

"Maladies des Céréales"

8 et 9 avril 2015

Clermont-Ferrand

Le potentiel toxinogène des souches de *Fusarium spp*: focus sur les mycotoxines émergentes

Laetitia Pinson-Gadais,

Christine Ducos, Françoise Turtaut, Gisèle Marchegay, Marie-Noëlle Bonnin-Verdal, Sophie Rolland, Vessela Atanasova-Penichon, Melisa Preignac, Florence Forget.

Unité de recherche INRA, Mycologie et Sécurité des Aliment_MycSA

http://www6.bordeaux-aquitaine.inra.fr/mycsa

lpinson@bordeaux.inra.fr



INRA
SCIENCE & IMPACT

Les principaux *Fusarium* présents sur blé et orge en Europe

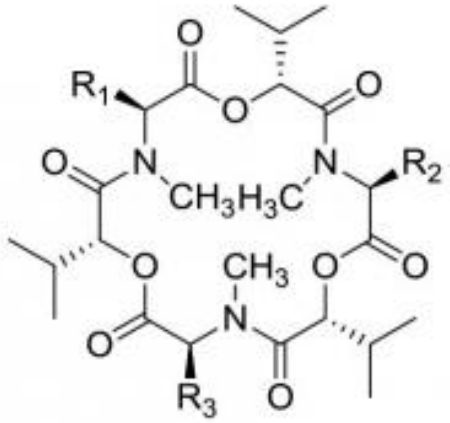
et leurs toxines associées

Sur blé et orge

F. graminearum	**TCTB, ZEA**
F. avenaceum	**MON,**BEA**,ENNs**
F. culmorum	**TCTB, ZEA**
F. poae	**TCTB (NIV),BEA,** ENNs
F. tricinctum	**MON**, BEA, **ENNs**
F. sambucinum	TCTA, ZEA, **BEA, ENNs**
F. sporotrichoïdes	TCTA,ENNs, **BEA**
F. equiseti	TCTA et B, ZEA, BEA, ENNs
F. acuminatum	TCTA, ENNs
F. crookwellense = cerealis	TCTB, ZEA
F. langsethiae	TCTA,ENNs, BEA
F. pseudograminearum	TCTB
F. heterosporum	ZEA
F. oxysporum	Fumonisine C, ENNs, BEA
F. verticilloides	Fumonisines B

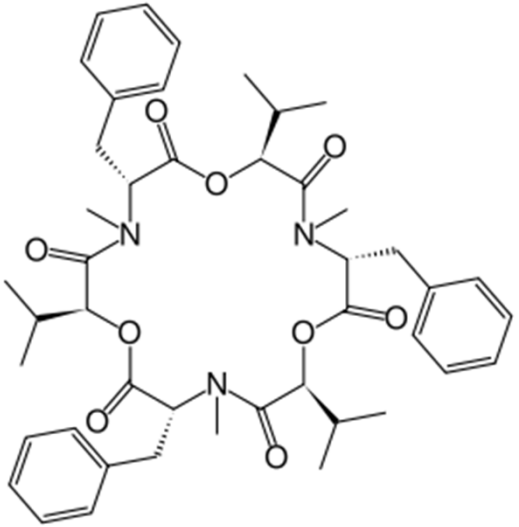
MON:Moniliformine, BEA:Beauvericine , ENNS:Enniatines, ZEA:Zearalenone, TCTA/B: Trichothécènes typeA/ B

Cyclodepsipeptides

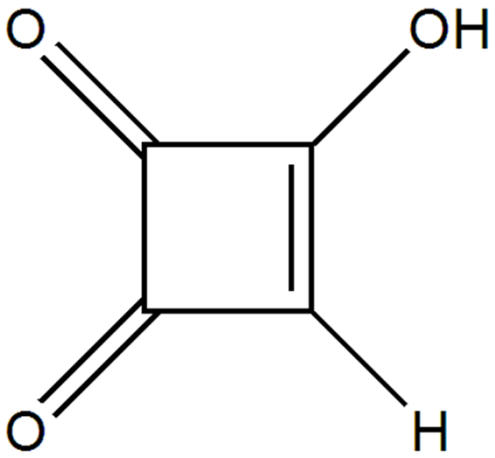


	R_1	R_2	R_3
A	s-Bu	s-Bu	s-Bu
A_1	i-Pr	s-Bu	s-Bu
B	i-Pr	i-Pr	i-Pr
B_1	i-Pr	i-Pr	s-Bu
C	i-Bu	i-Bu	i-Bu
D	i-Pr	i-Pr	i-Bu
E	i-Pr	s-Bu	i-Bu
F	s-Bu	s-Bu	i-Bu

Enniatines A, A1, B, B1, B2,B3,B4 → O3 (27)



Beauvericine



Moniliformine

Pas de réglementation sur ces mycotoxines dites "émergentes"

Peu d'études sur leur toxicité :

- La beauvericine inhibe spécifiquement la cholestérol acyltransferase considérée comme étant toxique pour les humains.
- Des travaux récents ont permis de mettre en évidence une cytotoxicité des enniatines et de la beauvéricine. Avec une action synergique avec d'autres mycotoxines comme le DON et la FB1.

  Ces travaux concluent que " La présence concomitante de mycotoxines pourrait augmenter le risque de myélotoxicité et d'immunotoxicité chez l'homme".

  "Évaluation de la toxicité de mycotoxines émergentes et de couples de mycotoxines sur les cellules humaines d'origine hématopoïétique - Anne-Sophie Ficheux (2010)"

- Les voies de biosynthèse de l'ENNs et BEA auraient une partie commune
- 1 seul gène est pour l'instant connu comme intervenant dans la voie de biosynthèse des ENNs : esyn 1

Occurrence de la beauvéricine et des enniatines en Europe

En Suède

Deoxynivalenol and other selected *Fusarium* toxins in Swedish wheat — Occurrence and correlation to specific *Fusarium* species

Mats Lindblad [a], Ann Gidlund [a], Michael Sulyok [b], Thomas Börjesson [c], Rudolf Krska [b], Monica Olsen [a], Elisabeth Fredlund [a,*]

International Journal of Food Microbiology 167 (2013) 284–291

Table 3
Median level and percentage of positive samples[a] of *Fusarium* spp. quantified by real-time PCR in 2009 (n = 31), 2010 (n = 28), and 2011 (n = 66) in Sweden.

Fusarium spp.	Median (pg DNA/mg grain)				Percentage of positive samples[a] (%)			
	Winter wheat		Spring wheat		Winter wheat		Spring wheat	
	2009	2011	2010	2011	2009	2011	2010	2011
F. graminearum	13	8	44	157	81	73	75	91
F. langsethiae	4	4	<LOQ	3	58	94	4	94
F. culmorum	4	8	100	25	90	91	96	88
F. poae	95	26	30	8	100	100	100	100
F. avenaceum	45	5	98	29	100	94	100	100
F. tricinctum	341	4	<LOQ	<LOQ	100	61	29	45
F. sporotrichioides	<LOQ	<LOQ	na	<LOQ	0	9	na	15

[a] All samples above LOQ (3 pg/mg grain for *F. tricinctum* and *F. graminearum*, 1 pg/mg grain for *F. langsethiae* and *F. sporotrichioides*, 0.2 pg/mg grain for *F. avenaceum*, and 0.1 pg/mg grain for *F. culmorum* and *F. poae*) were considered as positive samples.

