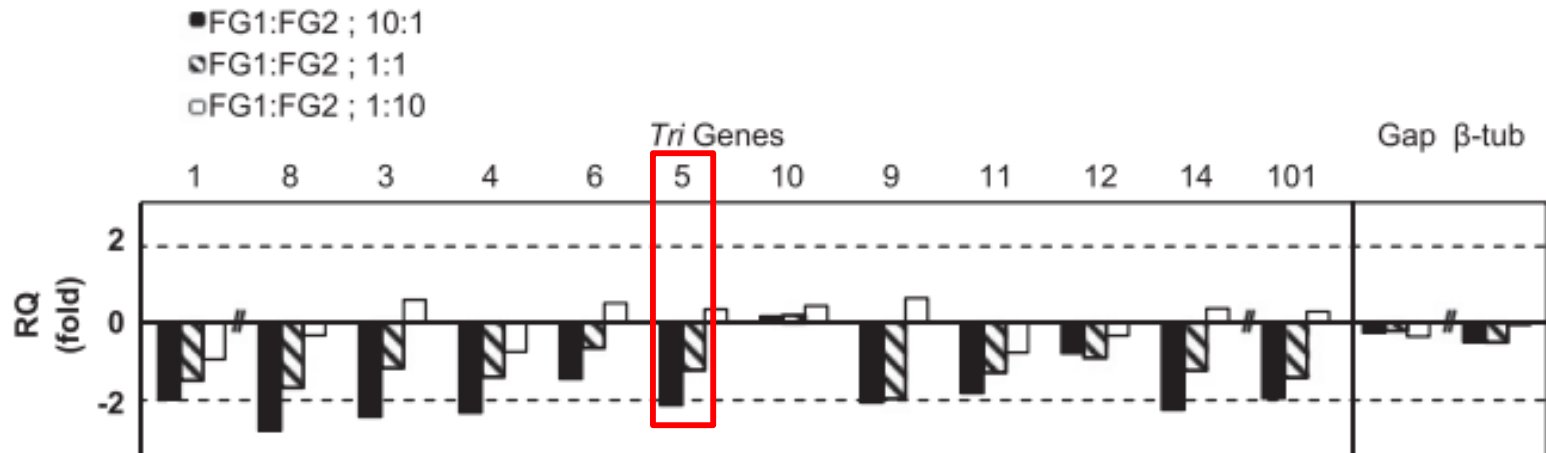
Pan Genome Identifies Core Genes

Species	Conserved Genes		Poorly Conserved Genes	
	Total ~15,300	Uniquely Present	Total	Uniquely Absent
<i>F. graminearum</i>	13959	105	1338	36
<i>F. asiaticum</i>	13974	184	1323	195
<i>F. meridionale</i>	14148	126	1149	72

