

*5th EC Forum on Fusarium toxins.
Brussels, January 10-11th, 2008*

T-2, HT-2 and deoxynivalenol (DON) in malting barley and malt

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Outline of presentation

- What is Euromalt
- Summary of evidence presented to 4th Fusarium Forum
- Data from 2007
 - Occurrence of T-2 and HT-2 toxins in malting barley
 - Changes in incidence
 - T-2 and HT-2 toxins in commercial malts
- Factors affecting leaching of T-2 and HT-2 from barley during steeping
- Questions remaining

Euromalt



- Euromalt was established in 1959 and represents the interests of the EU malting industry
- Around 8.8 million tonnes of malt (42% of the world total) are produced annually in the EU
- Around one quarter of this is exported
- 94% of total malt production is used for beer brewing
- 4% is used for whisky distilling
- 2% is used in the food industry



- Euromalt has been surveying mycotoxins in European malting barleys and malts since 2002

Euromalt mycotoxin survey

- 100 - 200 samples per year in total, from all EU member states with significant malt production
- Number of samples per country is proportional to malt production
- 10kg samples are collected and analysed according to EU protocol (Directive 2002/26/EC)
- Samples are collected as pairs: a barley sample and the malt produced from that barley
- Samples analysed for a wide range of Fusarium toxins by validated GC-MS or LC-MS/MS methods



Summary of data presented at 4th Fusarium Forum

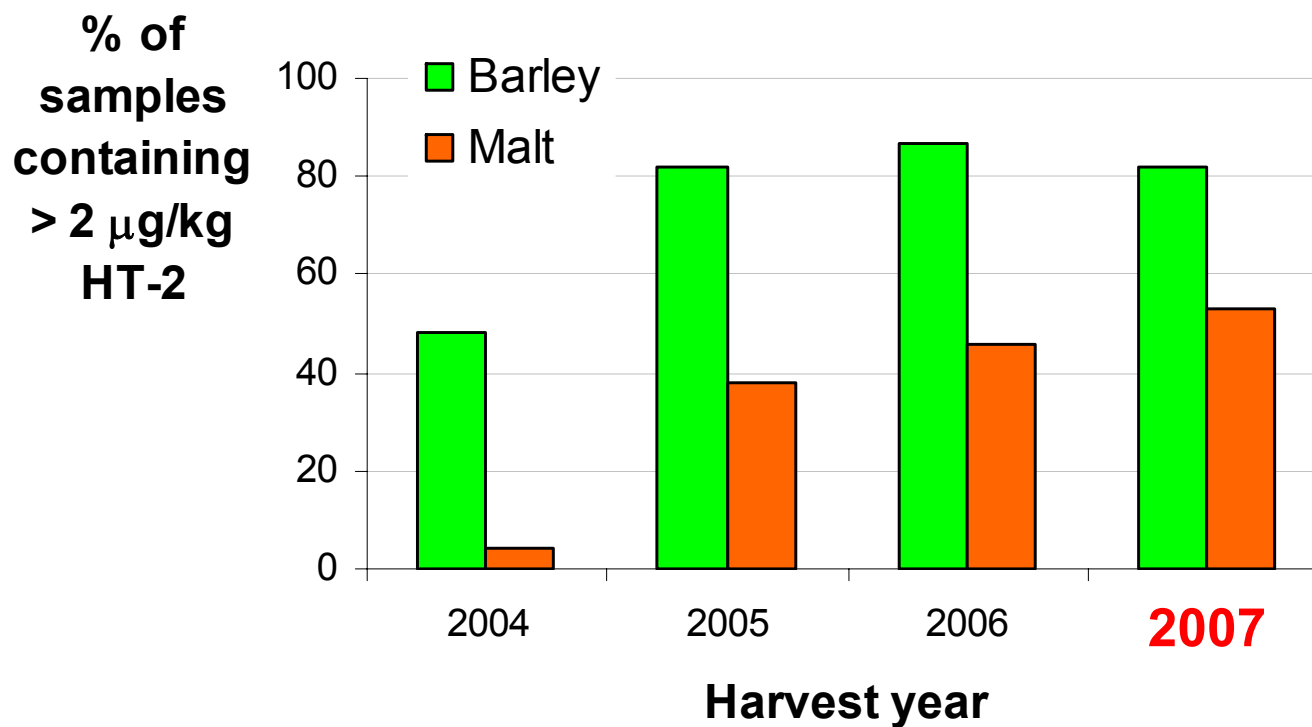
- Occurrence and concentrations of T-2 and HT-2 toxins in barley appear to be increasing across Europe
- Occurrence of toxins appears to be related to an increase in the incidence of *F langsethiae*
- Data from France data suggest that levels in Winter barley varieties may be lower than in Spring barleys
- Pilot malting studies suggest that T-2 and HT-2 can be leached out of barley during steeping
- Levels of T-2 and HT-2 in malt are almost always lower than in barley
- However, the relationship between levels in malt and those in barley is not constant