Table 4
Median levels, maximum levels and percentage of positive samples of *Fusarium* toxins quantified by LC–MS/MS in winter wheat from 2009 (n = 31) to 2011 (n = 33) in Sweden.

Mycotoxin	Median[a] (µg/kg grain)		Max (µg/kg grain)		Percentage of positive samples[a] (%)	
	2009	2011	2009	2011	2009	2011
Deoxynivalenol	38	31	303	1394	81	64
Zearalenone	20	<LOQ	86	25	100	9
Nivalenol	26	<LOQ	111	39	94	33
Moniliformin	53	<LOQ	497	255	74	36
Enniatins[b]	255	60	2499	1271	100	97
Beauvericin	3	<LOQ	12	9	77	52

[a] All samples above LOQ were considered as positive samples (LOQ = 13 µg/kg grain for DON and NIV, 5 µg/kg grain for ZEA, 10 µg/kg grain for T-2 and HT-2, 1.7 µg/kg grain for BEA, 15 µg/kg grain for MON, and 1.7 µg/kg grain for enniatin B, 1.0 µg/kg grain for B1, 1.0 µg/kg grain for A and B3, and 0.7 µg/kg grain for A1 and B2).
[b] Sum of enniatins A, A1, B, B1, B2, B3.

Table 5
Median levels, maximum levels and percentage of positive samples of *Fusarium* toxins quantified by LC–MS/MS in spring wheat from 2010 (n = 28) to 2011 (n = 33) in Sweden.

Mycotoxin	Median[a] (µg/kg grain)		Max (µg/kg grain)		Percentage of positive samples[a] (%)	
	2010	2011	2010	2011	2010	2011
Deoxynivalenol	150	298	1189	6460	93	91
Zearalenone	<LOQ	<LOQ	32	678	36	39
Nivalenol	<LOQ	<LOQ	39	50	50	27
Moniliformin	61	27	1066	2078	71	64
Enniatins[b]	269	220	4281	2635	96	100
Beauvericin	<LOQ	<LOQ	7	4	64	12

[a] All samples above LOQ were considered as positive samples (LOQ = 13 µg/kg grain for DON and NIV, 5 µg/kg grain for ZEA, 10 µg/kg grain for T-2 and HT-2, 1.7 µg/kg grain for BEA, 15 µg/kg grain for MON, and 1.7 µg/kg grain for enniatin B, 1.0 µg/kg grain for B1, 1.0 µg/kg grain for A and B3, and 0.7 µg/kg grain for A1 and B2).
[b] Sum of enniatins A, A1, B, B1, B2, B3.

Occurrence de la beauvéricine et des enniatines en Europe

En Suède

Deoxynivalenol and other selected *Fusarium* toxins in Swedish wheat — Occurrence and correlation to specific *Fusarium* species

Mats Lindblad [a], Ann Gidlund [a], Michael Sulyok [b], Thomas Börjesson [c], Rudolf Krska [b], Monica Olsen [a], Elisabeth Fredlund [a,*]

International Journal of Food Microbiology 167 (2013) 284–291

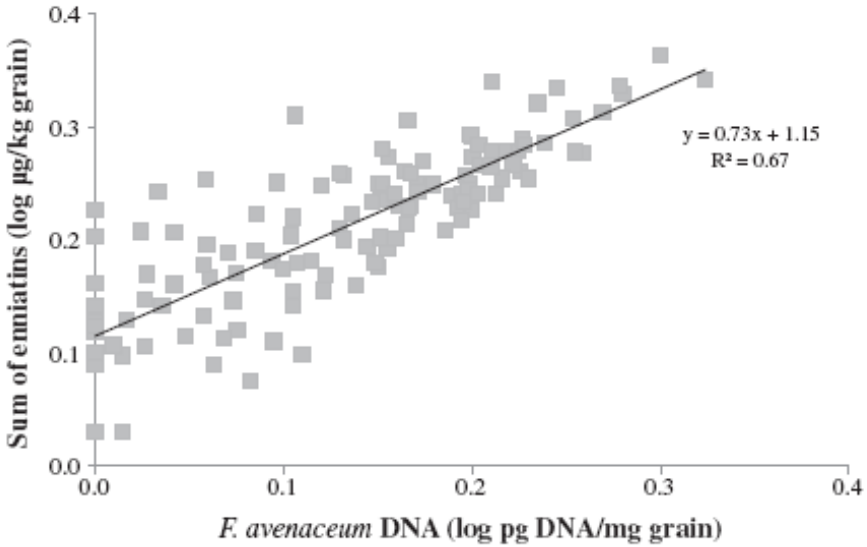


Fig. 2. Regression of the sum of levels of enniatins (A, A1, B, B1, B2, and B3) against levels of DNA from *F. avenaceum* in wheat samples collected in 2009, 2010 and 2011 (n = 125).

Table 6
Pearson's correlation coefficients (*r*) of the statistically significant correlations (*** $P < 0.001$) between mycotoxin and *Fusarium* DNA levels in wheat samples from 2009 through 2011 (n = 93).

Mycotoxin	*Fusarium* spp.	r[a]	r^2
Deoxynivalenol	*F. graminearum*	0.86	0.74
	F. avenaceum	0.54	0.29
	F. culmorum	0.41	0.17
Zearalenone	*F. tricinctum*	0.54	0.29
	F. avenaceum	0.41	0.17
	F. graminearum	0.40	0.16
Nivalenol	*F. tricinctum*	0.64	0.41
	F. poae	0.48	0.23
Moniliformin	*F. avenaceum*	0.79	0.66
	F. graminearum	0.54	0.29
Enniatins	*F. avenaceum*	0.82	0.67
	F. graminearum	0.51	0.26
Beauvericin	*F. poae*	0.69	0.48
	F. tricinctum	0.46	0.21

[a] Pearson's correlation coefficients <0.40 and with lower significance than $P < 0.001$ were not included in the table.