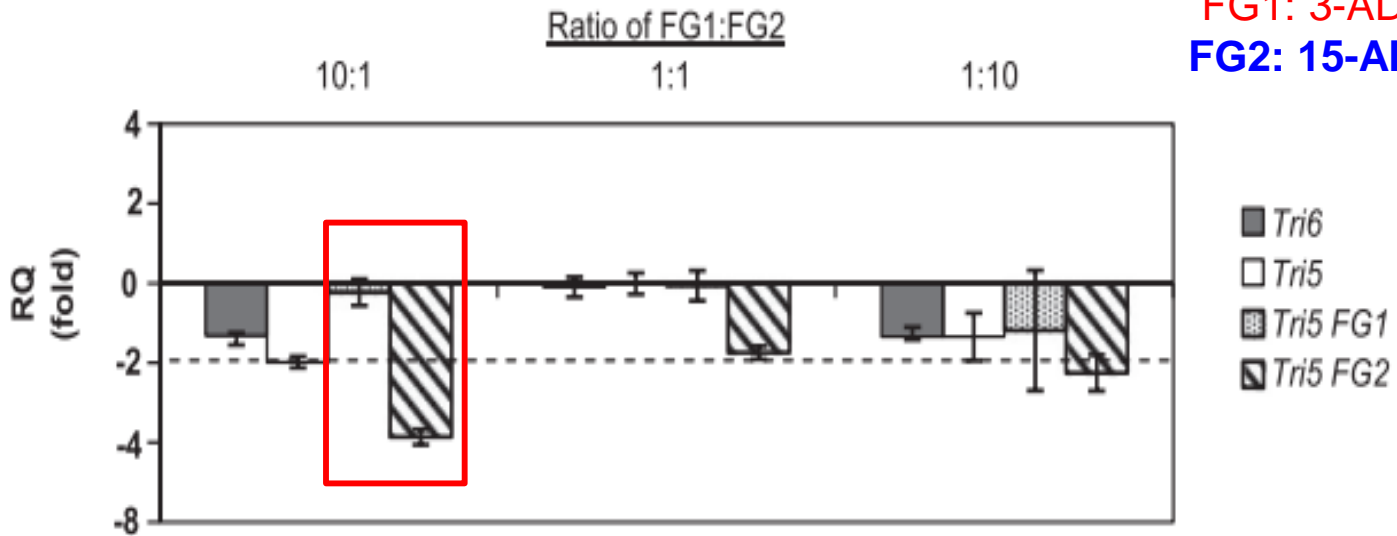
Pan Genome Identifies Core Genes

Isolate	Conserved Genes		Poorly Conserved Genes	
	Total	Uniquely Present	Total	Uniquely Absent
PH-1	14244	14	1053	13
DAOM180378	14254	18	1043	8
NRRL28336	14383	92	914	2
DAOM233423	14234	18	1063	10
DAOM241165	14307	12	990	1
CS3005	14274	14	1023	6
NRRL6101	14171	65	1126	34
NRRL28720	14151	72	1146	29
NRRL28721	14212	18	1085	2
NRRL28723	14218	22	1079	7

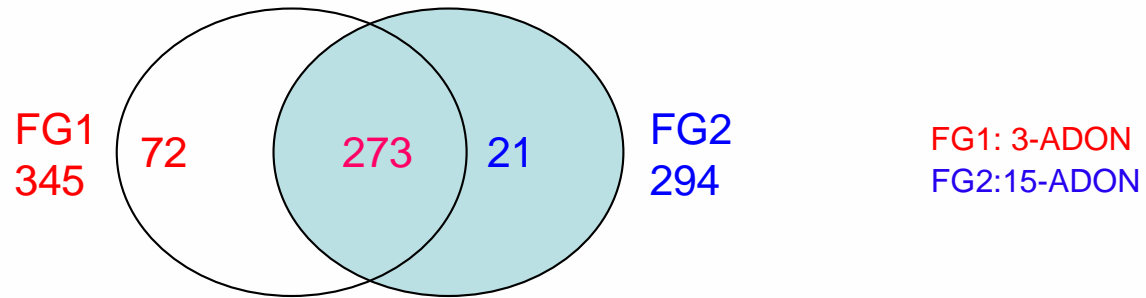
Validation of SNPs as Markers



FG1: 3-ADON
 FG2: 15-ADON



Pan genome Vs Pan Secretome Reveal Potential Function



Gene Name	Source/descriptor	SNPs per kb
FGSG_04546	related to serine-type carboxypeptidase f precursor	115
FGSG_12548	related to feruloyl esterase B precursor	114
FGSG_04527	related to carboxypeptidase	114
FGSG_11399	related to oxidoreductase	109
FGSG_07678	related to acid phosphatase Pho610	100
FGSG_00100	related to 6-hydroxy-d-nicotine oxidase	100
FGSG_02918	related to aspartic proteinase, pepstatin-sensitive	100
FGSG_16186	related to LPL lysophospholipase precursor	89
FGSG_02342	probable cutinase 1 precursor	86

Summary:

Interaction between Fusarium may be common

- › Cautious about interpreting phenotypes

Comparative genomics

- › Identify genetic differences that may account for some of the diversity within *Fusarium graminearum* genomes
- › Pan-Genome: fast/slow evolving genes
 - design markers
 - Virulence factors
 - PAMPs

Acknowledgements:



Agriculture and
Agri-Food Canada

Agriculture et
Agroalimentaire Canada



People. Discovery. Innovation.

