

« 6th Fusarium-toxin Forum »
Brussels, 9-10 February 2009

T-2 and HT-2 toxins in cereals in France

Bruno BARRIER-GUILLOT
INTERCEREALES
COPA-COGECA


b.barrier@arvalisinstitutduvegetal.fr

COPA-COGECA, INTERCEREALES

COPA-COGECA: the voice of European farmers and co-ops since 1958 - 76 Member Organisations - 15 million farmers - 40.000 co-ops

INTERCEREALES: professional organization of the cereal sector in France

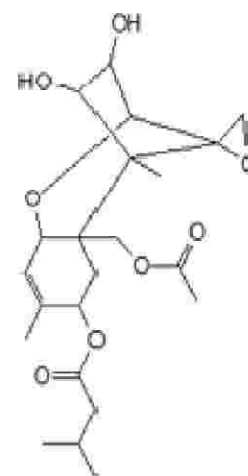
- Production
- Collectors (coops, merchants)
- International trade
- First processing industries (food and feed)

- 
- ① Toxicity of T-2 & HT-2
 - ② Estimated dietary exposure
 - ③ Questions on T-2 & HT-2 and first answers
 - Q1: levels found in cereals
 - Q2: correlations with other *Fusarium*-toxins
 - Q3/Q4/Q5: factors involved in formation of T-2 & HT-2 toxins / sources of variation / agricultural practices / possibilities to mitigate the risk
 - Q8: screening and confirmatory methods of analysis + sampling

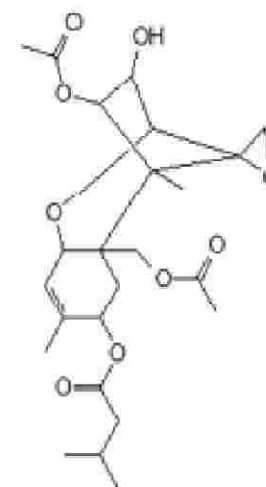
① Toxicity of T-2 and HT-2 toxins

- From the group of trichothecenes A
- Produced by *Fusarium langsethiae*, a newly identified species from *Sporotrichiella* section.
- LOAEL = 0.029 µg/kg-bw/d based on one experiment in pigs
- Safety factor = 10 x 10 x 5 due to less data available
- t-TDI = 0.06 µg/kg-bw/d

HT 2 Toxin



T 2 Toxin



SCF opinion, 30 May 2001

Toxicity of T-2 and HT-2 toxins

- « *The toxicity of T-2 toxin in pigs in only one short-term study is used as the basis for the safety assessment... Similar effects were not observed in other studies in pigs neither at this nor even at higher doses... »*
- « *...There are deficiencies on metabolism and toxicokinetic studies. To account for this and the use of a LOAEL, an extra uncertainty factor of 5 was included, giving an overall uncertainty factor of 500... »*
- « *...The toxicity induced by T-2 & HT-2 toxin should be investigated... »*

SCF Opinion, 30 may 2001

- ➔ **Necessity to have a recent, robust and scientific safety assessment on T-2 & HT-2**
- ➔ **Less toxicity of HT-2 toxin vs T-2 toxin ???**

② Dietary exposure

- « ...For T-2 and HT-2 toxins, the estimated dietary intake exceed in most cases the t-TDI. However, most occurrence data are obtained by making use of methods of analysis with high limit of detection... The dietary intake is strongly influenced by the limit of detection of the used analytical methods...»

856/2005 and 1881/2006 Regulations

⇒ Problems with scoop task 3.2.10 report (2003)

- Probably over-estimation of calculated exposure to T-2 & HT-2 toxins
- The lower the LoD, the lower the calculated exposure !

⇒ Necessity to have a recent dietary exposure study with low LoD

2006-2010: Second French Total Diet Study (TDS)

New

Aim: to get an accurate and realistic view of exposure to contaminants for the purposes of quantitative risk assessment

Method: Study based on a standardized methodology recommended by WHO; 20,280 different food products purchased in 2007-2008, making up 1,352 composite samples of core foods to be analyzed

Analysis: several contaminants including mycotoxins, Exposure of the population assessed by crossing Contamination data and consumption data from the INCA2 national survey (2006-2007)