Occurrence de la beauvéricine et des enniatines en Europe

En Italie Occurrence of Fusarium mycotoxins in Italian cereal and cereal products from organic farming

Cristina Juana, Alberto Ritienib, Jordi Mañesc - Food Chemistry 141 (2013) 1747-1755

93 échantillons issus de l'agriculture biologique: blé, avoine, orge, épeautre, seigle

41% des échantillons contiennent les 6 mycotoxines suivantes :

BEA, ENA, A1, B, B1 et B4

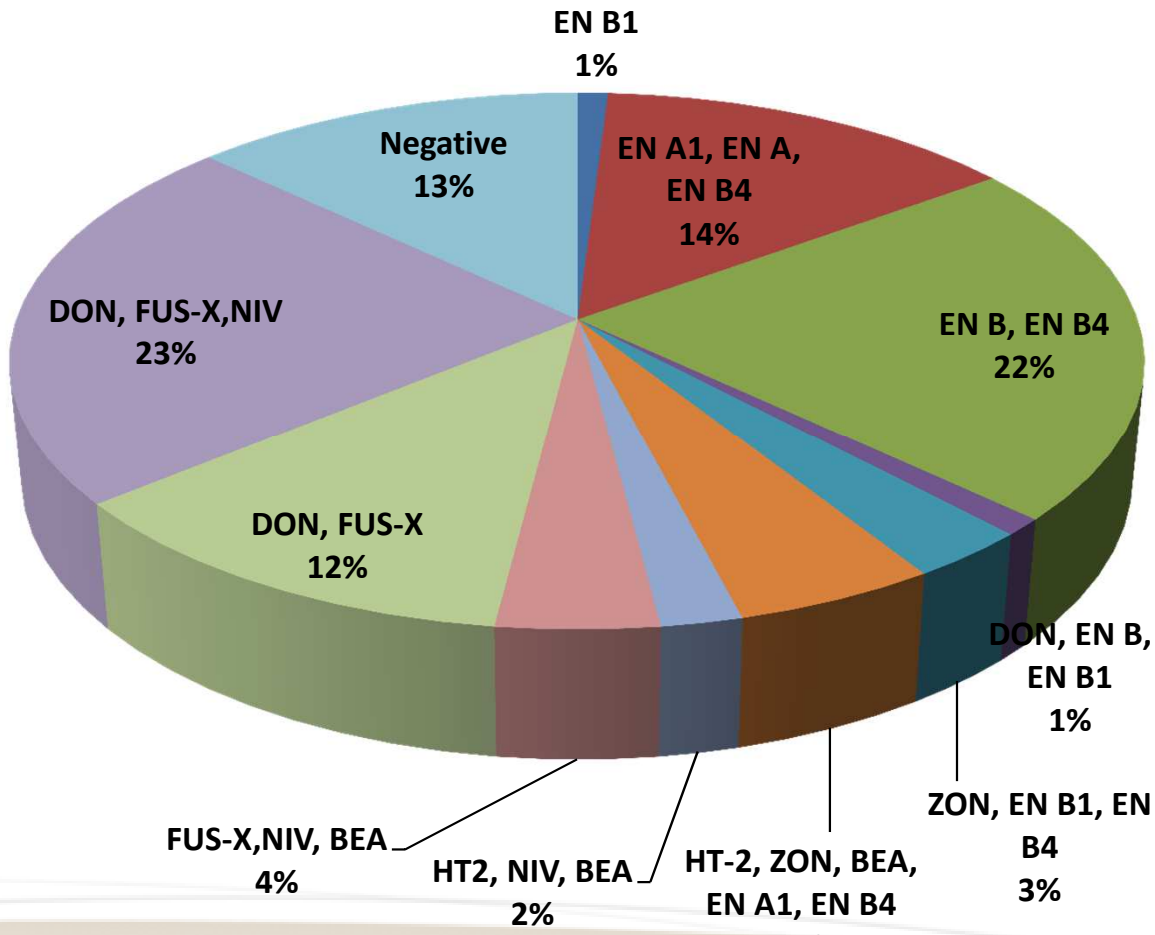
Blé

[TCTB] ° (DON, Fus-X, NiV) =< [BEA et/ou ENNS] °

Expl (µg/Kg)

DON	moy 10.96	(16/57)	=> max 99.6
FUS-X	moy 18.44	(14/57)	=> max 102
ENB	moy 20.20	(16/57)	=> max 97
ENB4	moy 38.44	(18/57)	=> max 110.2
BEA	moy 12.80	(5/57)	=> max 35

Co-occurrence of mycotoxins detected in analysed samples



Occurrence de la beauvéricine et des enniatines en Europe

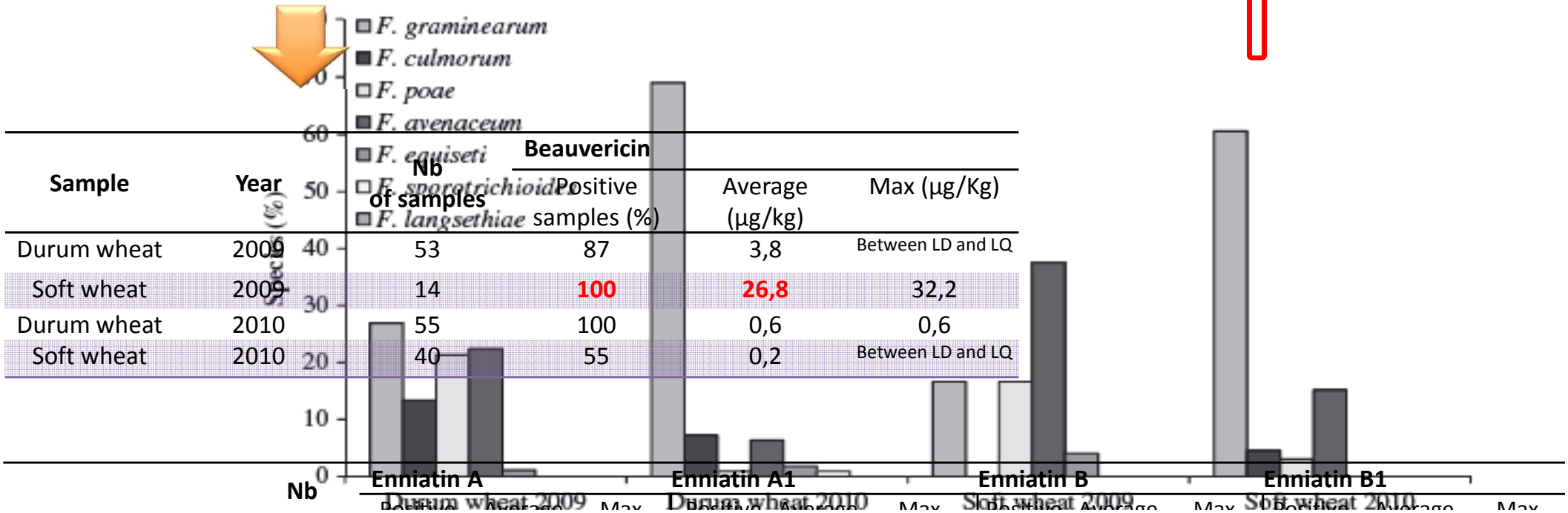
En Italie

L. Covarelli, G. Beccaria, A. Prodib, S. Generottia, F. Etruschia, G. Mecac, C. Juanc, J. Mañesc
***Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy-** Journal of the Science of Food and Agriculture Vol 95 (2014) 540-551
Biosynthesis of beauvericin and enniatins *in vitro* by wheat *Fusarium* species and natural grain contamination in an area of central Italy- Food Microbiology vol 46 (2015) 618-626

162 échantillons blé dur et blé tendre

2009 et 2010=> centre de l'Italie (région Umbria)

Sample	Year	Nb of samples	Beauvericin Positive samples (%)	Average (µg/kg)	Max (µg/Kg)
Durum wheat	2009	53	87	3,8	Between LD and LQ
Soft wheat	2009	14	**100**	**26,8**	32,2
Durum wheat	2010	55	100	0,6	0,6
Soft wheat	2010	40	55	0,2	Between LD and LQ



En 2009 => Quantité de pluie et humidité inférieure à 2010 pendant la phase floraison

➲ ↘ présence des principaux agents responsable de la fusariose (F.graminearum) remplacés par F. avenaceum et F. poae .

