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THE REFERENCE IN THE BARLEY TO THE BEER

Last findings on T2/HT2 on malting barley and behaviour from malting barley to malt

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Aim

- ⇨ *Fusarium* contamination occurs in the field: control has to be setup within the field
- ⇨ Growth can occur during malting process (favourable humidity and temperature)
- ⇨ Better knowledge of Fusariotoxins from field to end products and by-products
- ⇨ Recommendation to reduce mycotoxin level in field and process

The project

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⇨ **BARSAFE : *Fusarium langsethiae*, from barley culture (*Hordeum vulgare*) to the finished products (beer) and by-products: study of the biology and the epidemiology of the pathogen, the conditions of T2/HT2 toxins production, of their transfer, biological breakdown and toxicity, for a better sanitary risk management**

PARTNERS

Institut Français de la Brasserie et de la Malterie (IFBM)
Arvalis Institut du Végétal
Laboratoire des Sciences du Génie Chimique
Laboratoire Génie chimique
Laboratoire de Pharmacologie-Toxicologie
Laboratoire de Pathologie Végétale et Epidémiologie

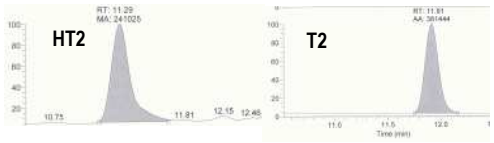
Study

- Study of *Fusarium* contamination & mycotoxins production in field
- Impact of *Fusarium* strains viability during storage
- Mycotoxins behaviour during malting & brewing process
- Mycotoxins behaviour from feed to end products

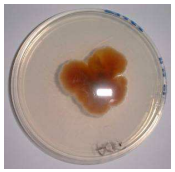
T2 / HT2 Toxins

Analysis method : barley & malt

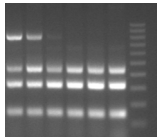
Multitrichothecene analysis : HPLCMSMS, LOD = 1 ppb)



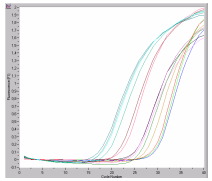
Isolation and identification of species by culture on agar media



Identification of species by specific primer DNA analysis



Quantification of species by real time PCR DNA analysis



Moulds

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Barley *Fusarium species*

Fusarium species actually found on brewing barley

F. graminearum

F. culmorum

F. langsethiae

F. tricinctum

F. avenaceum

F. poae

F. sporotrichioides (rare)

F. heterosporum
F. acuminatum
F. sambucinum
F. equiseti
F. lateritium
F. crookwellense (rare)
+ Microdochium nivale

Mycotoxin

type B TCT (DON, NIV), zearalenone

TCT A, TCT B

TCTA

Fusaric acid, beauvericine, fusarine C

Moniliformine (F. avenaceum)

Enniatines, Beauvericine

Other genera closely studied : Penicillium, Alternaria

Fusarium Identification

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Visual identification	PCR identification	PCR quantification
Fusarium spp	*Fusarium spp*	*Fusarium spp*
F. graminearum	*F. langsethiae*	*F. langsethiae*
F. tricinctum	*F. sporotrochioides*	*F. sporotrochioides*
F. culmorum	*F. graminearum*	*F. graminearum*
F. avenaceum	*F. culmorum*	*F. culmorum*
F. langsethiae	*F. poae*	*F. poae*
F. poae	*F. tricinctum*	*F. tricinctum*
F. sporotrichioides	*F. avenaceum*	*F. avenaceum*
F. heterosporum	*Microdochium nivale*	*Microdochium nivale*
F. solani	*Microdochium majus*	*Microdochium majus*
F. oxysporum	*F. heterosporum*	
F. acuminatum	*F. solani*	*F. verticillioides*
F. sambucinum	*F. moniliforme*	*F. proliferatum*
F. equiseti	*F. oxysporum*	*F. subglutinans*
F. lateritium	*F. acuminatum*	
F. proliferatum	*F. sambucinum*	
F. subglutinans	*F. equiseti*	
F. anthophyllum	*F. lateritium*	
F. verticillioides		
F. crookwellense		
F. chlamydosporum		
F. scirpi		
Microdochium nivale		
Microdochium majus		

Harvest analysis

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From 2003 to 2008, 150 to 200 malting barley samples are analysed every year

- Multitrichothecenes (LC-MS/MS)