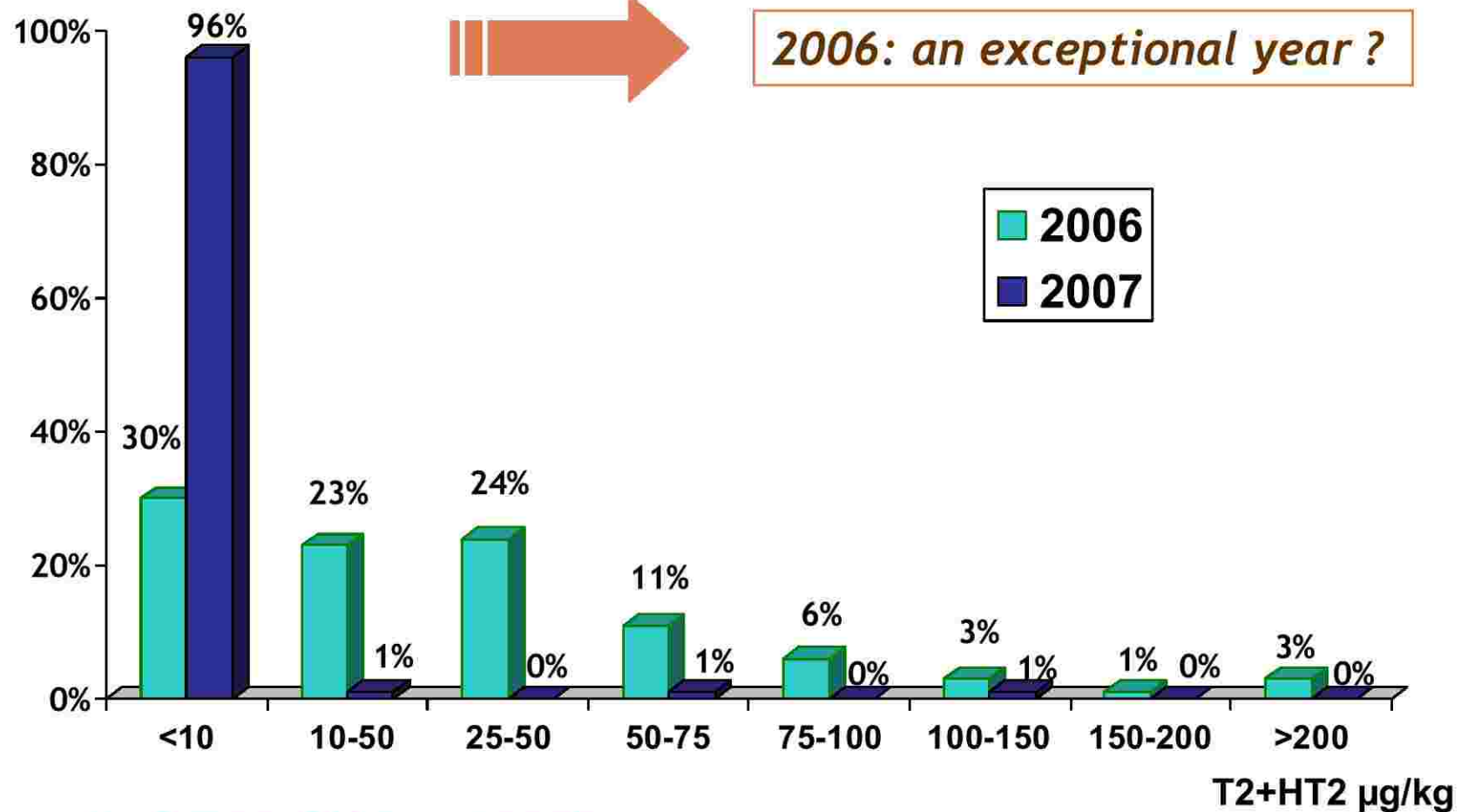
Results: 2009-2010

AFSSA

③ Q1: Occurrence found in cereals

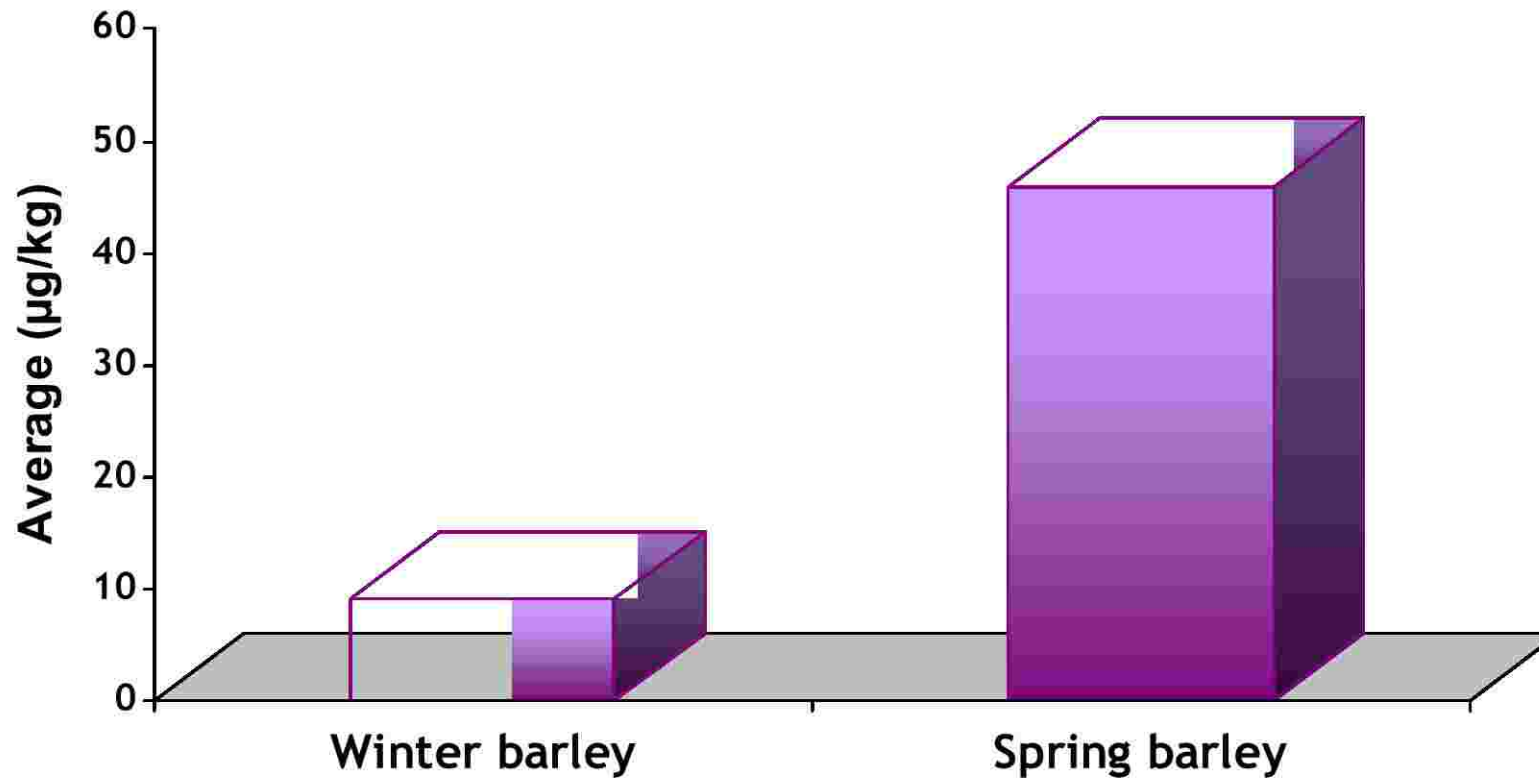
- Wheat and durum wheat seem to accumulate low T-2 and HT-2 toxins, but sporadic high levels can occur
- T-2 and HT-2 toxins levels in maize are uncertain (2006: an exceptional climate ?)
- Spring barley and oats are more susceptible and must be carefully examined

T-2 and HT-2 in maize



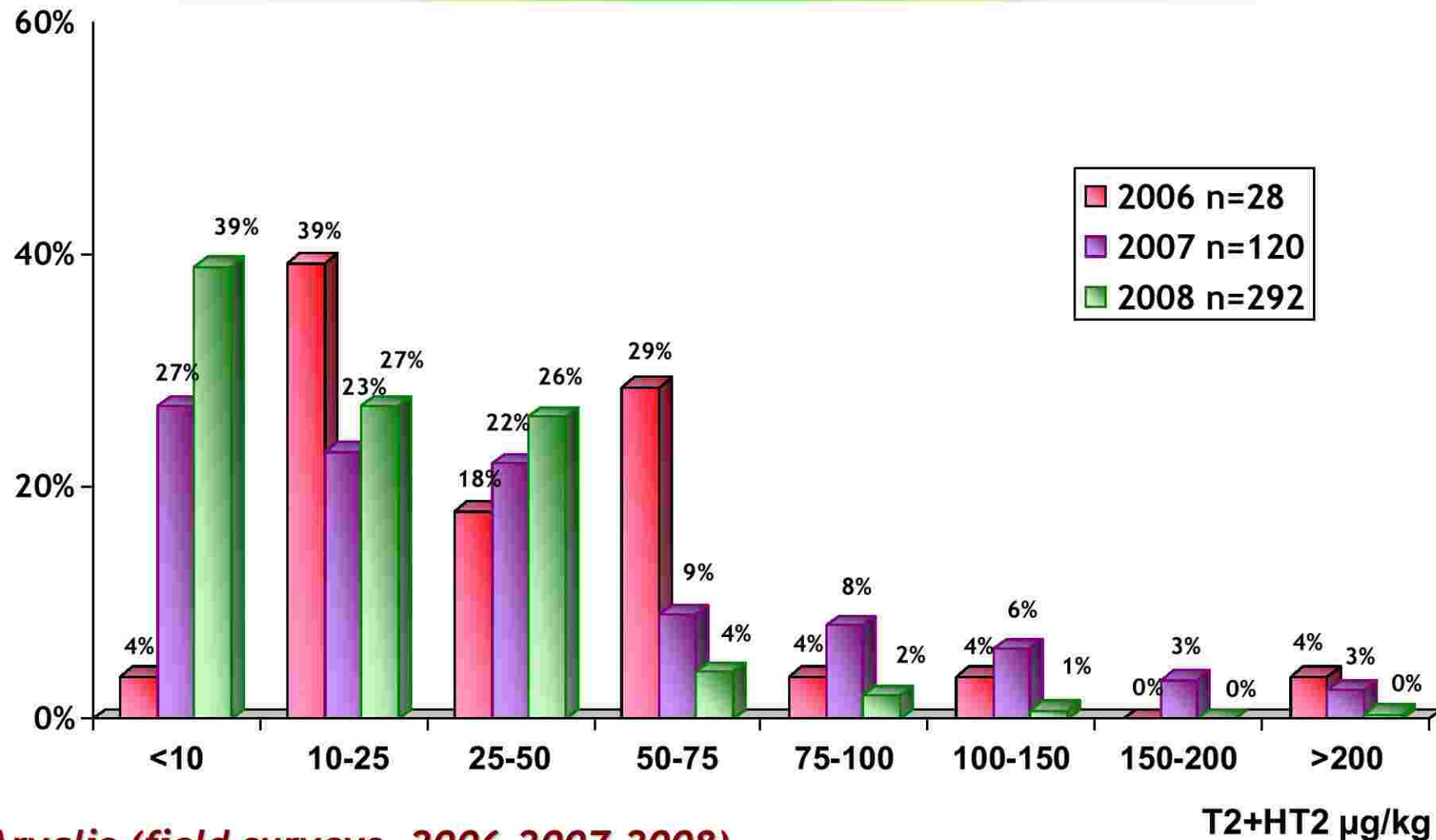
Arvalis-ONIGC (2006 and 2007 surveys)