T° mini basse et T°maxi plus élevées en 2009 /2010 ➲ influence la composition des espèces fusariennes

Occurrence de la beauvéricine et des enniatines en Europe

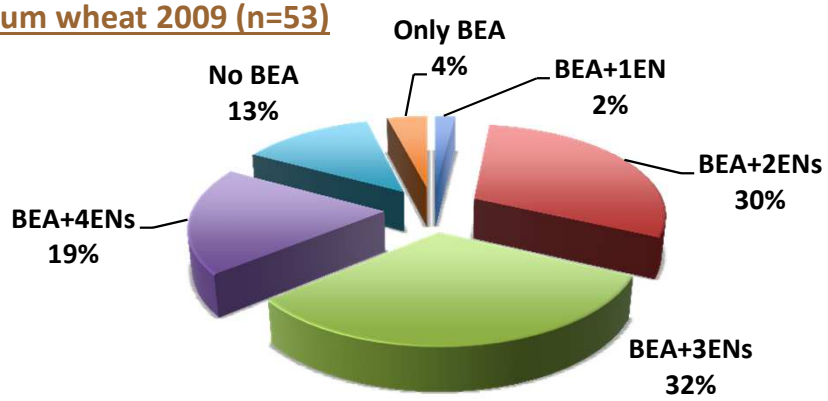
En Italie

L. Covarelli, G. Beccaria, A. Prodib, S. Generottia, F. Etruschia, G. Mecac, C. Juanc, J. Mañesc
***Fusarium* species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy-** Journal of the Science of Food and Agriculture Vol 95 (2014) 540-551
Biosynthesis of beauvericin and enniatins *in vitro* by wheat *Fusarium* species and natural grain contamination in an area of central Italy- Food Microbiology vol 46 (2015) 618-626

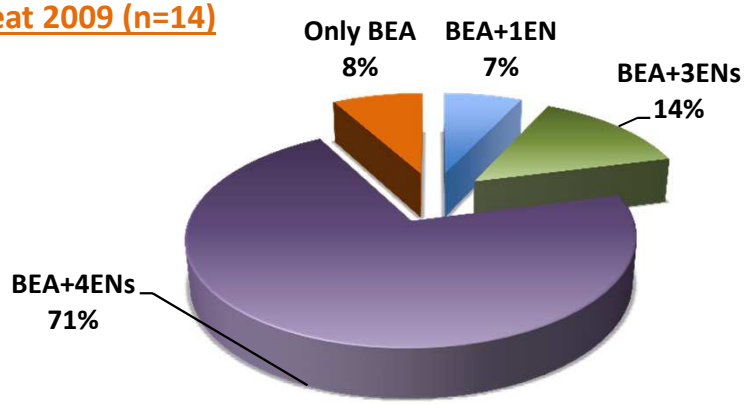
162 échantillons blé dur et blé tendre 2009 et 2010=> centre de l'Italie (région Umbria)

Echantillons + → Co-occurrence BEA et ENs

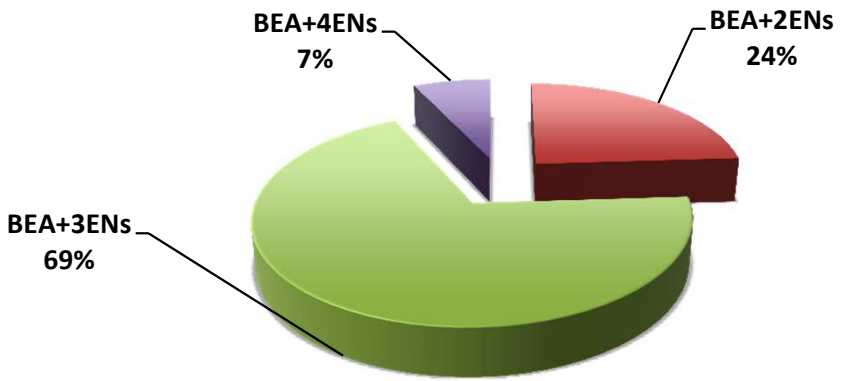
Durum wheat 2009 (n=53)



Soft wheat 2009 (n=14)



Durum wheat 2010 (n=55)



Soft wheat 2010 (n=40)

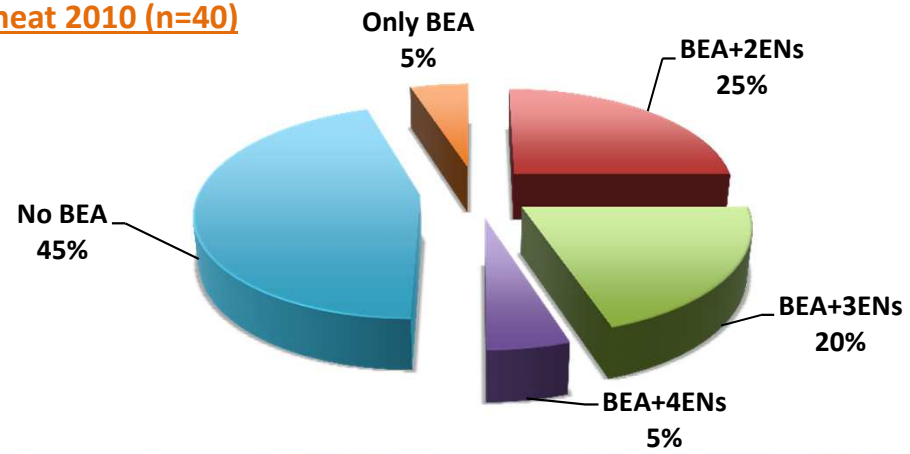


Fig. 2. Co-occurrence (positive samples %) of beauvericin and enniatin analogues on durum and soft wheat cultivated in the Umbria region (central Italy) in 2009 and 2010.