New data from 2007 Euromalt survey

Incidence of T-2 and HT-2 toxins in European malting barleys and barley malts:

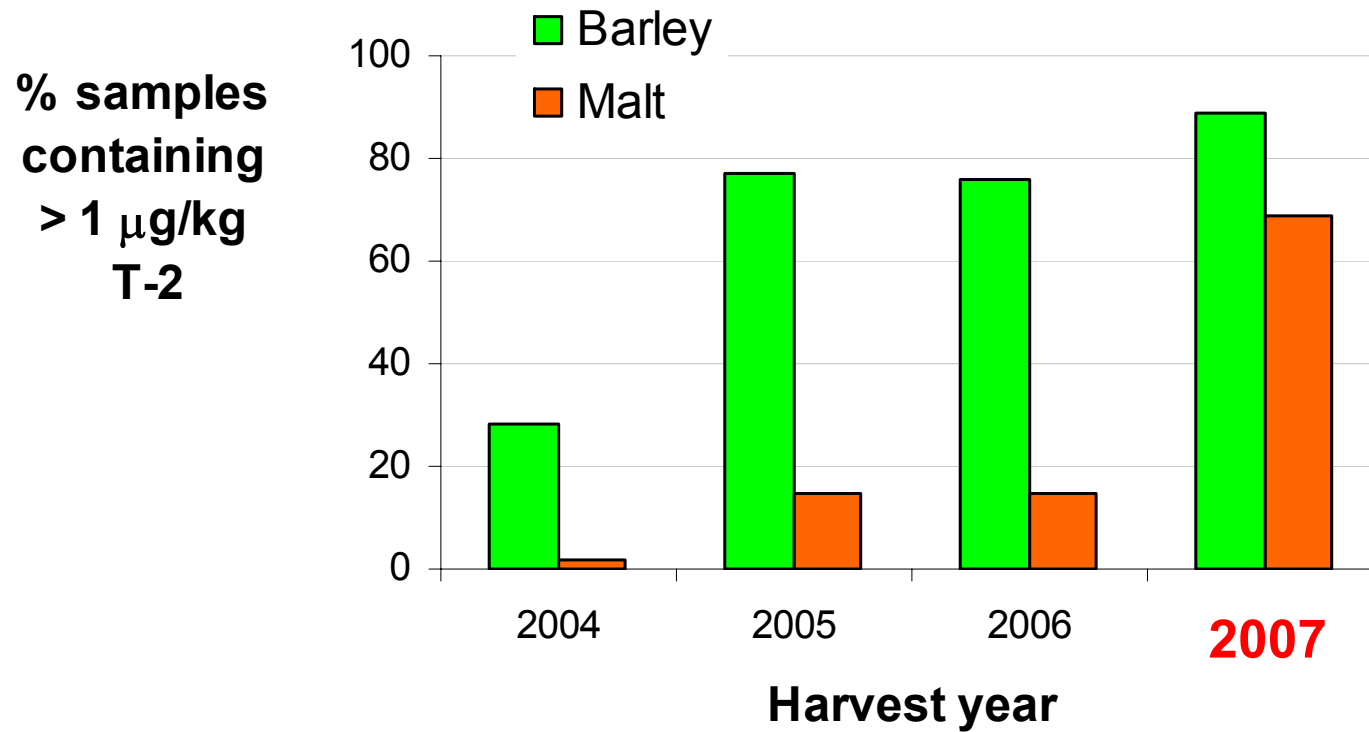
- Has the increased incidence observed in 2005 and 2006 been sustained?