T-2 and HT-2 toxins in barley



Arvalis (field surveys, 2006 and 2007)

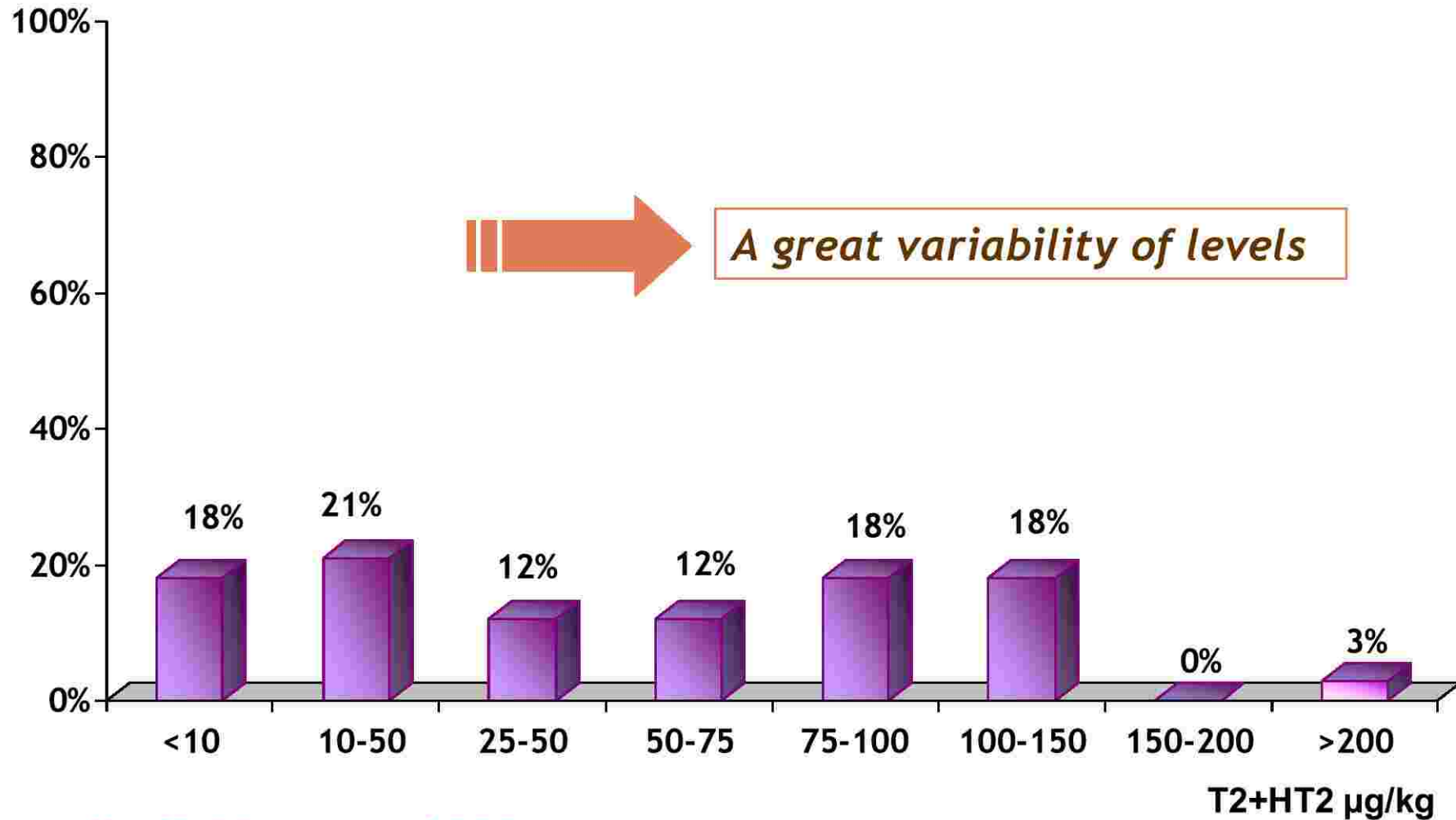
T-2 and HT-2 toxins in spring barley



Arvalis (field surveys, 2006,2007,2008)

New

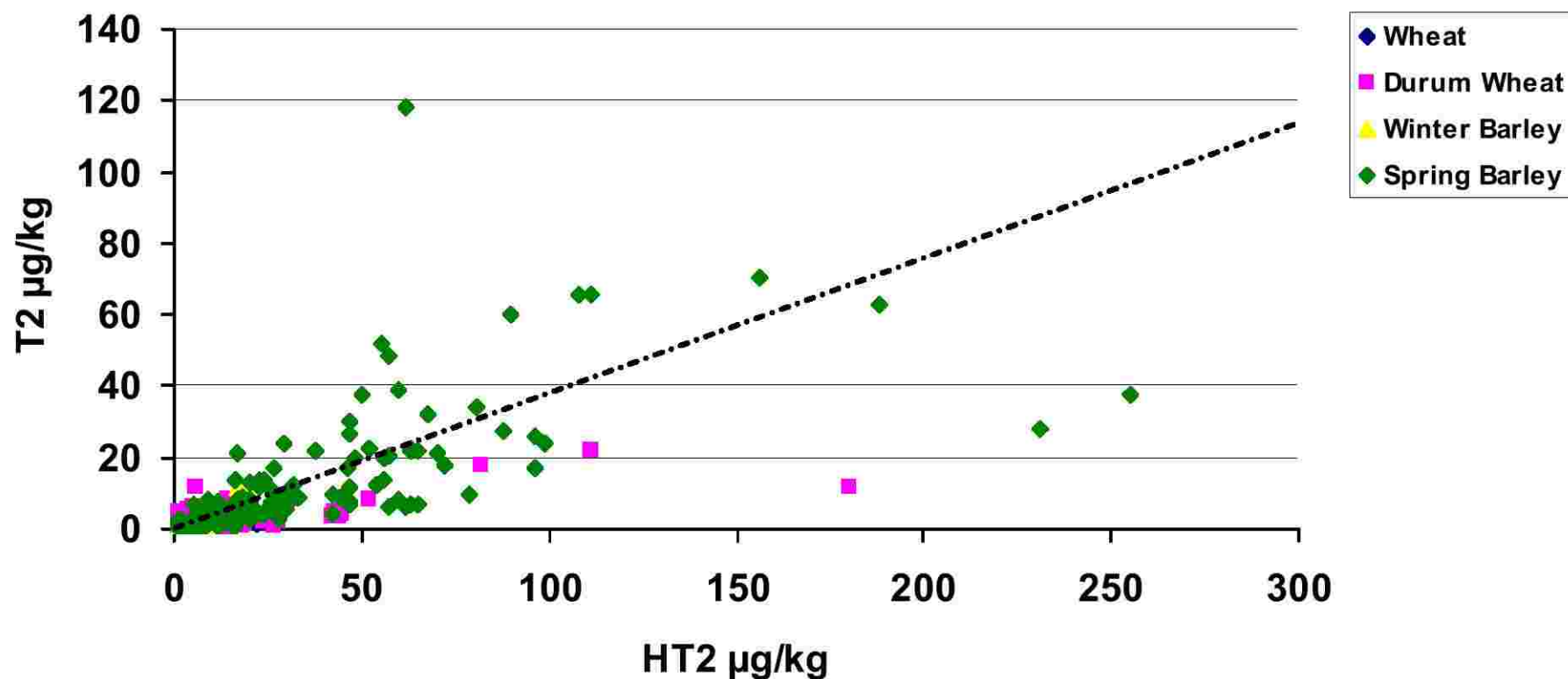
T-2 and HT-2 toxins in oats



Arvalis (field surveys, 2008)