Subramaniam Lab:
Sean Walkowiak
Li Wang
Armand Mirmiran
Chris Bonner

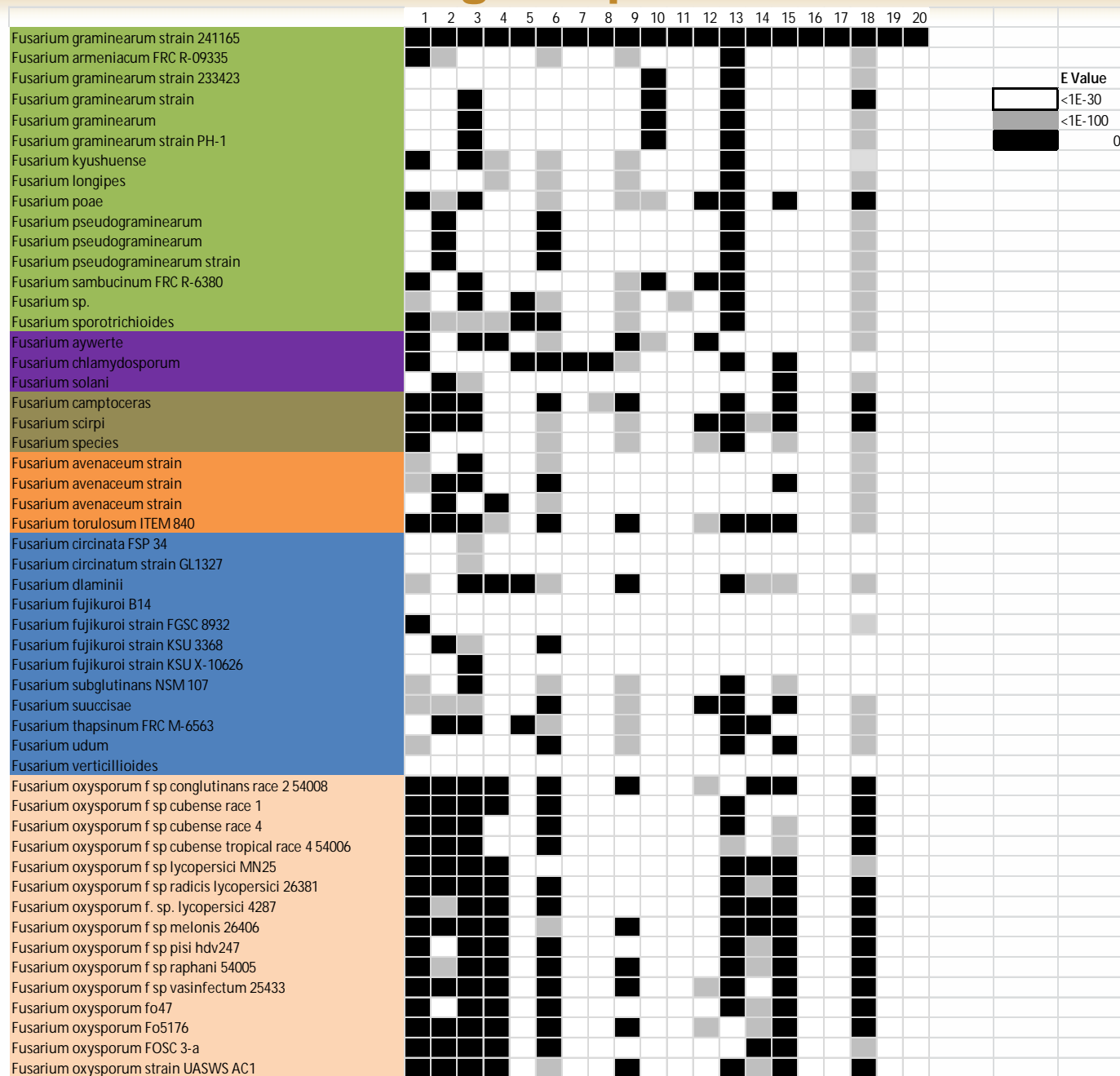
Linda Harris Lab:
Danielle Schneiderman

Chris Rampitsch Lab

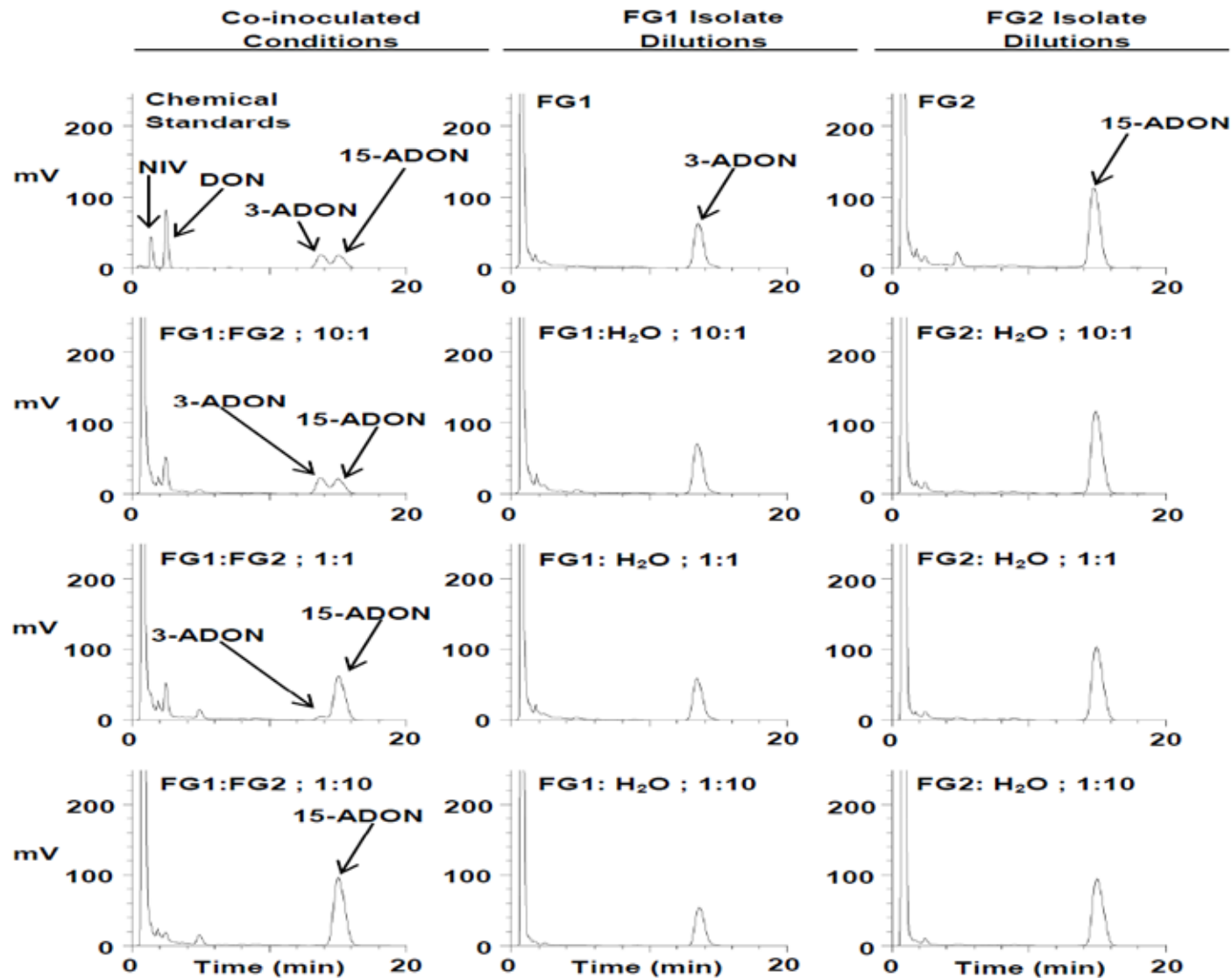


**Collaborators at the,
University of Toronto:**
Darrell Desveaux
David Guttman

Genes within Contig 207 present in other Fusarium



3-ADON and 15-ADON during competition



Supplementary Fig. S1. Sample HPLC chromatographs that show abundance of 3-ADON and 15-ADON in FG1 and FG2 in axenic and co-inoculated cultures.