Incidence of HT-2 Euromalt survey



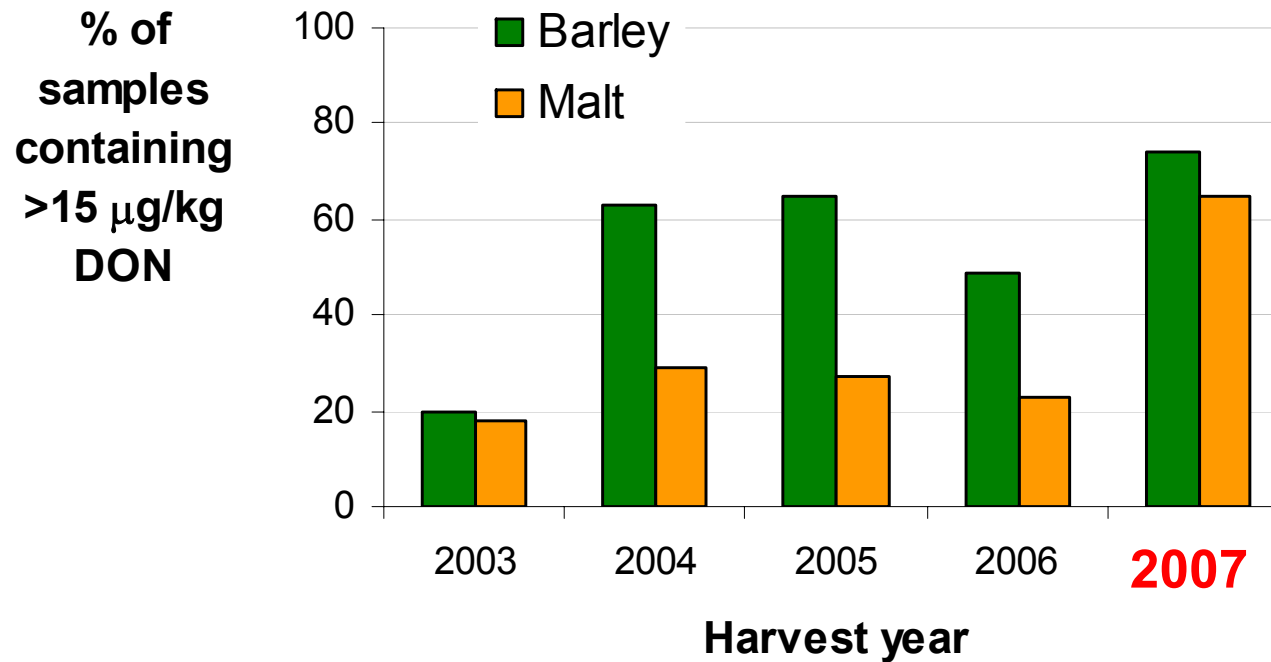
Data from 88 sample pairs

Incidence of T-2 Euromalt survey



***Increased incidence observed in 2005 and 2006 sustained in 2007;
(Data from 88 sample pairs)***

Incidence of DON Euromalt survey



***No sustained trend in incidence of DON.
Data from 88 sample pairs***

Incidence: summary of results

- Incidence of both T-2 and HT-2 in barley appears to have stabilised at about 80% of harvest samples
- Incidence in malt continues to rise
- Trends for T-2 and HT-2 are different from that of the related trichothecene DON

Concentrations of T-2 and HT-2 toxins :

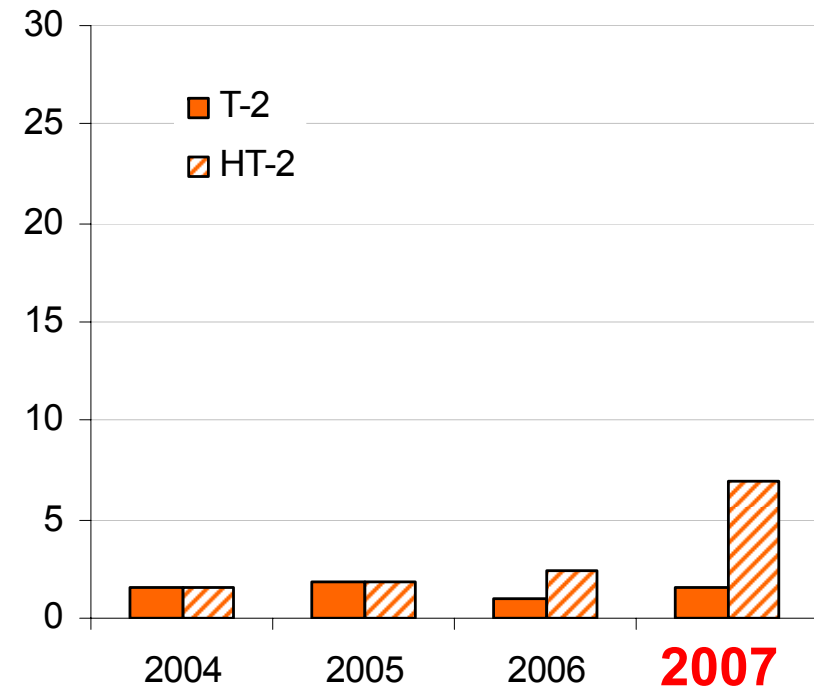
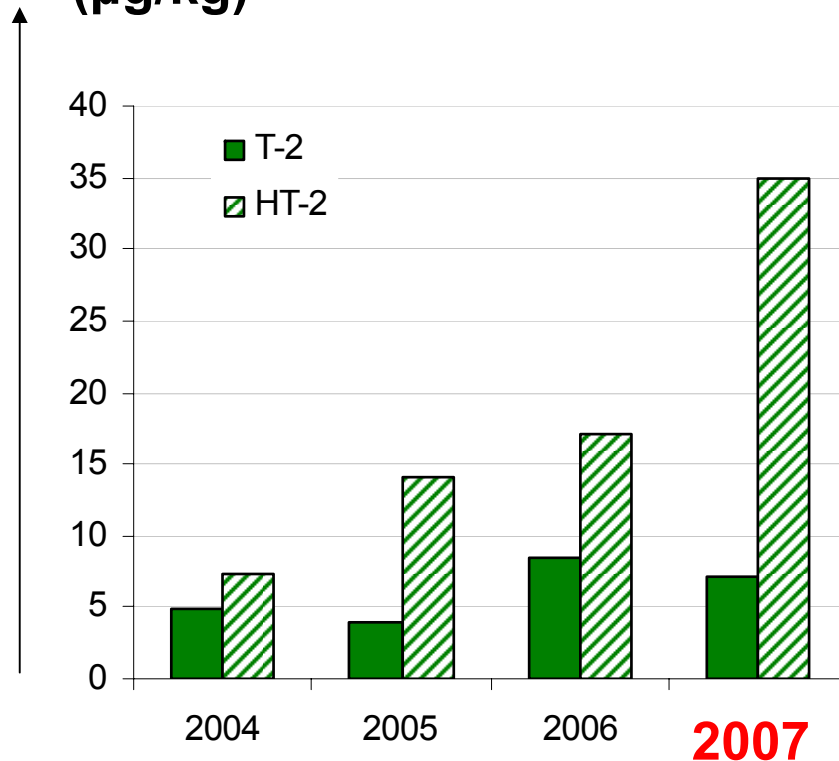
- Have these increased in 2007?
- Are levels in malts consistently lower than those in barley?
- Is there a difference between Winter and Spring varieties?