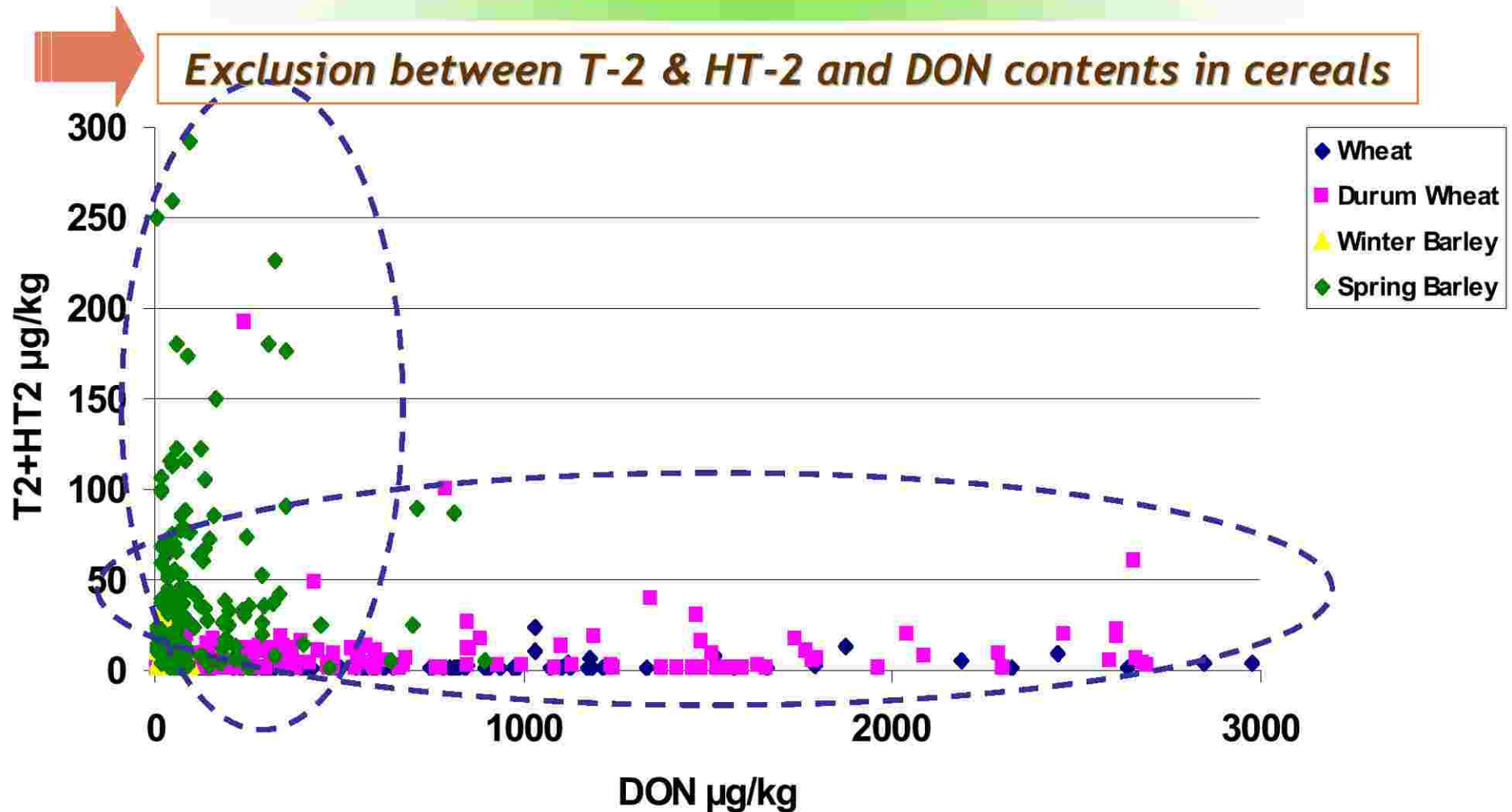
Relationship between T-2 and HT-2 toxins in cereals

~ 2.5 times more HT-2 toxin than T-2 toxin



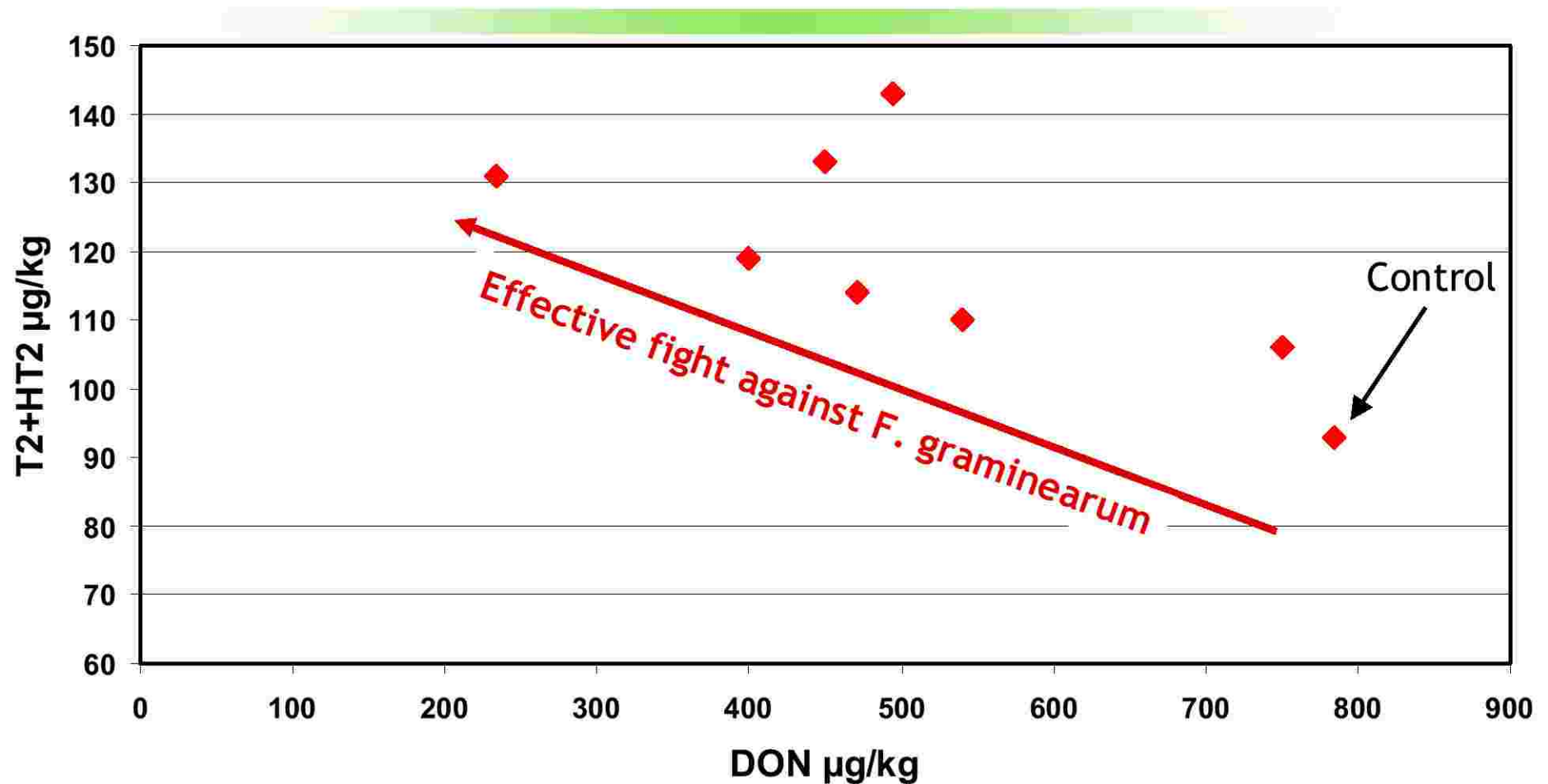
Arvalis (field surveys, 2006 and 2007)