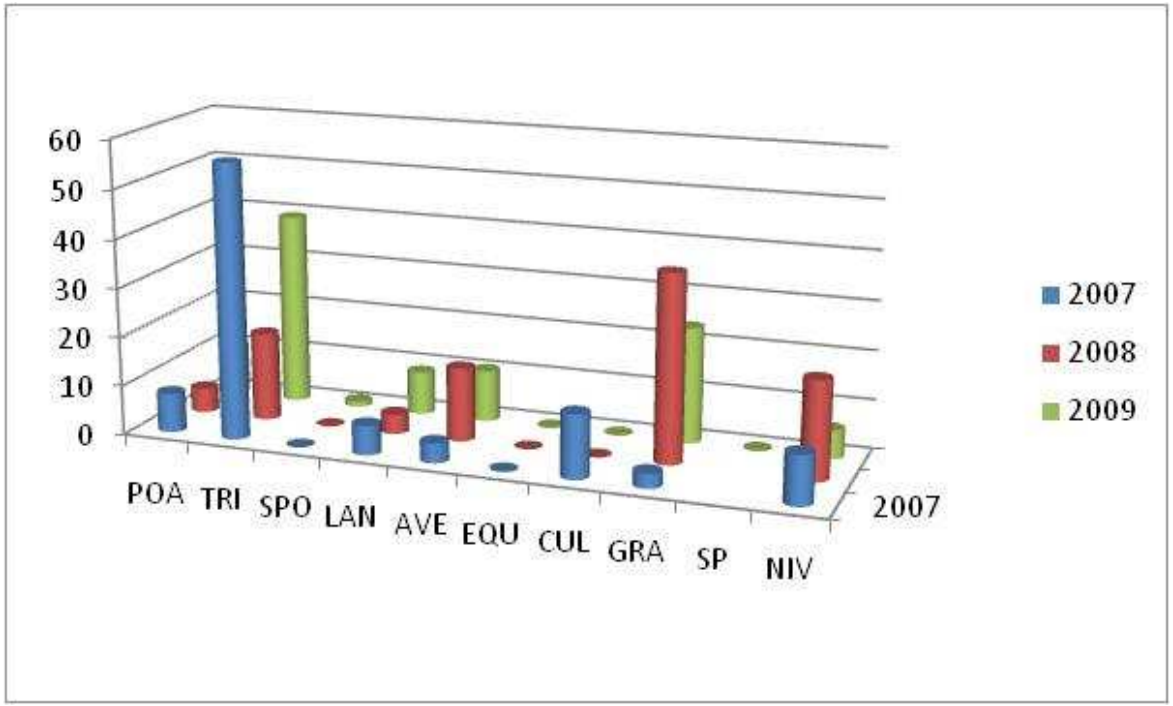
- Visual identification

- Identification by PCR

Fusarium : evolution from 2007 à 2009

Fusarium strains solated from calibrated French malting barley

Fusarium species proportion (%)



- **TCT A**
  - *F. langsethiae*
  - *F. poae*
  - *F. sporotrichioides*
- **Autres**
  - *F. tricinctum*
  - *F. avenaceum*
  - *M. nivale*
- **TCT B**
  - *F. graminearum*
  - *F. culmorum*
  - *F.poae*

Fusariotoxins: Harvest 2006-2008

Barley		T2+HT2	DON	NIV
		Harvest 2006		
Spring	mean	73	60	20
	max	708	508	135
Winter	mean	5	80	nd
	max	41	660	
		Harvest 2007		
Spring	mean	59	121	83
	max	489	957	576
Winter	mean	2	26	8
	max	63,5	176	136
		Harvest 2008		
Spring	mean	35	187	24
	max	146	1082	202
Winter	mean	12	122	7
	max	29	416	69
		Harvest 2009		
Spring	mean	46	89	18
	max	687	409	229
Winter	mean	22	242	4
	max	306	1226	20

Toxinogenic Potential of *Fusarium Langsethiae*

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Isolated from malting barley
Harvest 2005, 2006, 2007

	T2/HT2	TCT B
Fusarium poae	0-100 ppb	0-700 ppb
Fusarium sporotrichioides	59-668 ppb	None
Fusarium langsethiae	50 000 - 300 000 ppb	None

Harvest 2008

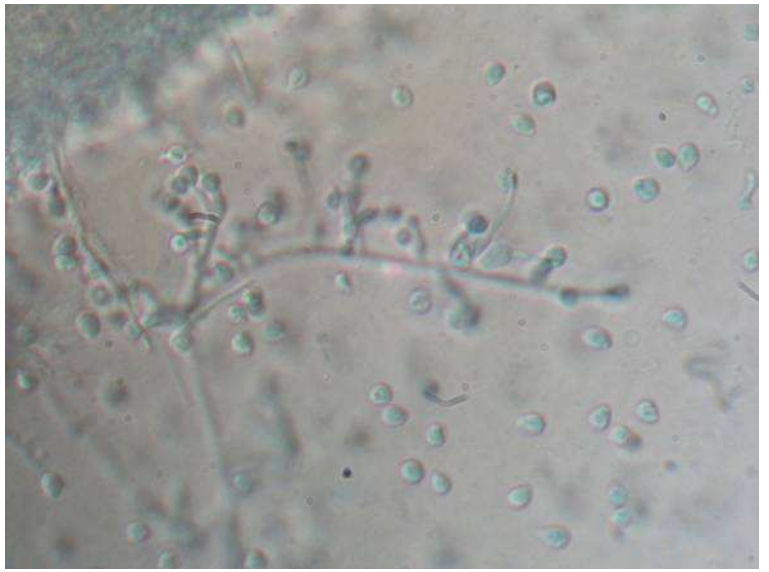
	T2/HT2	TCT B
Fusarium sporotrichioides	300 ppm	nd