Occurrence de la beauvéricine et des enniatines en Europe

En Espagne Further data on the presence of Fusarium emerging mycotoxins enniatins, fusaproliferin and beauvericin in cereals available on the Spanish markets

Giuseppe Mecaa, Abdellah Zinedineb, Jesus Blesaa, Guillermina Fonta, Jordi Mañesa- Food and Chemical Toxicology 48 (2010) 1412-1416

64 échantillons issus de supermarché : blé, maïs, orge principalement

75% + EN A, A1, B, B1

32% + BEA

Samples	Nb	Enniatins ENNs (mg/Kg)								
		+ samples	EN A		EN A1		EN B		EN B1	
			Mean	Max level	Mean	Max level	Mean	Max level	Mean	Max level
Wheat	21	13	ND	ND	225.37	634.85	ND	ND	ND	ND
Corn	28	25	ND	ND	167.70	813.01	4.47	6.31	4.34	4.34
Barley	4	2	ND	ND	148.16	361.57	21.37	21.37	45.94	45.94
Other:										
Mix cereals	5	4	ND	ND	191.03	268.64	ND	ND	ND	ND
Bread mill	4	4	ND	ND	37.47	149.90	ND	ND	23.94	23.94
Oat flour	1	1	ND	ND	388.38	388.38	ND	ND	ND	ND
Rice	1	1	ND	ND	814.42	814.42	7.95	7.95	ND	ND

Samples	Nb	Beauvericin (mg/Kg)		
		+ samples	BEA	
			Mean	Max level
Wheat	21	9	2.30	3.50
Corn	28	6	5.72	9.31
Barley	4	2	ND	6.94
Other:				
Mix cereals	5	1	3.12	3.12
Bread mill	4	1	0.51	0.51
Oat flour	1	1	4.18	4.18
Rice	1	1	11.78	11.78

Occurrence de la beauvéricine et des enniatines au Canada

Au Canada Fusarium Damage in Small Cereal Grains from Western Canada. 2. Occurrence of Fusarium Toxins and Their Source Organisms in Durum Wheat Harvested in 2010

TITTLEMIER S ; ROSCOE M; TRELKA R; GABA D; CHAN J; PATRICK S;SULYOK M; KRSKA R; MCKENDRY T; GRÄFENHAN T. J. Agric. Food chem. 2013, vol. 61, n°23, pp. 5438-5448

54 Echs 2010 blé Dur ➔ F. avenaceum et F. graminearum sont les deux espèces les plus fréquentes

75% échantillons analysés contenaient des [ ]° quantifiables d'ENs, BEA, DON, et MON

Enniatines + Beauvericine : de **0,011 à 34,2 mg/kg**,
Déoxynivalénol : jusqu'à **4,7 mg/kg,**
Moniliformine : jusqu'à **6,36 mg/kg**

Table 3. Occurrence and Concentrations (mg/kg) of Mycotoxins in Canadian Durum Samples Harvested in 2010 from Crop Districts in Alberta and Saskatchewan

ENs+BEA

crop district	*n* samples	MON mean[a]	MON median[a]	MON range	DON mean	DON median	DON range	∑Depsi[b] mean	∑Depsi median	∑Depsi range
				Alberta						
1	3	0.01	0	0.04	0.14	0.09	nd–0.32	0.10	0.17	0.078–0.20
2	3			nd[c]	0.14	0.17	nd–0.25	0.20	0.24	0.066–0.28
3	1	0.11	0.11	0.11			nd	2.4	2.4	2.4
				Saskatchewan						
1A	1			nd			nd	0.035	0.035	0.035
2B	6	0.82	0.70	0.04–2.51	1.3	0.48	0.10–4.7	7.3	7.4	0.74–17.2
3A	2			nd	0.025	0.025	nd–0.05	0.013	0.013	0.011–0.015
3BN	3	0.41	0.49	0.08–0.65	1.1	0.06	nd–3.3	4.1	5.7	0.83–5.77
3BS	2			nd			nd	0.014	0.014	0.012–0.016
4A	1			nd			nd	0.012	0.012	0.012
4B	3	0.30	0.064	nd–0.84	0.017	0	nd–0.05	1.9	0.52	0.17–4.9
5A	3	0.65	0.59	0.51–0.85	0.83	0.78	0.60–1.1	7.7	5.7	3.8–13.4
6A	2	1.4	1.4	0.04–2.75	1.6	1.6	0.37–2.8	11.0	11.0	3.35–18.7
6B	5	0.99	0.67	0.19–2.45	0.34	0.11	0.06–3.3	7.7	6.6	1.82–15.9
7A	16	2.1	1.7	nd–6.36	0.30	0.14	nd–2.7	14.9	13.2	1.23–34.2
7B	2	0.29	0.29	nd–0.58			nd	2.1	2.1	0.015–4.26
8B	1	0.10	0.10	0.10	0.06	0.06	0.06	0.87	0.87	0.87

[a]Results less than the limit of quantitation were set to 0 for the calculation of means and medians. [b]Sum of enniatin A, enniatin A1, enniatin B, enniatin B1, enniatin B2, enniatin B3, and beauvericin concentrations. [c]Not detected above limit of quantitation.

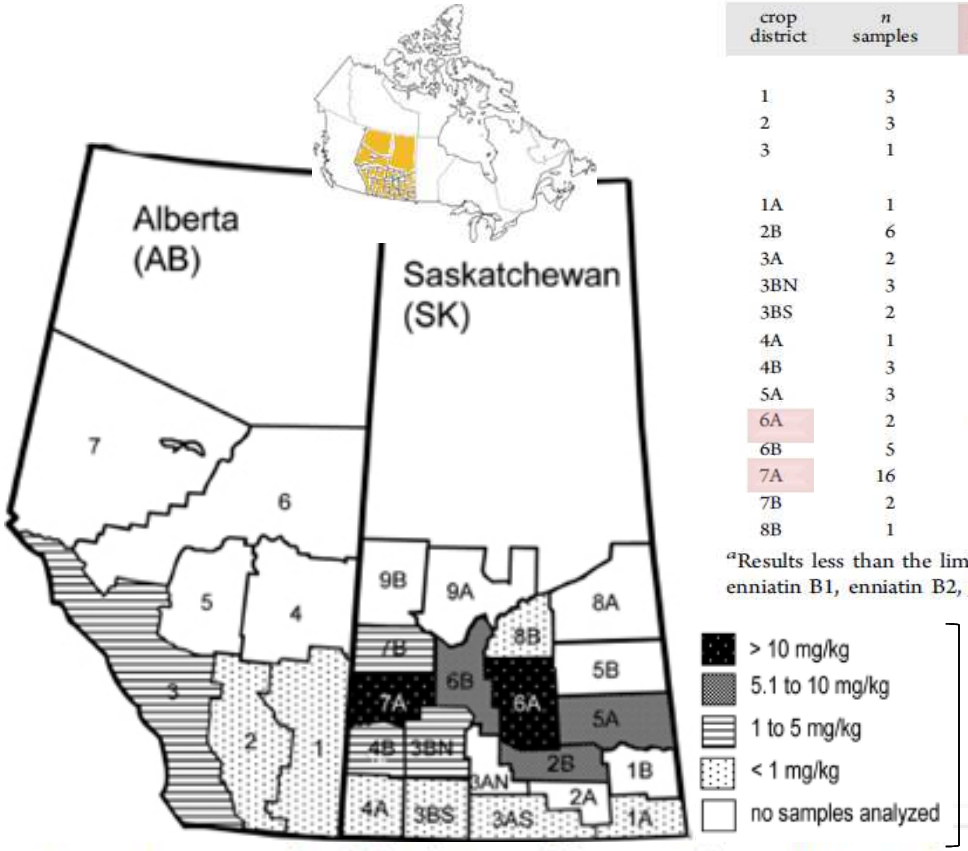


Figure 1. Geographical distribution of depsipeptides in 2010 Canadian durum samples from crop districts in Alberta and Saskatchewan.