Q2: Correlation with other Fusarium-toxins



Arvalis (wheat and barley field surveys)

Experimental confirmation on durum wheat



Fungicide trial on durum wheat, Léméré

Arvalis 2008 (CT durum wheat Centre-Ile de France)

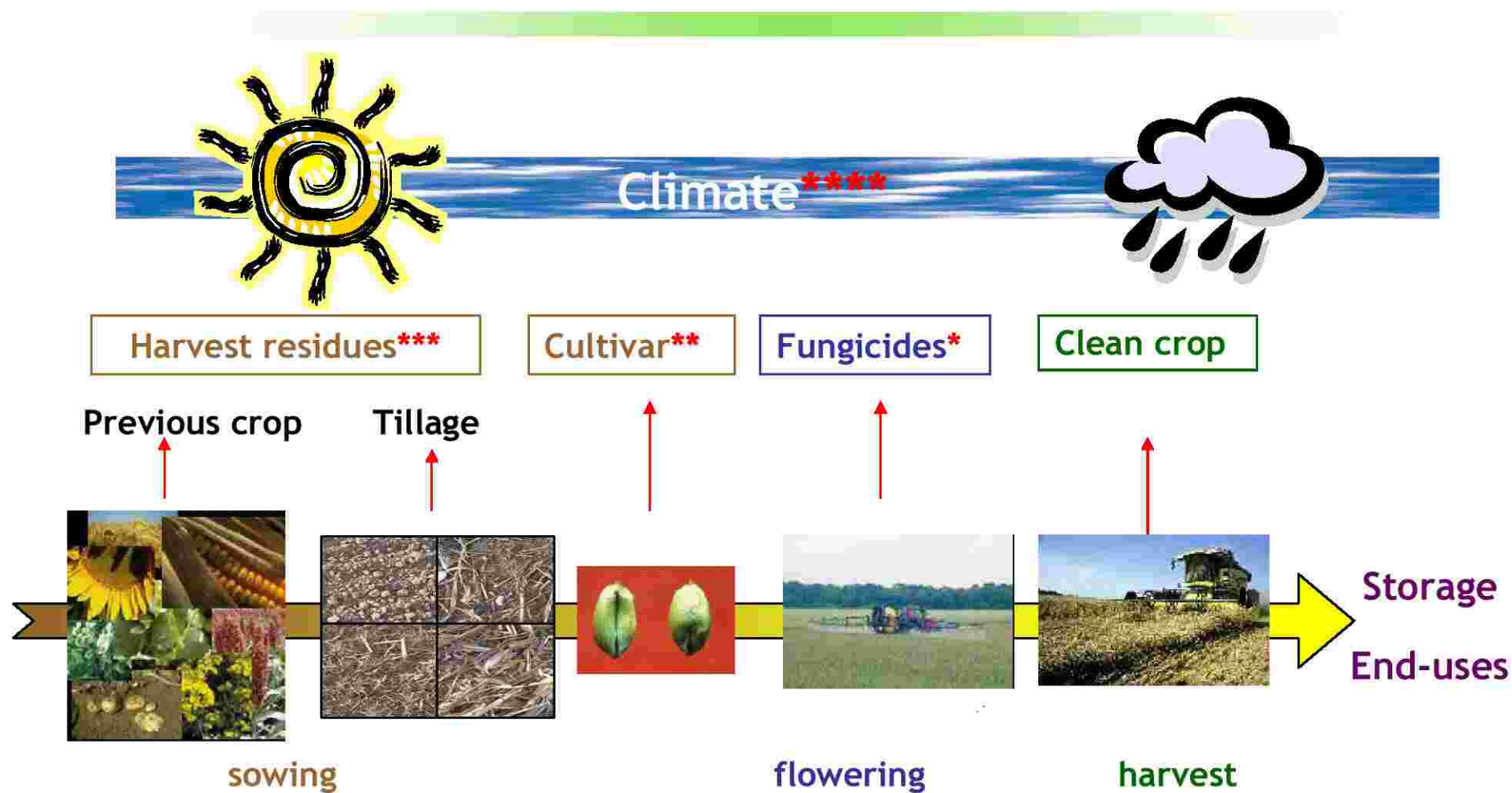
Why an apparent opposition between T-2 & HT-2 toxin and DON levels ?

- Competition between different *Fusarium* species ?
- Different optimal conditions of growth for the different species of *Fusarium* producing T-2 & HT-2 or DON ?
- Spore release (and head blight contamination) later for species producing T-2 & HT-2 (than for DON), and more in phase with the period of susceptibility to infection of spring barleys ?

Q3/Q4/Q5 Factors involved ? Agricultural practices ?

- A network of field surveys in France undertaken by Arvalis since 2006 on spring barleys
- Characterize the Fusarium-toxin contents (DON, T2 & HT2) of barley in collaboration with agrosupply distributors (coops and merchants)
- Analyse the situation of each region
- Identify involved factors, weigh the importance of these factors and study their interactions
- Improve knowledge, identify the actions of prevention and disseminate good practices