Conditions: sterilised barley, 2 weeks at 25°C

T2/HT2 producer

Fusarium langsethiae

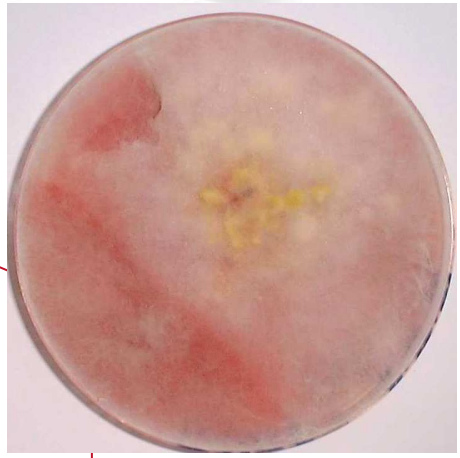
***F. langsethiae* on PDA**

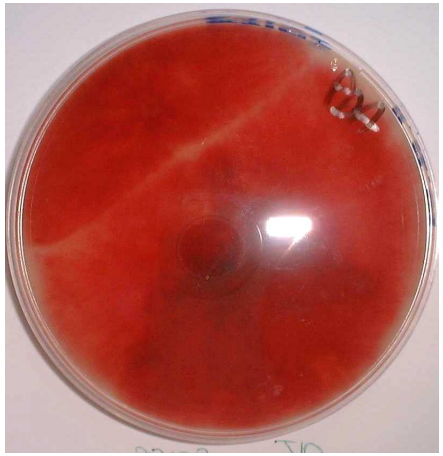


Microconidies of *F. langsethiae*

T2/HT2 producer

Fusarium sporotrichioides

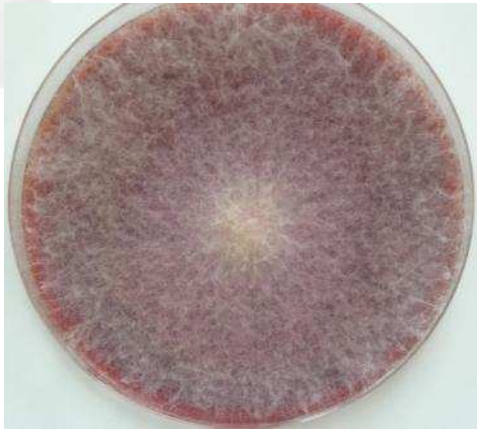


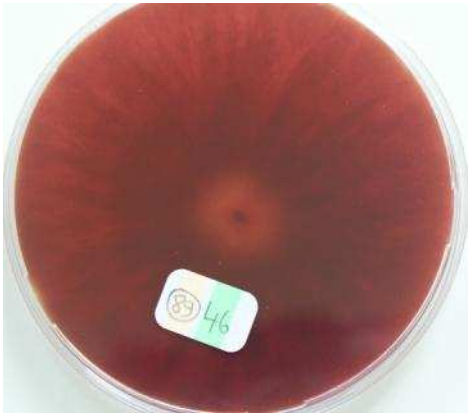


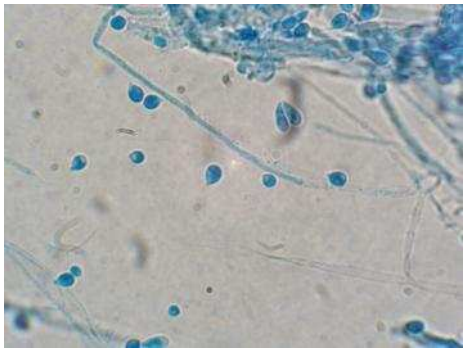
***F. sporotrichioides*on PDA**

Microconidies of *F. sporotrichioides*

Fusarium poae

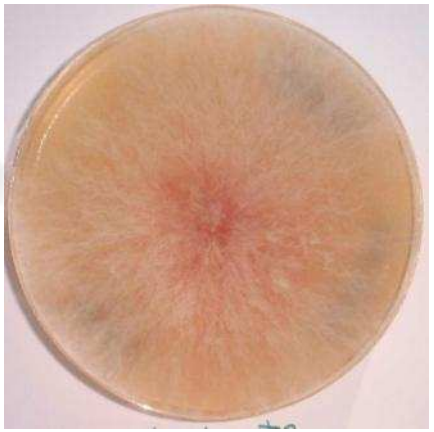






	MYCOTOXINS								
Strains	Eniatin B (ppb)	Beauvericin (ppb)	T2triol (ppb)	T2 (ppb)	HT2 (ppb)	3-DON (ppb)	DON (ppb)	NIV (ppb)	GUSHING
E-488 -08-088	0	**1200**	0	2	26	0	0	**80**	0
E-488 -0-089	0	**450**	0	0	0	0	0	**480**	0
E-488 -08-092	0	**980**	0	0	0	0	0	**80**	0
E-488 -07-094	0	**1900**	0	0	0	0	0	**600**	0
E-488 -07-008	0	**1100**	0	2	26	0	0	**78**	0

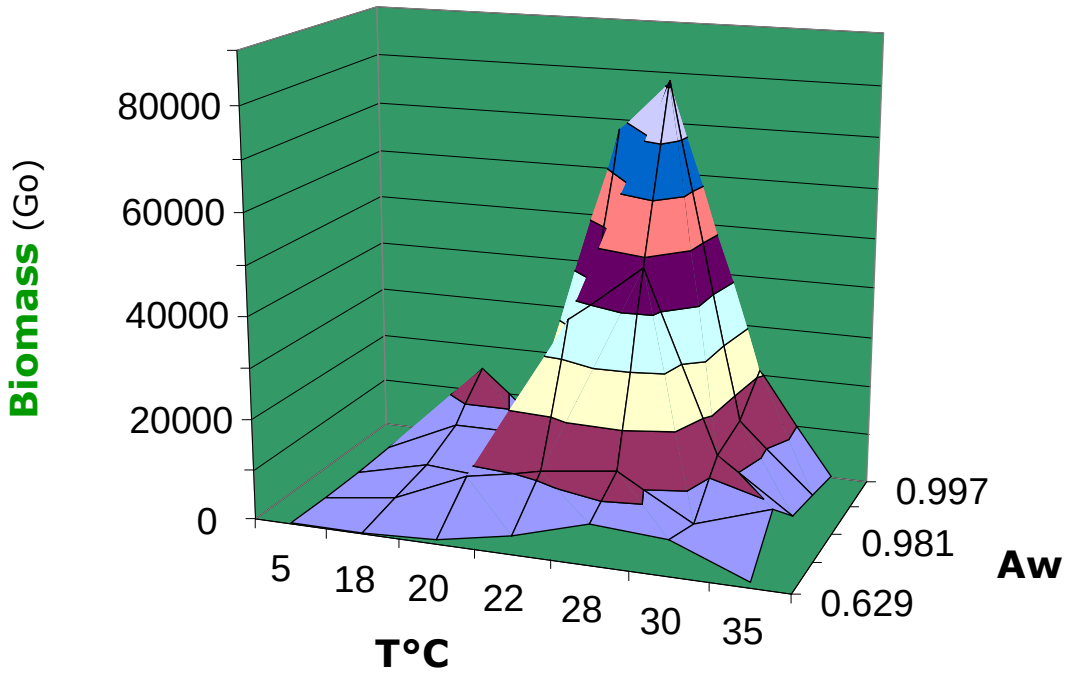
Fusarium graminearum



	MYCOTOXINS								
Strains	Eniatin B (ppb)	Moniliformin (ppb)	T2triol (ppb)	T2 (ppb)	HT2 (ppb)	3-DON (ppb)	DON (ppb)	NIV (ppb)	GUSHING
E-488 -07-071	0	0	0	0	0	0	**100**	0	0
E-488 -07-073	0	0	0	0	0	0	**64**	0	0
E-488 -07-075	0	0	0	0	0	0	**189**	0	0
E-488 -07-076	0	0	0	0	0	0	**117**	0	0
E-488 -07-079	0	0	0	0	0	0	**90**	55	0