Occurrence de la beauvéricine et des enniatines au Canada

Au Canada

Fusarium Damage in Small Cereal Grains from Western Canada. 2. Occurrence of Fusarium Toxins and Their Source Organisms in Durum Wheat Harvested in 2010

TITTLEMIER S ; ROSCOE M; TRELKA R; GABA D; CHAN J; PATRICK S;SULYOK M; KRSKA R; MCKENDRY T; GRÄFENHAN T. Journal of agricultural and food chemistry 2013, vol. 61, no23, pp. 5438-5448

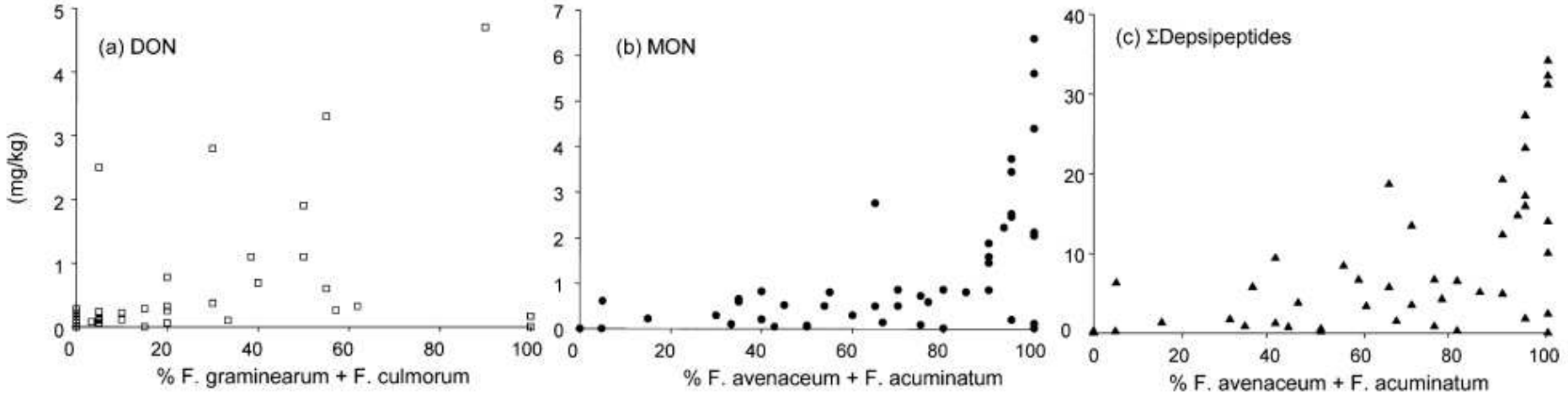


Figure 3. Relationship between mycotoxin concentrations (mg/kg) and presence of *Fusarium* species on Canadian durum harvested in 2010.

➲ **Dispersion des concentrations de DON / pourcentages F.graminearum et F.culmorum**

➲ **Schéma différent pour la moniliformine et Ens+ BEA ➔ pour les teneurs les plus élevées on observe un taux d'infection de 90% de F. avenaceum et F. acuminatum**

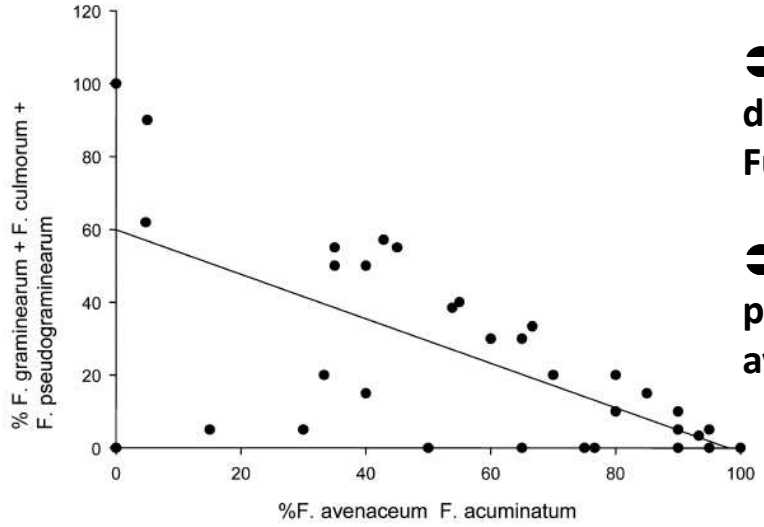


Figure 4. Relationship between the occurrences of *Fusarium* species on single kernels of Canadian durum harvested in central Saskatchewan in 2010.

➲ **une relation linéaire inverse entre les pourcentages de grains infectés par ces deux groupes d'espèces de Fusarium.**

➲ **F. graminearum et F. culmorum comme ayant un haut pouvoir pathogène par rapport à la faible pathogénicité de F. avenaceum.**

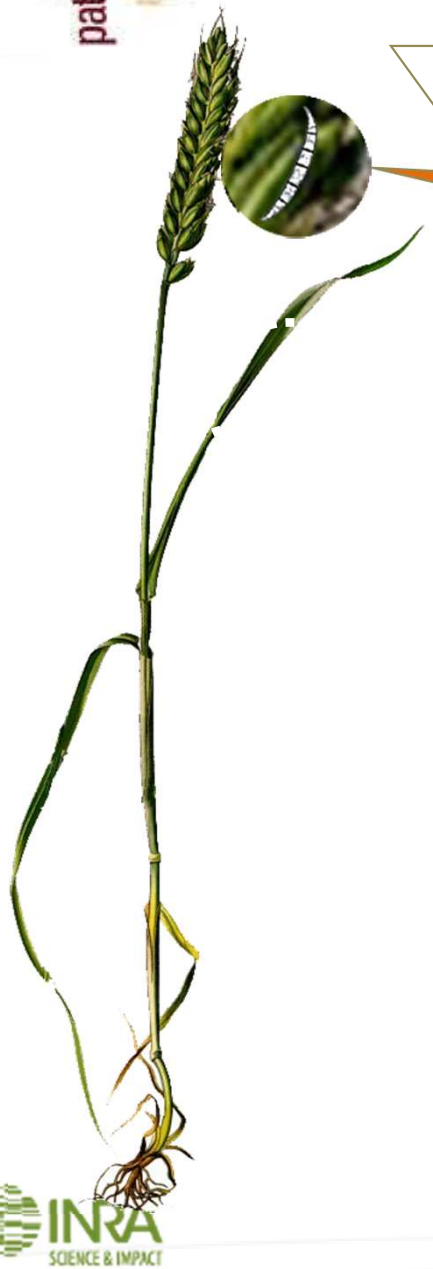
Occurrence de la beauvéricine et des enniatines en France