DON contamination in wheat is plurifactorial

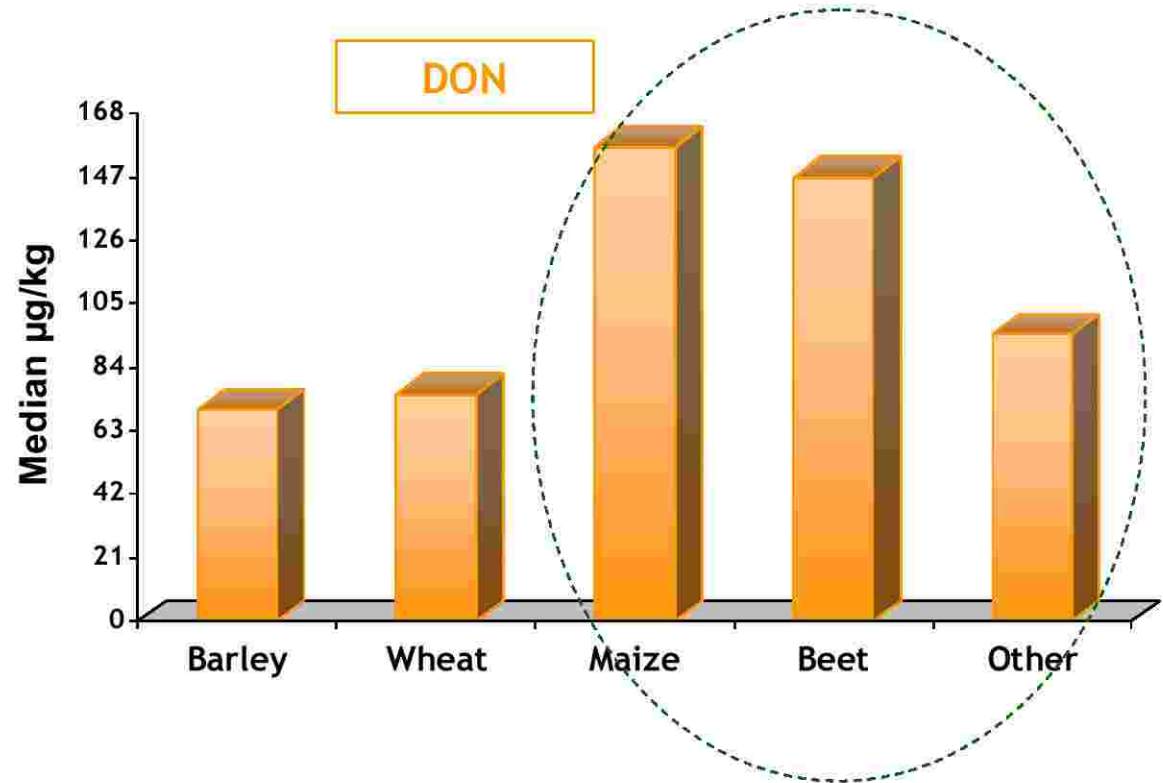
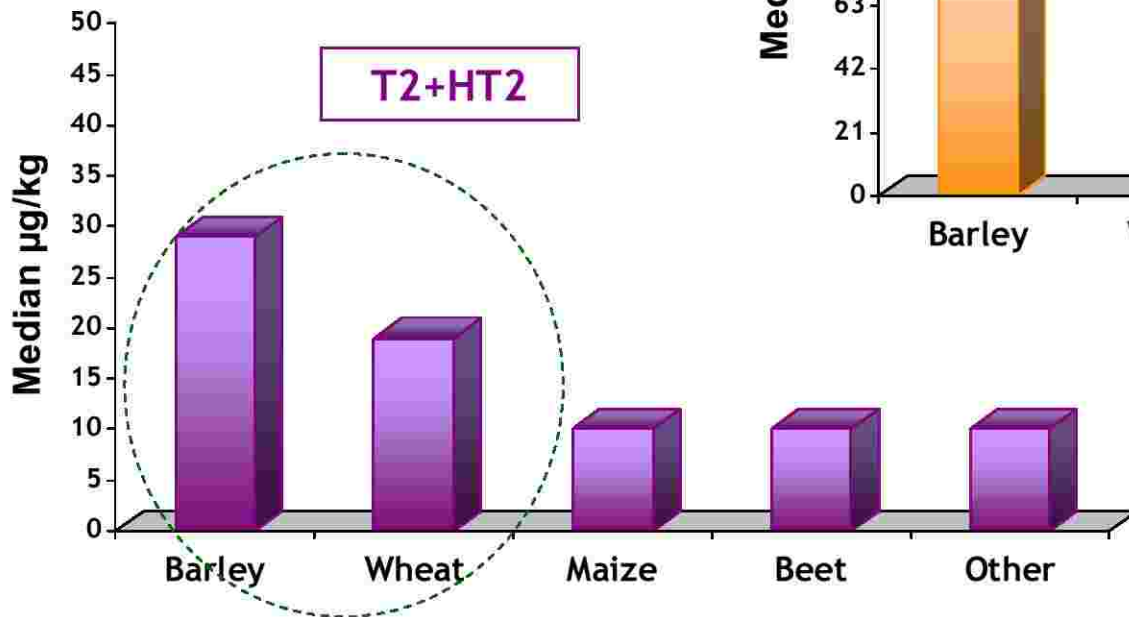


Factors : from the highest (****) to the lowest (*) of importance

ARVALIS-Institut du végétal, 2005

T-2 & HT-2 contents in spring barley according to previous crop

Difference with DON !
 Small grain cereals as a previous crop lead to higher T-2 & HT-2 contents in following spring barley

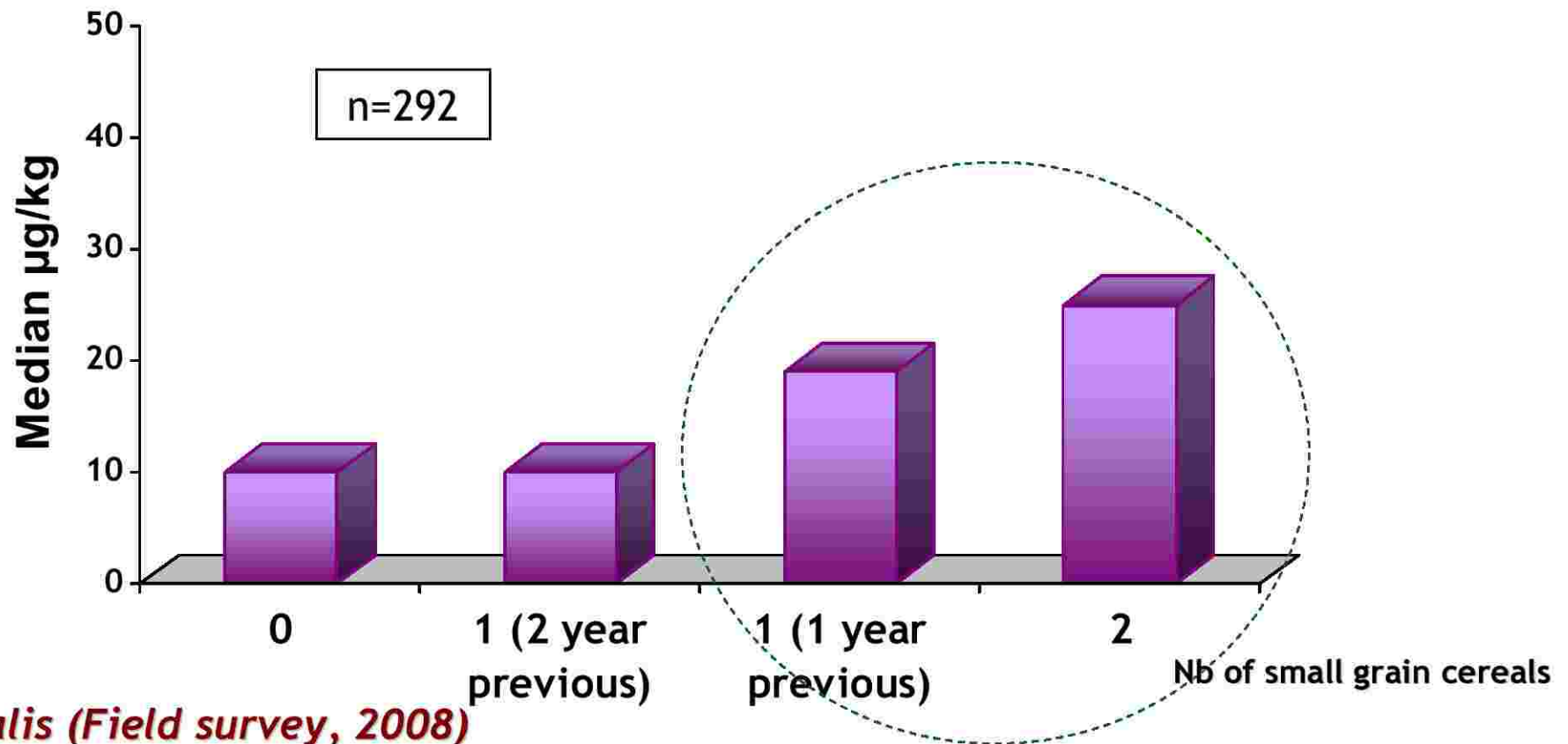


n=292

Arvalis (Field survey, 2008)