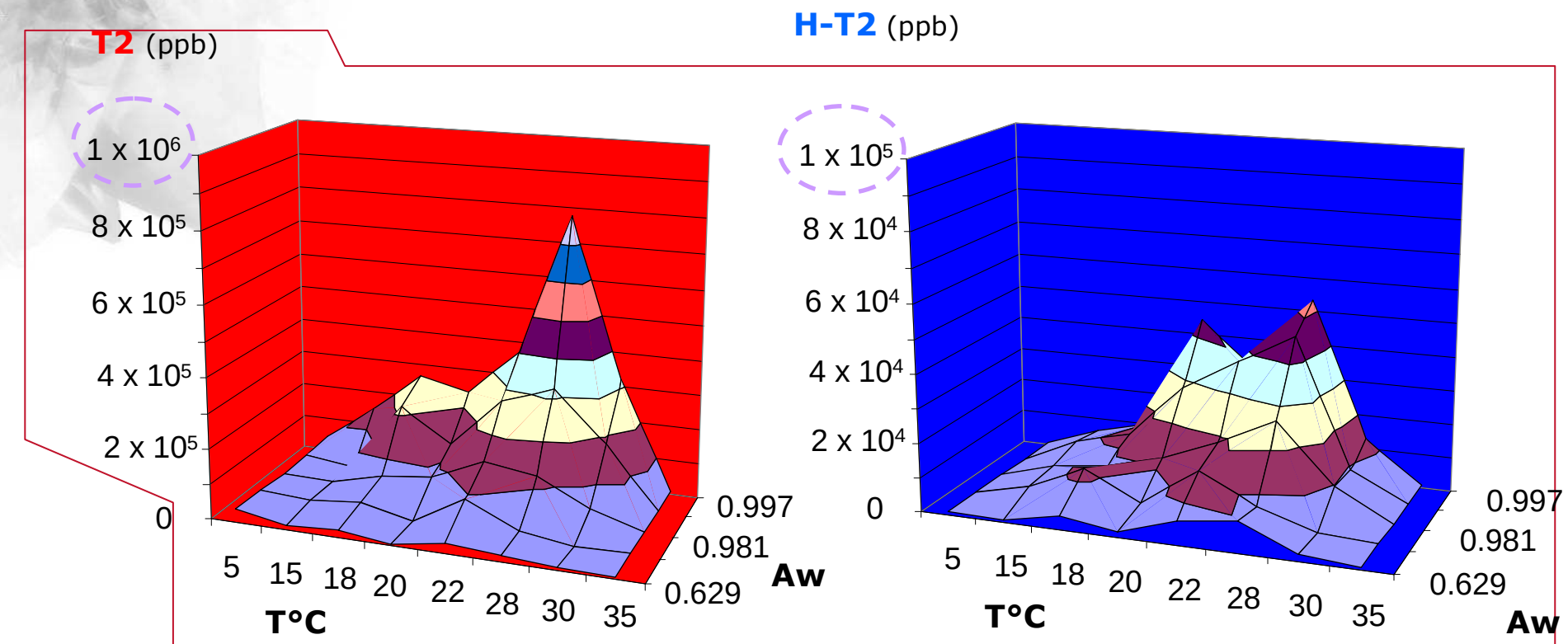
Ecotoxicogenesis conditions : Growth conditions

F. langsethiae (2008)
Culture on autoclaved barley



- Optimal growth temperature : 28°C
- Optimal growth Aw 0.992
- No growth at 5 and 35°C