Mean concentrations of toxins

Mean concentration
($\mu\text{g}/\text{kg}$)

Barley

Malt



Harvest year

Mean concentration of HT-2 continues to rise. Data from 88 sample pairs

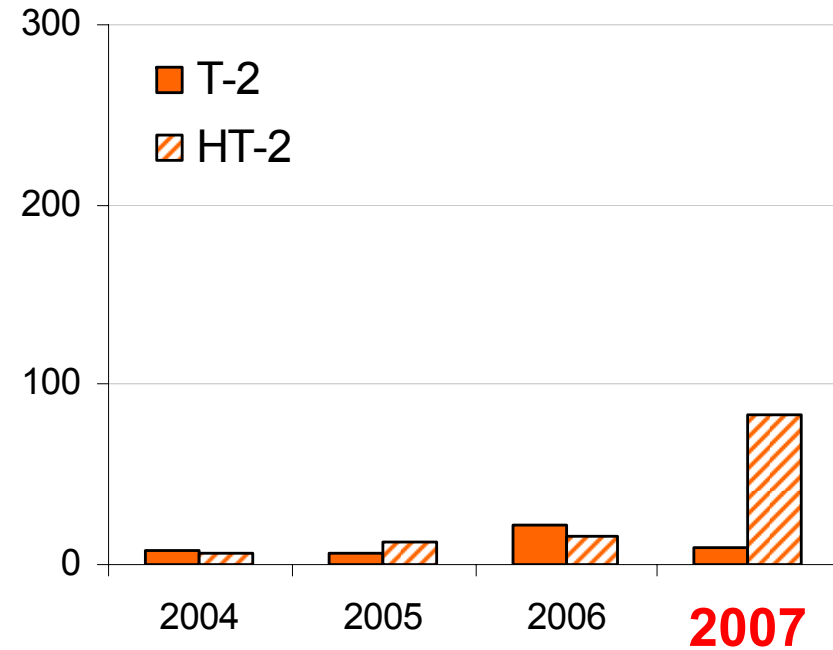
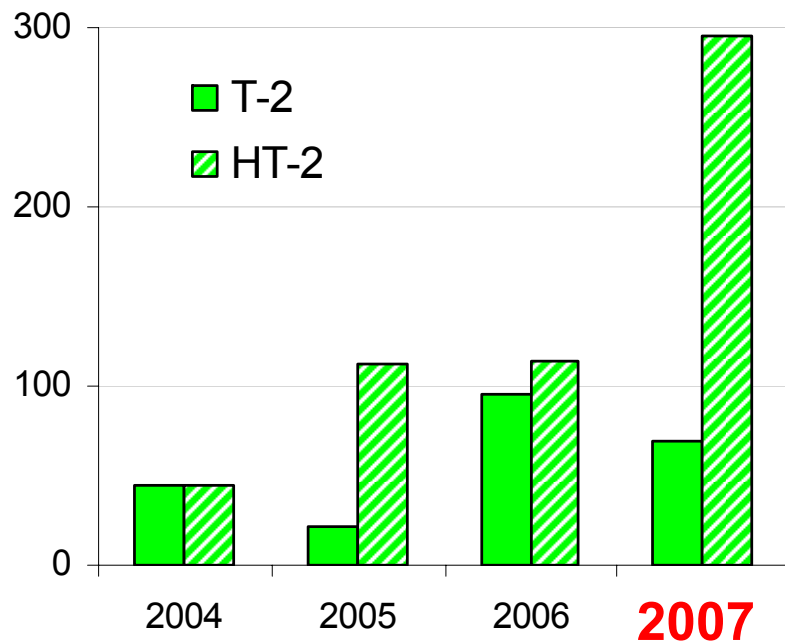
Maximum concentrations

Maximum concentration
($\mu\text{g}/\text{kg}$)