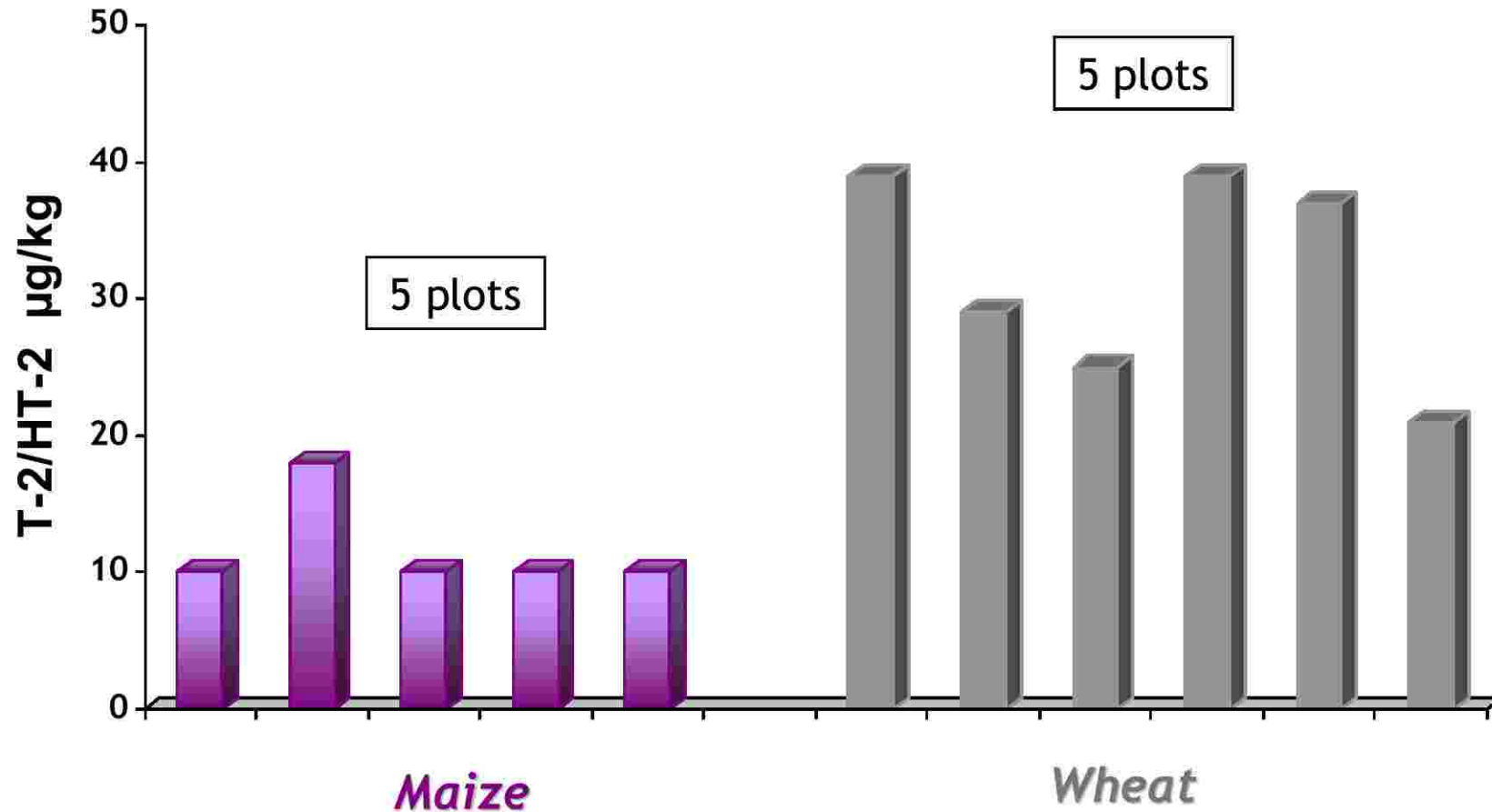
T-2 & HT-2 contents in spring barley according to previous crops

T-2 and HT-2 contents increase with the number of small grain cereals during the two previous years



Arvalis (Field survey, 2008)

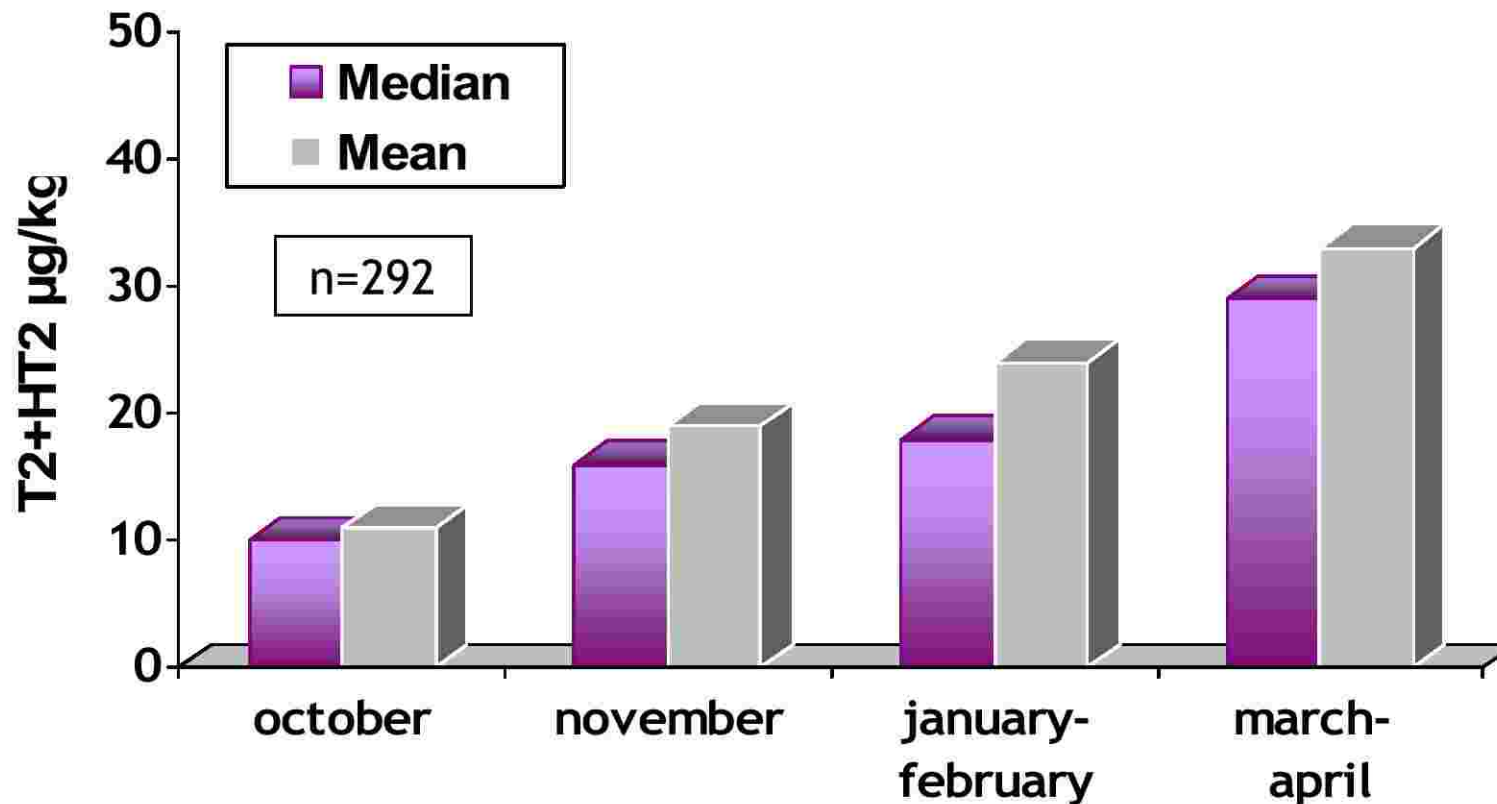
Experimental confirmation in wheat T-2 & HT-2 contents according to previous crop