Ecotoxicogenesis conditions : Toxin production

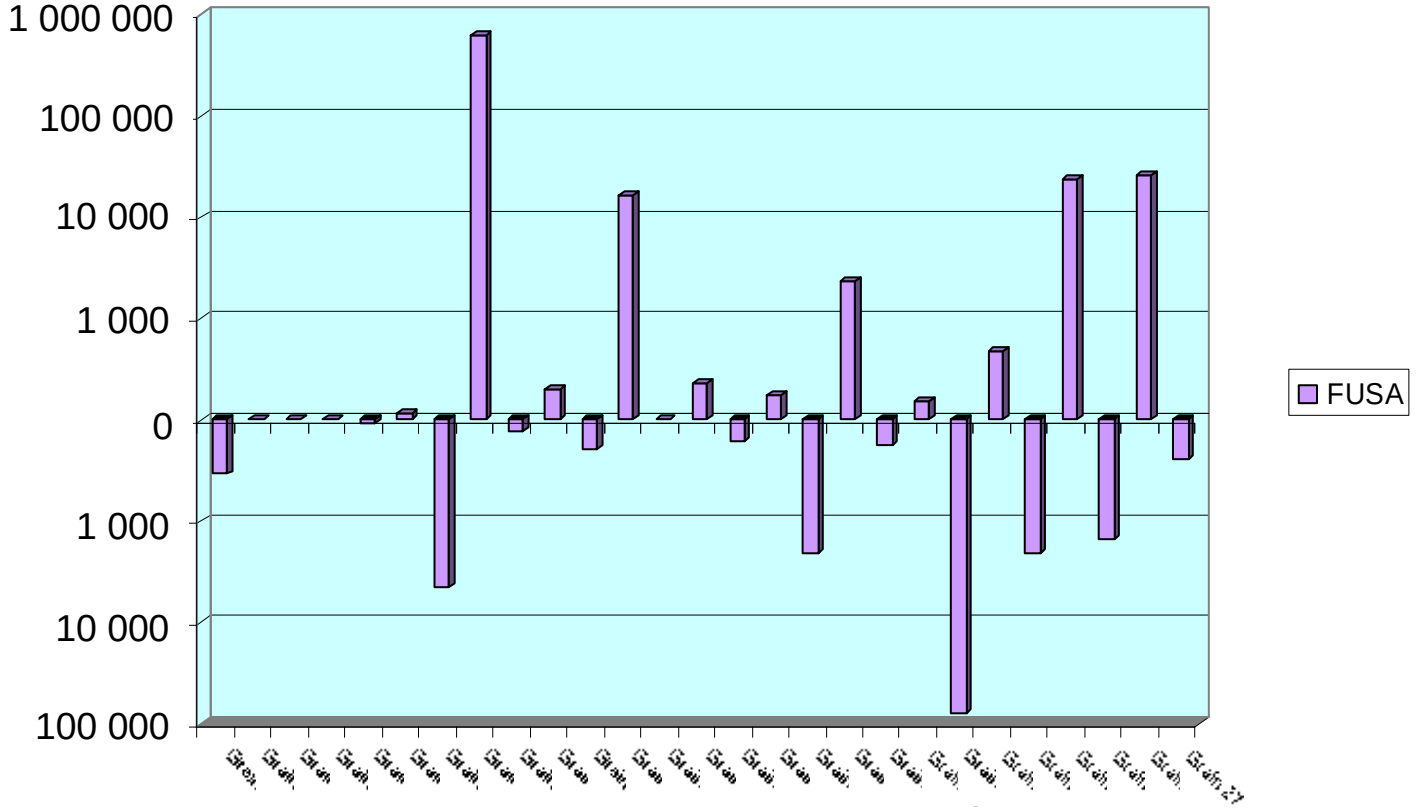


- [T2] > [H-T2]
- Optimal conditions for production : 28°C ; Aw 0.997
- No production at 5 and 35°C

LGC Toulouse

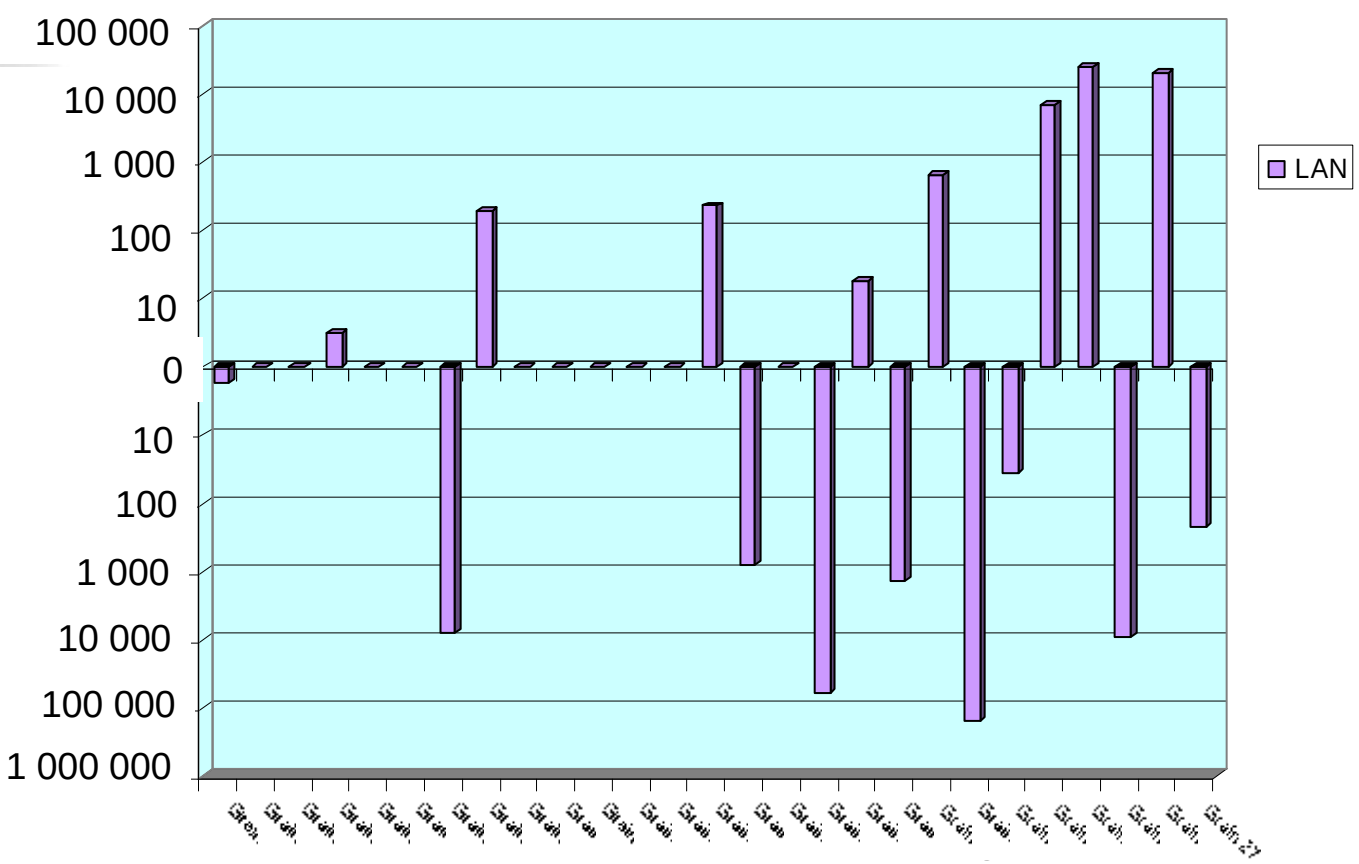
Symptom infection

Fusarium **genome amount /1000 barley genomes**



Symptom infection

***F. langsethiae* genome amour /2500 barley genomes**



Symptom infection

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⇨ **Other Fusarium species**