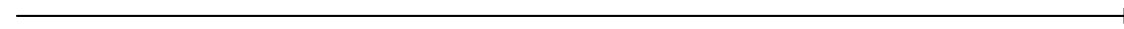


Barley

Malt



Harvest year



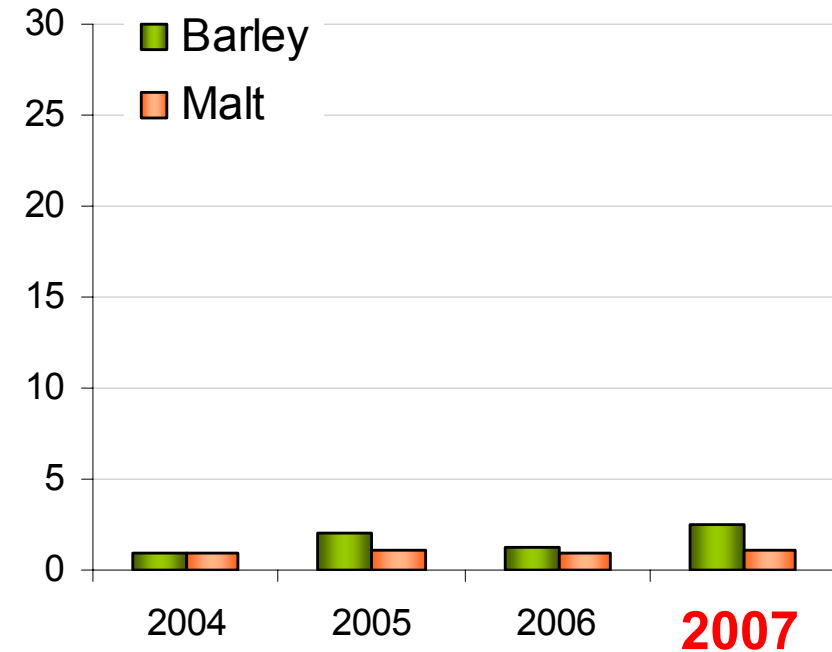
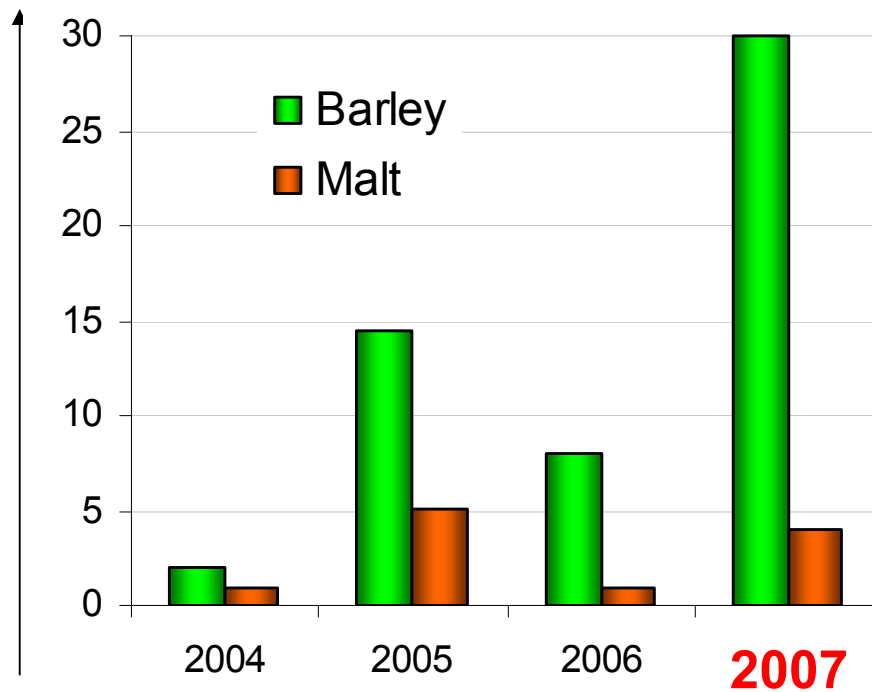
Significant rise in maximum concentration of HT-2. Data from 88 sample pairs

T-2 Triol

concentration
($\mu\text{g}/\text{kg}$)