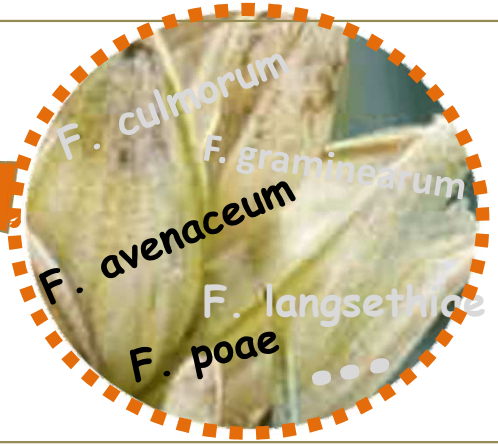


?

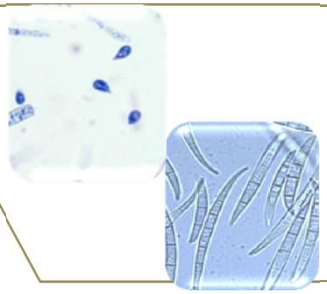
Connaissance de la flore Fusarienne productrice de ces toxines



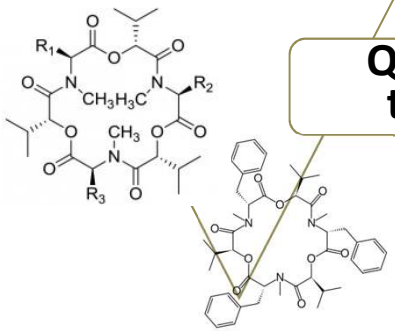




Quelles espèces *Fusarium* ?



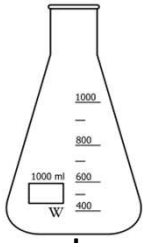
Quelles souches ? Diversitée ?



Quel potentiel toxinogène?

Caractérisation des potentiels toxinogènes des souches de Fusarium biochimiquement