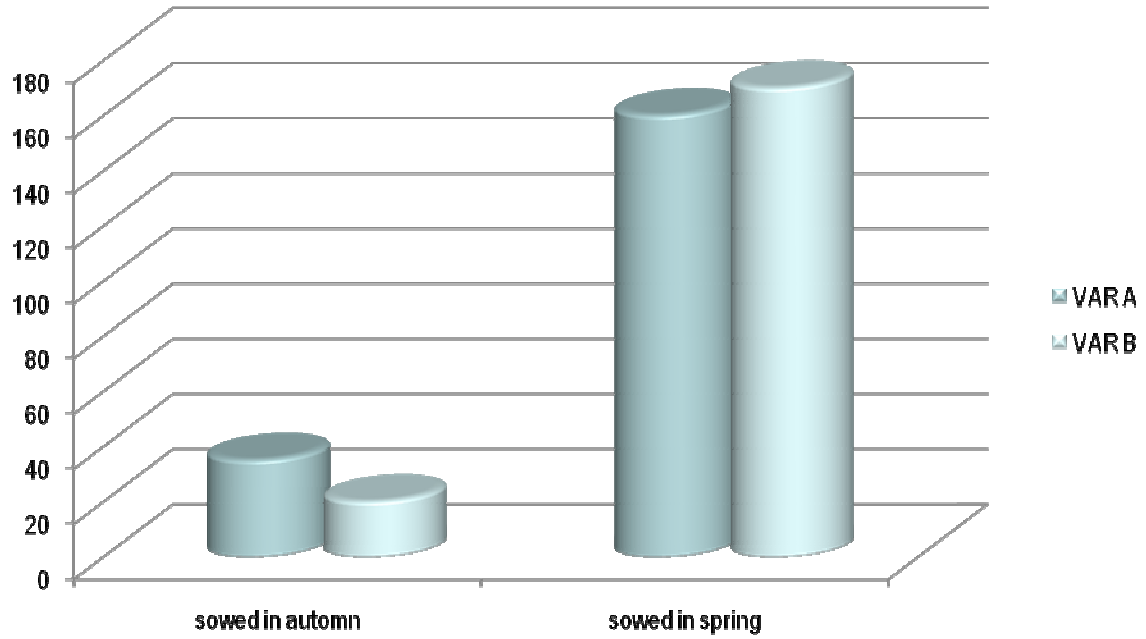
FIELD EXPERIMENTS

Influence of sowing date

YEAR 2007

Spring Barley : same site, different sowing date (autumn and spring)

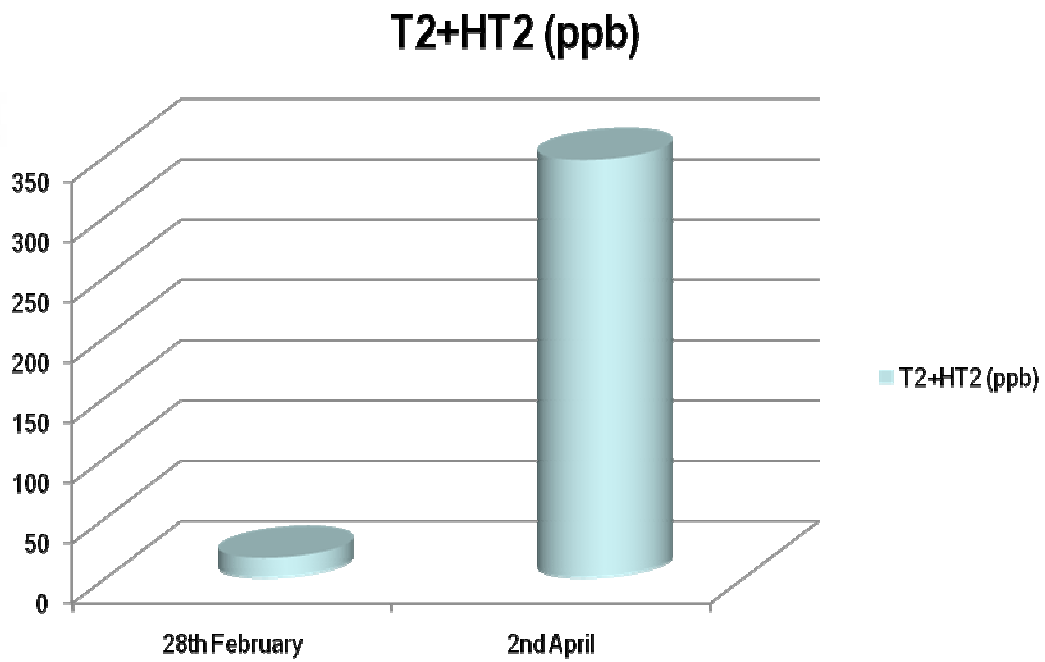
T2+HT2 (µg/kg)



Spring Barley: the sowing date is important

Influence of sowing date

HARVEST 2008 **Spring Barley**
same variety, same site, different date (same year)