Maximum

Mean



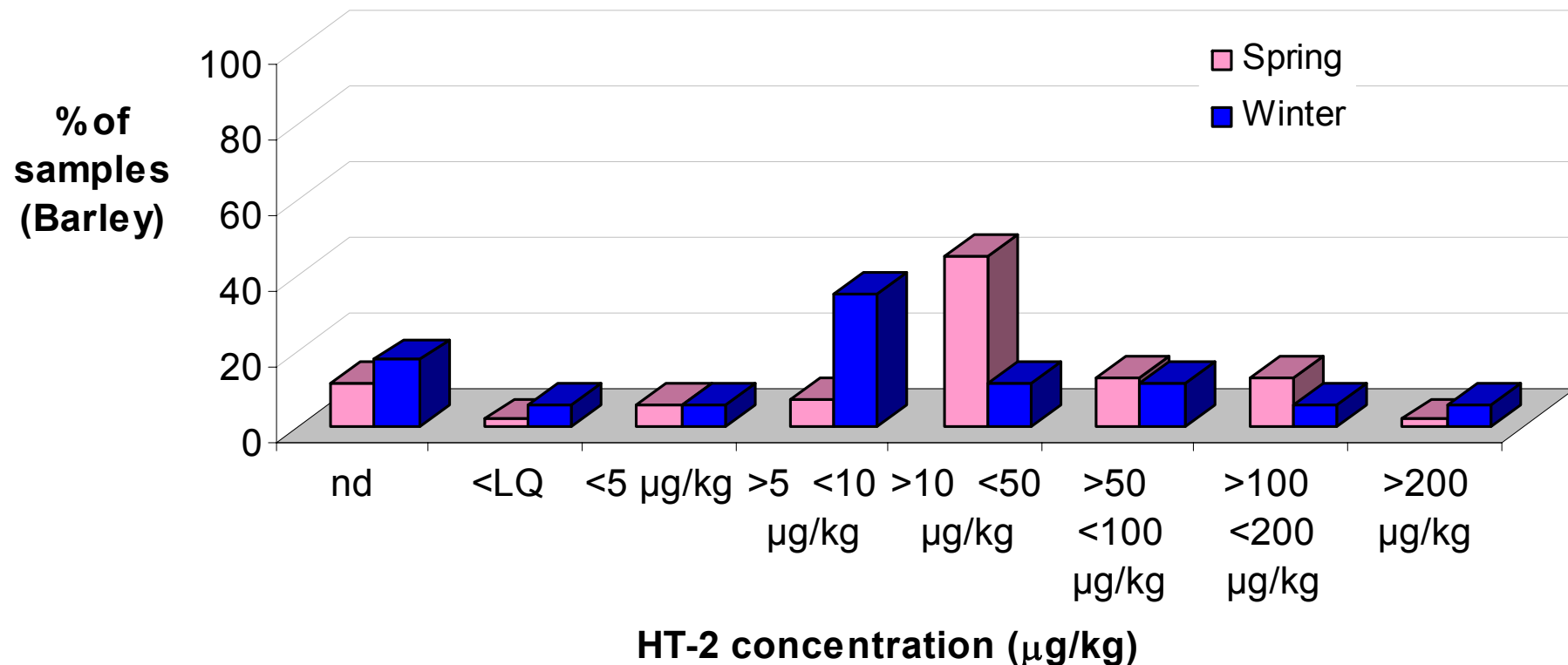
Harvest year

Triol remains low in malt despite increase in barley. Suggests disappearance in malting NOT due to conversion to triol. Data from 73 sample pairs

HT-2 in Spring v Winter barleys

	Spring barley	Winter barley	Spring malt	Winter malt
Number of samples	53	17	53	17
Incidence (%>LOD)	89	82	68	53
Mean HT-2 ($\mu\text{g}/\text{kg}$)	44	41	9.7	4.1
Max. HT-2 ($\mu\text{g}/\text{kg}$)	205	295	83	14