Arvalis (Boigneville tillage system trial, 2008)

T-2 & HT-2 contents in spring barley according to date of sowing

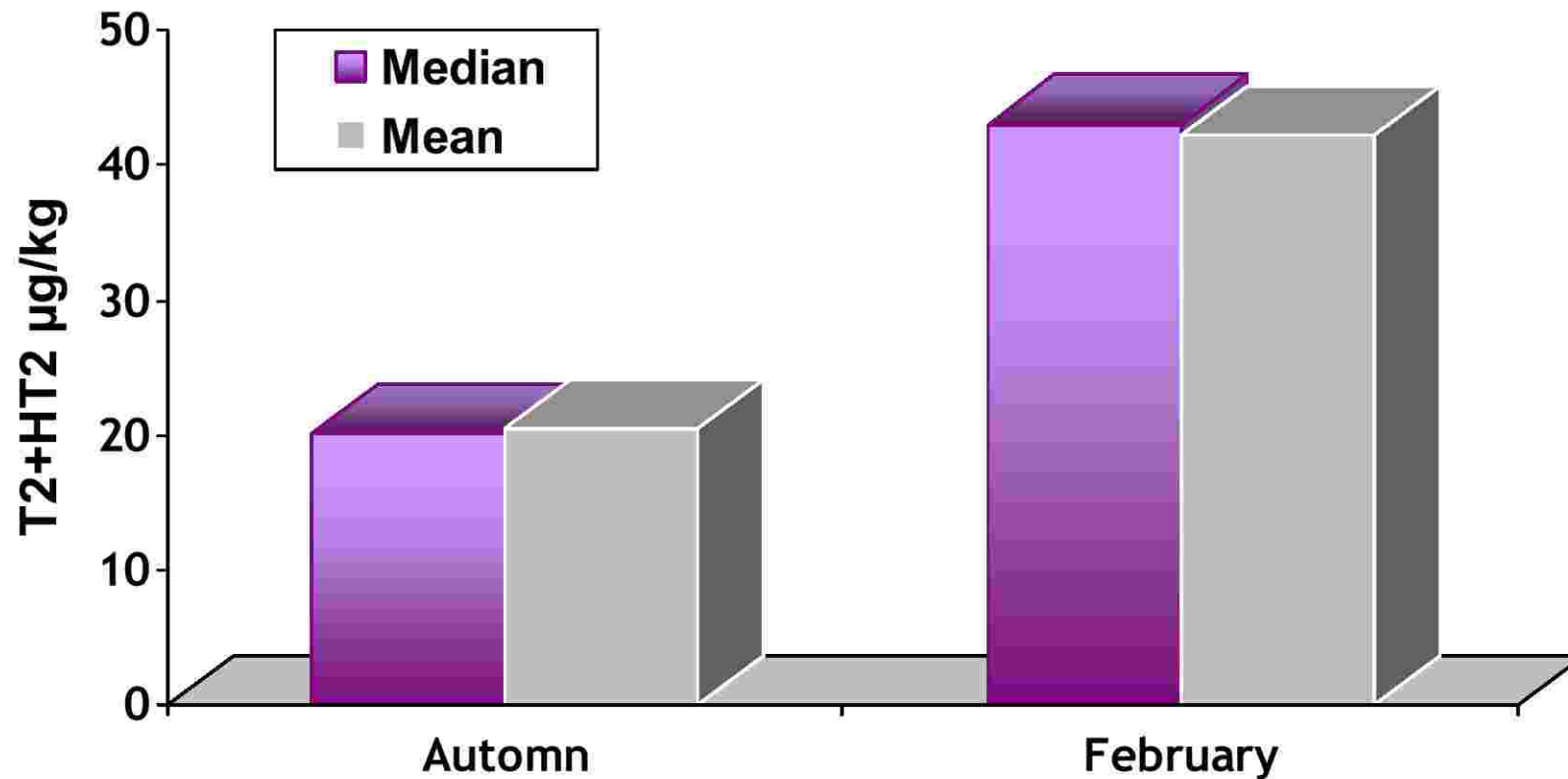
The later the date of sowing the higher the T-2 and HT-2 toxin levels



Arvalis (Field survey, 2008)

T-2 & HT-2 contents in spring barley according to date of sowing

2 times less T-2 & HT-2 toxin levels with autumn sowing



6 cultivars x 2 dates of sowing

Arvalis 2008 (Ouzouer trial)

What about cultivar and fungicide effects ?

- Problem : inability to inoculate cereals with *F. Langsethiae* producing T-2 and HT-2 toxins to mimic natural infection!
- Necessity of improving knowledge
 - On the biology of the *Fusarium langsethiae*
 - On competition between the different *Fusarium* (TCT B vs TCT A producers)
- Only after it will be possible to evaluate the cultivar susceptibility to accumulate T-2 & HT-2 and the fungicide effect

Q3/Q4/Q5 Factors involved ? Agricultural practices ?

- ⇒ What is known about DON prevention seems to be not applicable for T-2 & HT-2 toxins...
- ⇒ Commission recommendation on 17 august 2006 (2006/583/EC) on the prevention and reduction of *Fusarium* toxins in cereals is not suited for T-2 and HT-2 toxins...

Q8: Screening and confirmatory methods of analysis

● Reference analyses

- Complex analysis, very expensive equipment, expensive analyses
- Question of LoD
- Not appropriate for the screening of batches
- Developed methods : LC/MS/MS - GC/MS - HPLC/Fluorescence

New

Improvement of analytical methods for the determination of T-2 and HT-2 by LC/MS/MS on cereals

Project managers : IRTAC / Arvalis / Capinov

Partners : ≈ 10 laboratories

Beginning of the study : February / March 2009 (for 30 months)

Aims :

- Studies and improvement of critical points
- Ring Test
- Project of a method for European normalization

Q8: Screening and confirmatory methods of analysis

- Rapid detection methods
 - Necessity to have rapid, accurate and cheap methods for a first screening of batches
 - **Focus on ELISA Test KITS :**
 - ELISA test kits for T-2 are available
 - They must be evaluated (cross-reaction between T-2 and HT-2 toxins ?)
 - ELISA test kits for HT-2 ?
 - **Focus on lateral flow devices :**
 - The first tests for T-2 / HT-2 toxins have just been available
 - They must be evaluated

A research program 2008-2010: BARSAFE

New

Fusarium langsethiae, from barley culture to the finished products (beer) and by-products: study of













- The biology and the epidemiology of the pathogen,
- The conditions of T-2 & HT-2 toxins production,
- Their transfer, biological breakdown and toxicity,

for a better sanitary risk management

**Partners: IFBM (coordinator), ARVALIS, INRA,
CNRS-INPL-ENSAIA**

with funding of French Research National Agency

Conclusion : a lack of knowledge at all stages

		  	
	Situation for T-2 &HT-2		DON
Dietary exposure (with updated LoD)	Not available		
Biology of <i>F. langsethiae</i>	Not available		-
Cultivar, fungicide evaluation	Not available		
Risk assessment matrix	Not available		
Sampling method	No data		
Normalized method of analysis	Not available	