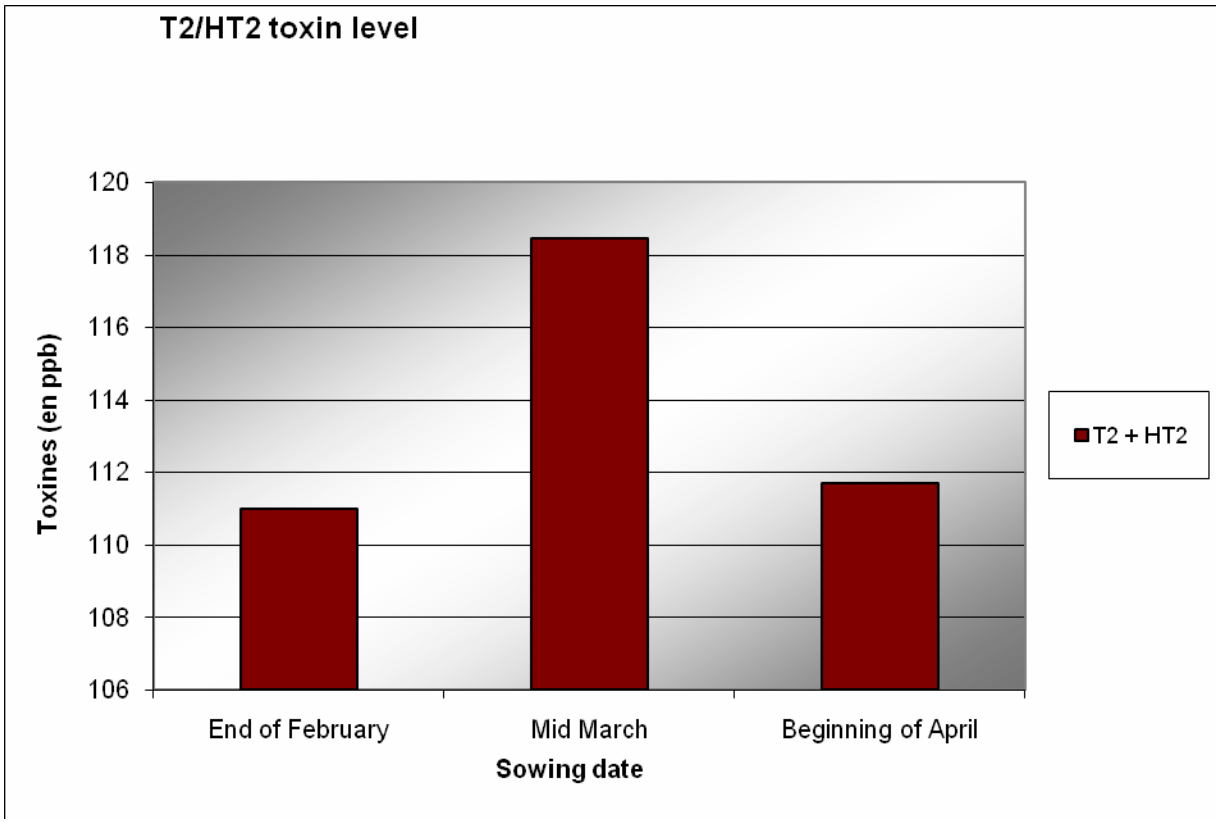
The later the sowing takes place, the higher the contamination

Sowing date trial

- **3 sowing dates of a Spring Barley (Tipple):**
  - End of february/ Mid March / beginning of April
- **3 repetitions**

- No difference observed for T2 + HT2 level

- Climatic influence



MALTING PROCESS

Calibration

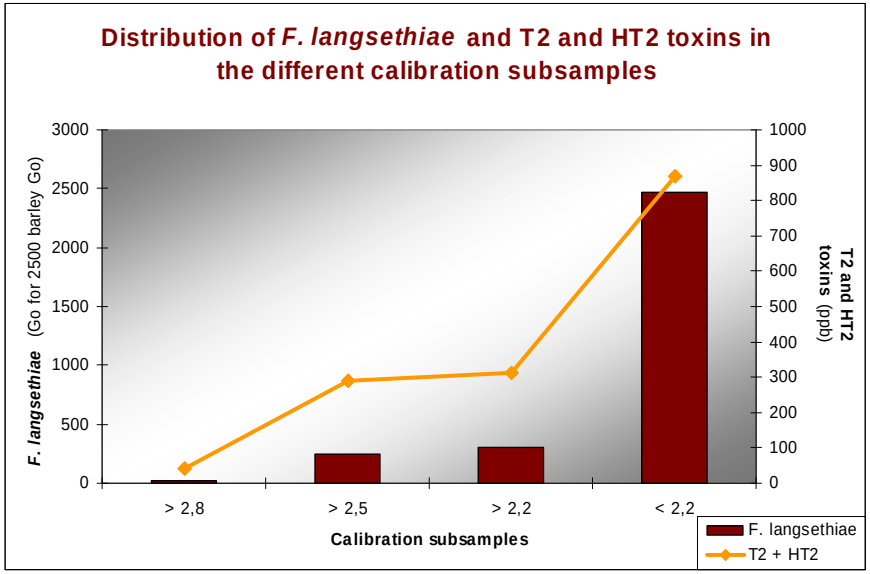
T2+HT2 toxin (µg/kg)

Barley	>2.8	2.8-2.5	2.2-2.5	<2.2
HT2	74	136	1103	5000
T2	26	61	379	917
HT2+T2	100	197	1482	5917

Calibration: impact for the malting industry

➢ 1st step = Elimination during harvest : smallest grains including those presenting *Fusarium langsethiae* symptoms are not recovered and a great part remains in the field