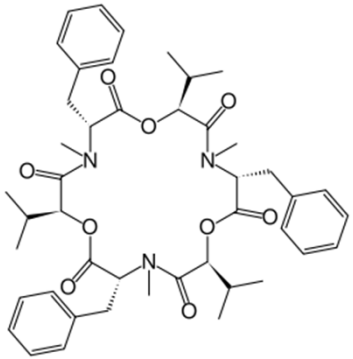
Maïs  Riz

Inoculation de la souche sur grains stérilisés

A 25°C à l'obscurité pendant 21 jours

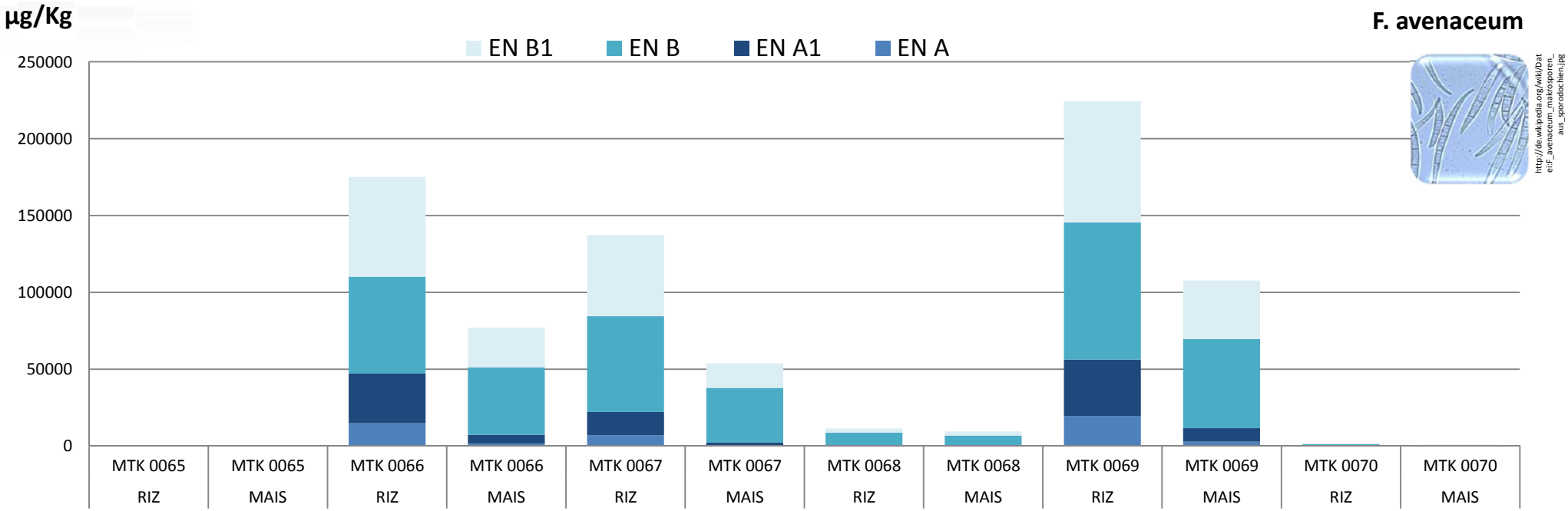


Quantification et identification des Toxines

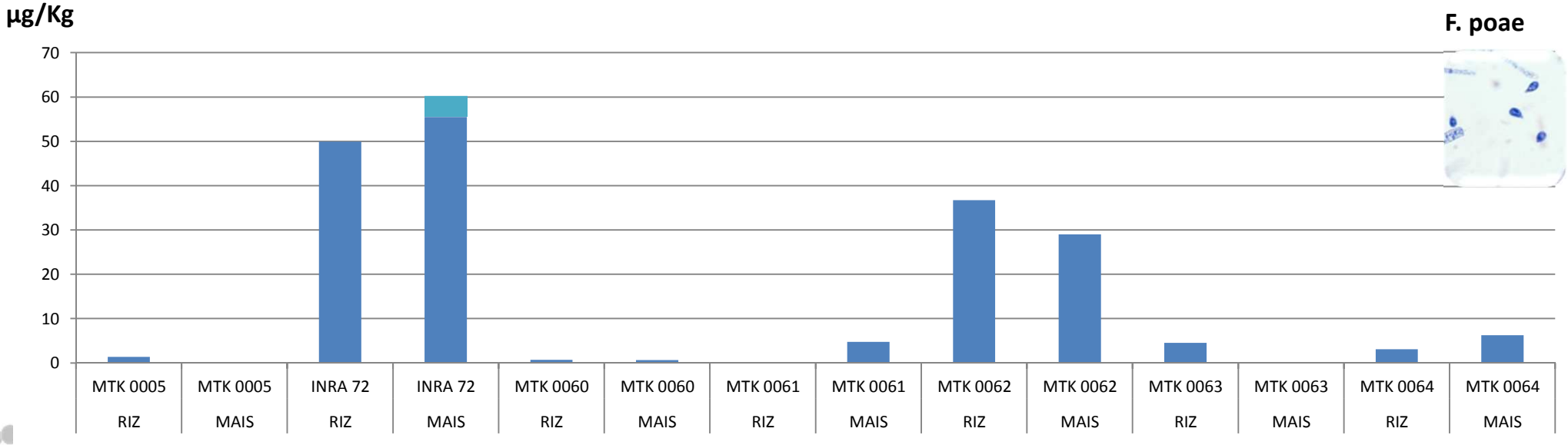


Caractérisation des potentiels toxinogènes des souches de Fusarium biochimiquement

Production d'enniatines par F. poae et F. avenaceum sur riz et maïs



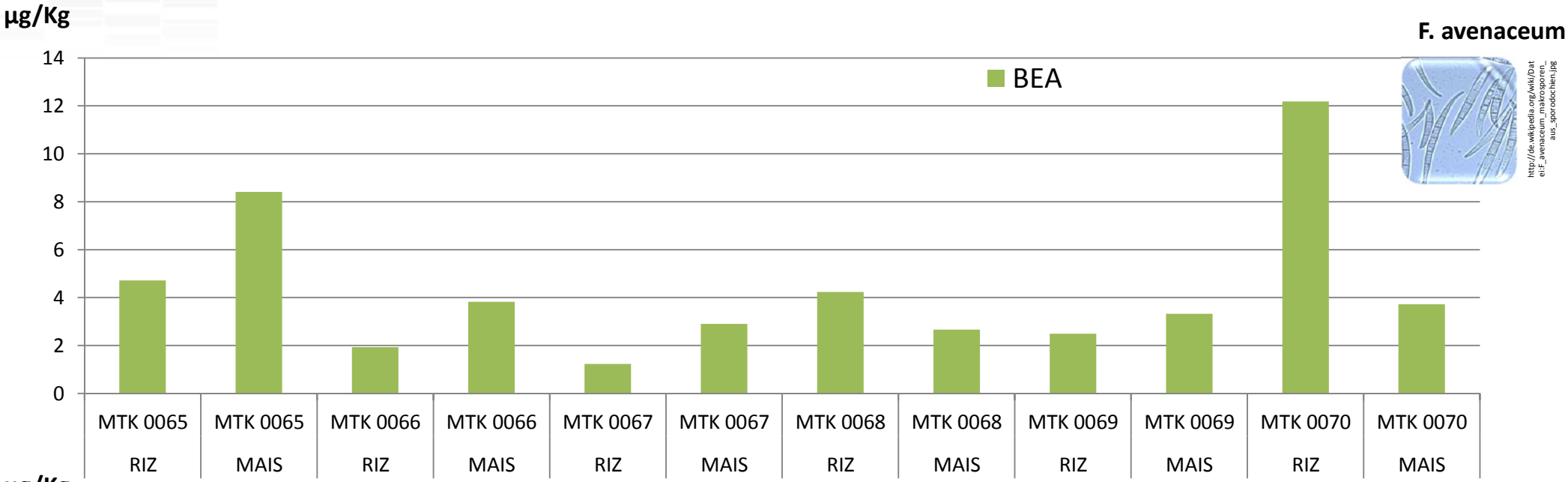
F. avenaceum

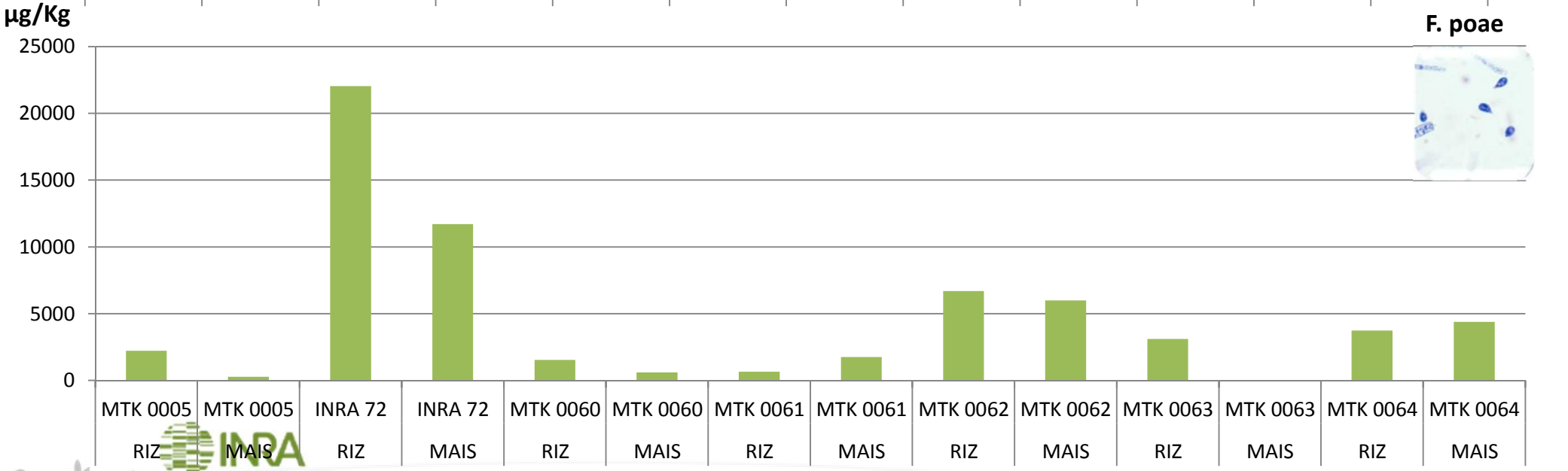


F. poae

Caractérisation des potentiels toxinogènes des souches de Fusarium biochimiquement

Production de beauvéricine par F. poae and F. avenaceum sur riz et maïs





- **Production de Moniliformine** par F. poae et F. avenaceum sur riz et maïs
  - **Aucune souche de F. avenaceum et F. poae ne produit de moniliformine**

En résumé

Espèces	ENNS				Nb souches produisant	BEA	Nb Souches produisant	MON
	A1	A	B	B1				
F.poae	0	+	0	0	5/6	++++	6/6	0
F.avenaceum	++	++	++++	++++	3/6	+	6/6	0

Meilleur expression du potentiel toxinogène sur Riz

Merci pour votre attention