Concentration profile of HT-2 in Spring and Winter barleys

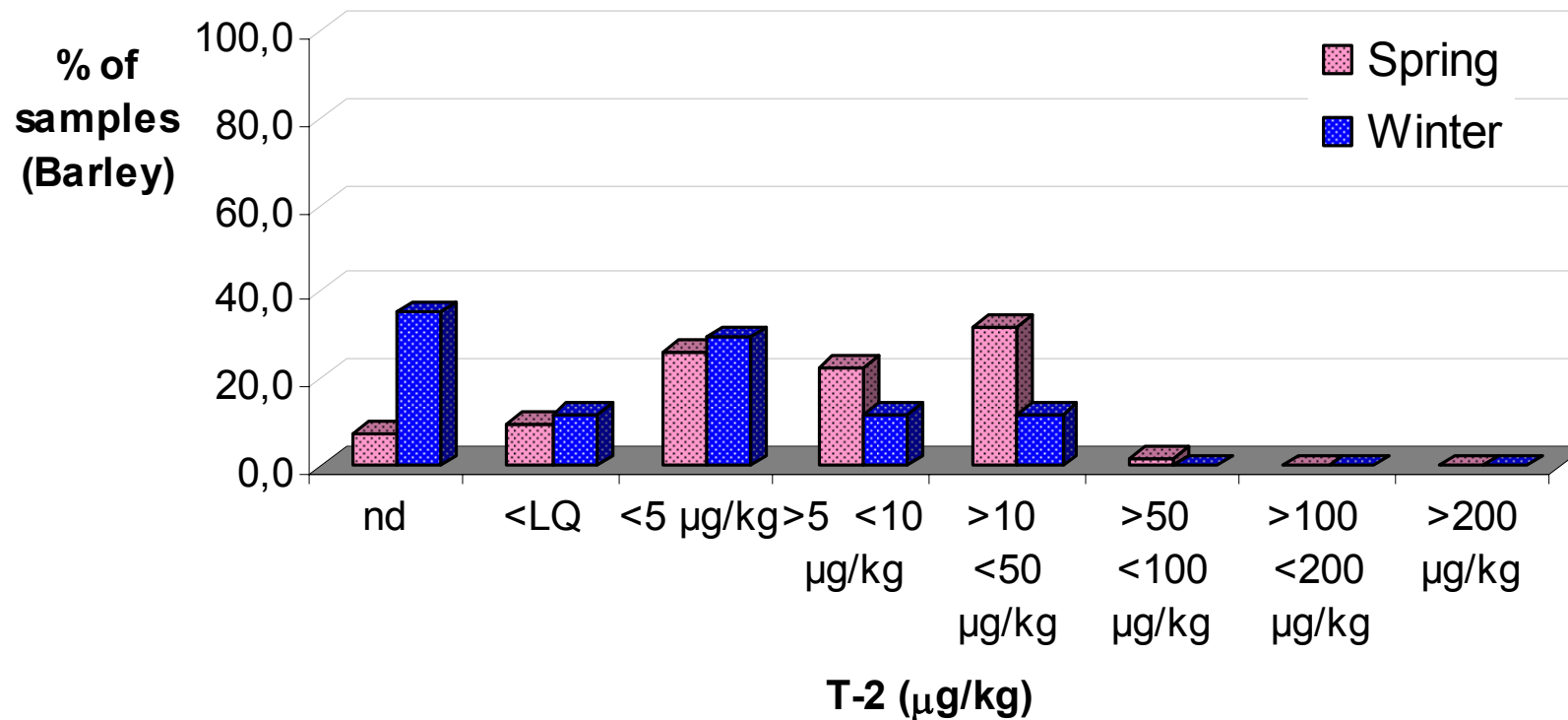


More Winter varieties in 5-10 range: more spring varieties in 10-50 range: difference probably not significant given measurement uncertainty.

T-2 in Spring v Winter barleys

	Spring barley	Winter barley	Spring malt	Winter malt
Number of samples	53	17	53	17
Incidence (%>LOD)	92	65	66	59
Mean HT-2 ($\mu\text{g}/\text{kg}$)	9.7	4.1	2.0	0.7
Max. HT-2 ($\mu\text{g}/\text{kg}$)	69	29	8.7	2.8

Concentration profile of T-2 in Spring and Winter barleys



Spring v Winter varieties

- French data from the 2006 harvest presented at the 2007 Fusarium Forum suggested that T-2 and HT-2 might be lower in Winter varieties compared with Spring varieties
- Europe-wide data from the 2007 harvest suggest little difference between winter and spring barleys in occurrence and levels of HT-2
- T-2 is slightly less prevalent in winter varieties and occurs at lower concentrations

Conclusions from survey

- Continuing upward trend in the incidence of T-2 and HT-2 toxins observed since 2004
- Incidence of DON does not parallel that of T-2 and HT-2, but varies more randomly from year to year
- Mean and maximum concentrations of T-2 generally lower than those of HT-2
- Mean and maximum concentrations of both T-2 and HT-2 are significantly lower in malt than in the starting barley
- There is no increase in T-2 triol during malting, suggesting that reduction of toxins during malting is not due to conversion to triol
- Winter barley varieties may have slightly less T-2, but HT-2 levels probably not significantly lower

Losses of T-2 and HT-2 during malting

- Preliminary work (reported at 4th Fusarium forum) suggests that T-2 and HT-2 are washed out during steeping
- Survey data indicates that the amount lost is not consistent