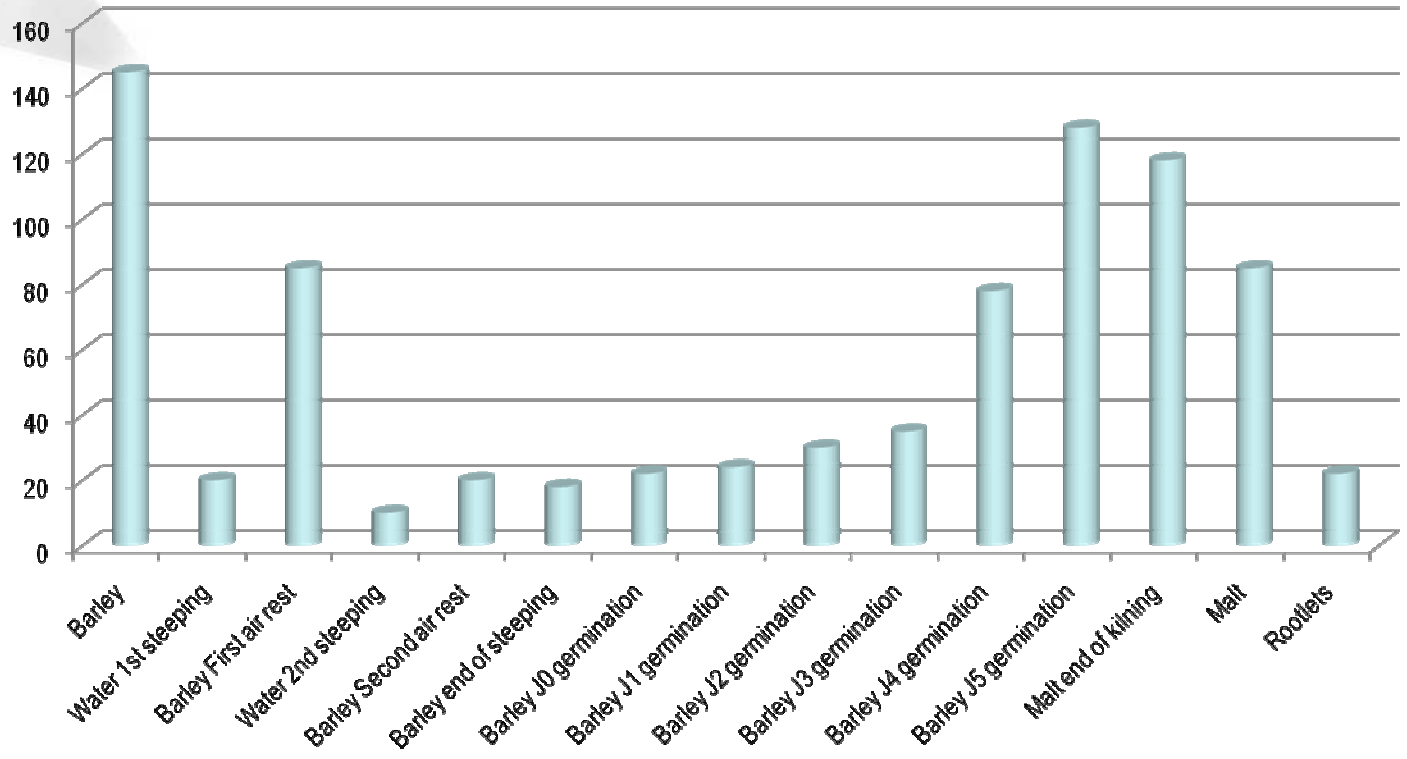
➢2nd step = Elimination due to calibration : according to *Fusarium langsethiae* symptom described above, the most contaminated fraction for both type A trichothecenes and *Fusarium langsethiae* is the smallest fraction < 2,2. Calibrating over 2,8 allows a maximal elimination of both mycotoxins and fungi.



T2+HT2 toxins are more concentrated in the smallest fraction (dust, small kernels, broken kernels…)

Malting process

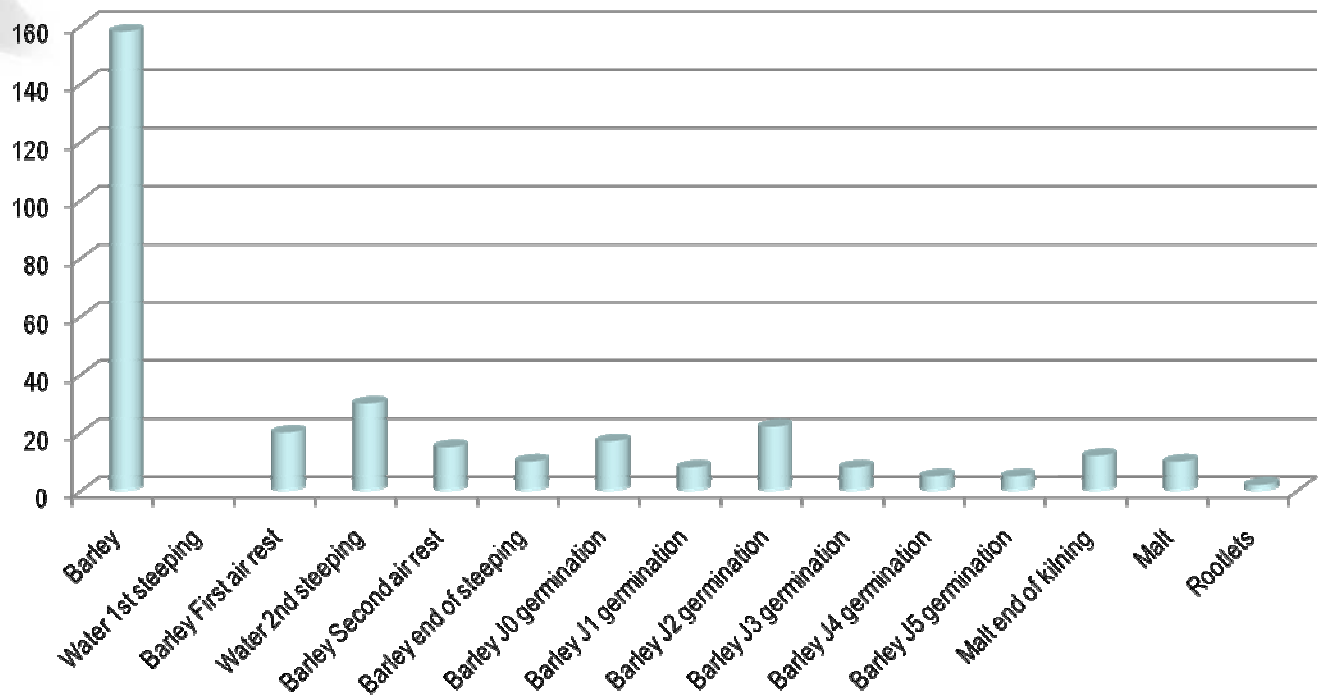
T2+HT2 (µg/kg) follow up in a 2008 sample malted in November 2008



Reaccumulation of T2+HT2 toxins during the germination steps

Malting process

T2+HT2 (µg/kg) follow up in a 2008 sample malted in March 2009



No re-accumulation of T2+HT2 toxins during the germination steps

F. Langsethiae did not survive during storage

General conclusion

- The *Fusarium langsethiae* and T2/HT2 toxin contaminations are still present in 2009
- *Fusarium sporotrichioides*, high T2/HT2 producer, has appeared in 2009 in French Malting Barley
- Harvest calibration, barley calibration prior to malting process and steeping step during malting process allow an consequent elimination of the toxins

General conclusion

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⇨ Thanks for your attention