**The
malting
process**

Raw barley grain



Steeping



Germination

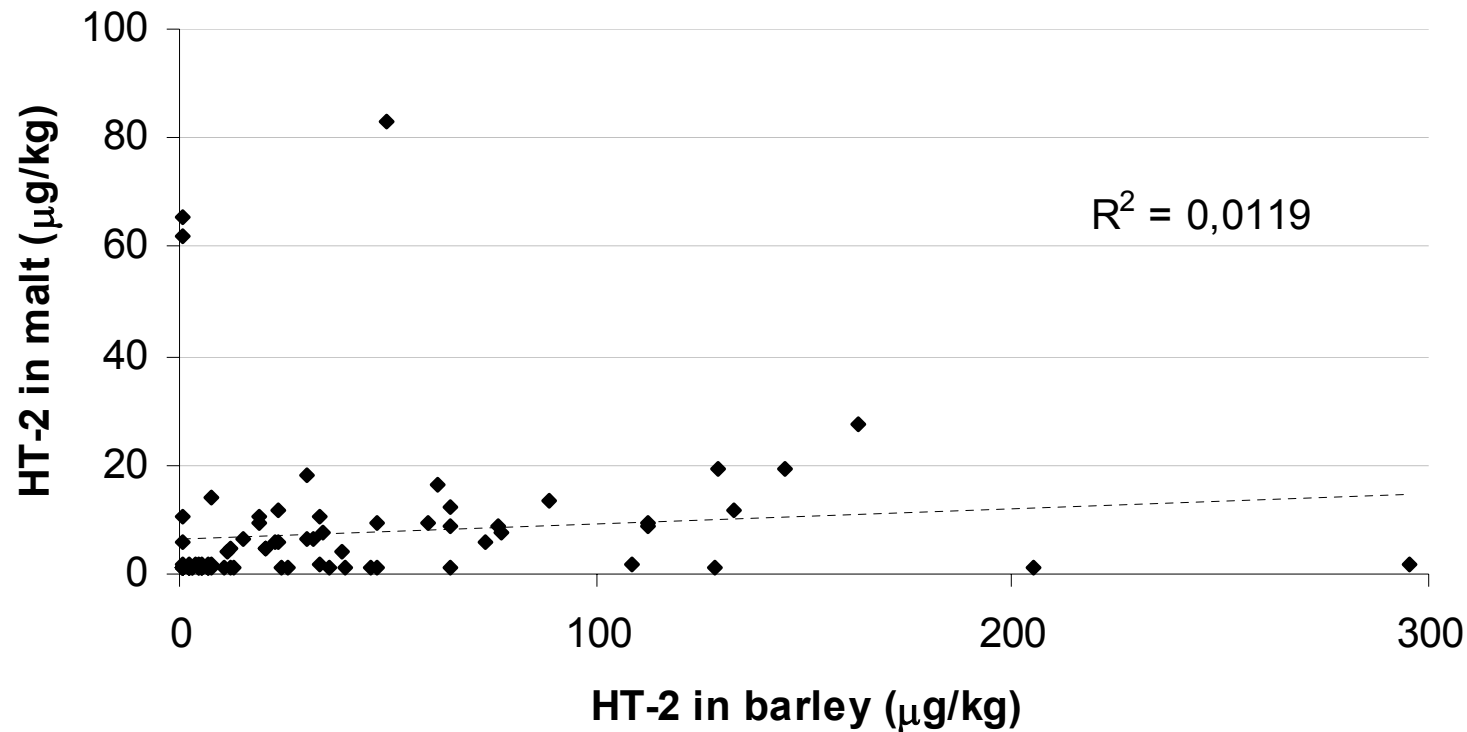


Kilning



MALT

Relationship between toxin concentrations in starting barley and in finished malt



No consistent relationship between toxin concentrations in starting barley and those in finished malt (2007 data)

Factors which could affect loss of T-2 and HT-2 during steeping

- Amount of water used
- Temperature of steep water
- pH of steep water
- Number of steeps
- Duration of steeps
- Extent of mixing / turbulence during steeping
- Thickness of husk

Preliminary results from research commissioned by Euromalt

*NB: these results are preliminary
and from single small scale experiments
only.*

Effects of steeping conditions on the amount of T-2 and HT-2 leached out of barley

- Steep water temperature
 - No significant difference over the range from 10 to 20°C
- Steep water pH
 - No apparent significant difference over the range from pH4 to pH9
 - No advantage in using deionised water
- Number of steeps
 - 70% of losses occur in first steep; minimal further loss in subsequent steeps

Questions which still need answering

- What is the toxicity of T-2 and HT-2 in relation to levels in malting barley and malt?
- What are the agronomic factors relating to the incidence of T-2 and HT-2?

Toxicity of T-2 and HT-2

- A temporary TDI of 0.06 µg/kg body weight/day was established by the EU SCF in 2001
- based on a single 3 week study with pigs showing a 10% reduction in feed intake and slight effects on immune system at a dose of 0.03 mg/kg body weight (*Rafai et al, 1995*)
- other studies showed no effects or only at higher doses:
 - No effects seen at higher doses in a more recent 7 week pig study, which also specifically investigated effects on the immune system (*Bernhoft et al, 2000*).
 - Doses of up to 0.64mg/kg bw/day for 5 – 8 weeks had no effects on the blood in two other studies (*Friend et al, 1992; Weaver et al, 1978*).
 - A 16-month mouse study, carried out according to the relevant OECD guidelines, found only a mild degree of toxicity at the lowest dose tested, 0.21mg/kg bw/day (*Schiefer et al, 1987*).

Toxicity of T-2 and HT-2

- An uncertainty factor of 500 rather than the more usual 100 was used because of deficiencies in the knowledge database
- TDI was temporary because a trichothecene group evaluation was planned
- A group evaluation was carried out by SCF in 2002
- A higher TDI of $1\mu\text{g}/\text{kg bw}/\text{day}$ was confirmed for DON
- The TDI for T-2+HT-2 remained temporary at $0.06\mu\text{g}/\text{kg bw}/\text{day}$
- Are any more recent toxicity data available?
- Are any more studies planned or in progress to address the deficiency of data or the large uncertainty factor?

Agronomic factors

- Why is incidence of *F langsethiae* in Europe increasing?
- Are any specific agronomic factors contributing to this increase?
- What can be done to reduce *F langsethiae* infection and toxin production in cereals in the field?
- Are there regional differences in the Fusarium species which produce T-2 and HT-2 in Europe?
- Are any resistant varieties available?

these questions should be
answered before legal limits are set
for grain.

