



Fusariotoxins in the cereal chain

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**Bühler mycotoxin day
Zuzwill-Switzerland, 07/10/2011**

Outline



- Presentation of CRAW
- Definitions, description
- Regulation
- Risk of contamination in the field (winter wheat)
- Detection of mycotoxins
 - ELISA tests
 - Analytical methods
- Reducing mycotoxins through the chain
- Conclusion

CRAW : Presentation

Centre wallon de Recherches agronomiques

La recherche au service de l'agriculture
et de l'environnement.

4 départements de recherche, une approche pluridisciplinaire



Walloon agricultural research centre

- Funded in 1872
- Organism of public interest (OIP)
- Walloon region

<http://www.cra.wallonie.be/>

Definitions, description

Mycotoxins - definition

- Mycotoxins are secondary metabolites produced by fungi on different products (cereals, fruits, coffee, meat, eggs,...)
- They are toxic for human and animals
 - Chronic intoxication (carcinogenic, mutagenic, teratogenic, immuno-suppressive)
 - Acute intoxication
- They are not eliminated by processing

In the field

- Trichothecenes
 - Fumonisin
 - Zearalenone
 - Ergot alkaloid
- } *Fusarium* sp.
- Claviceps purpurea*

During storage

- Ochratoxin A
 - (Aflatoxins)
- } *Penicillium* sp.,
Aspergillus sp.

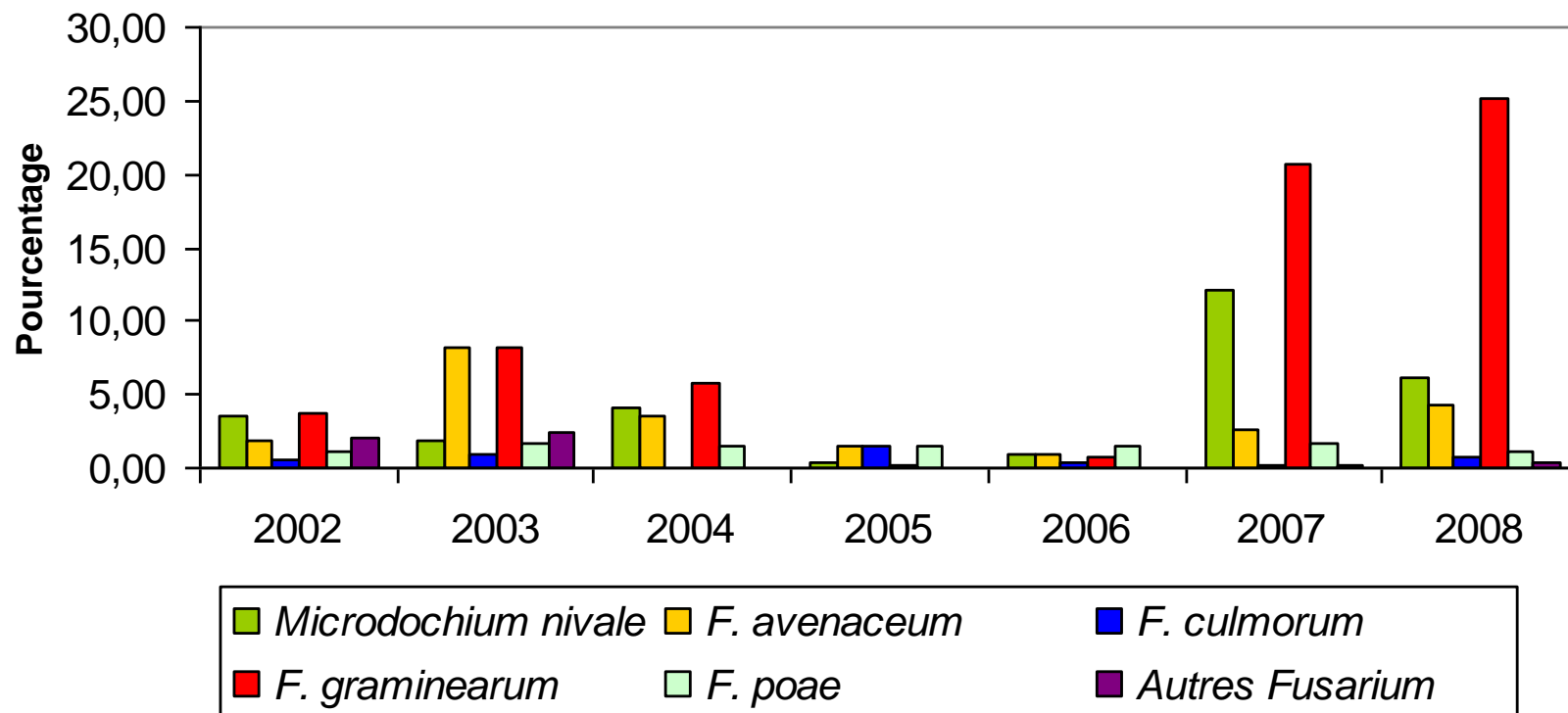
Potential contamination of wheat by mycotoxins

Several fungi species are responsible of the head blight disease

	Trichothecenes			ZEA	Mon/Enni	Fusarin C	Beauverecin
	DON	NIV	T2/HT2				
<i>F. graminearum</i>	+	+	-	+	-	-	-
<i>F. culmorum</i>	+	+	-	+	+	-	-
<i>F. poae</i>	-	++	(+)	-	-	+	+
<i>F. avenaceum</i>	-	-	(+)	-	+	+	+
<i>F. tricinctum</i>	-	-	-	-	+	+	-
<i>F. sporotrichoides</i>	-	-	+	+	+	+	+
<i>F. langsethiae</i>	-	-	++	+	+	+	-
<i>F. crockwellense</i>	-	+	-	+	-	+	-
<i>Microdochium nivale</i>	-	-	-	-	-	-	-

- The grains can be infected by several species
- Toxinogen species (*Fusarium*) can produce different toxins
- Some molecules are very toxic (T2/HT2; NIV; Fusarin C)
- Some molecules are not (yet) in the regulations

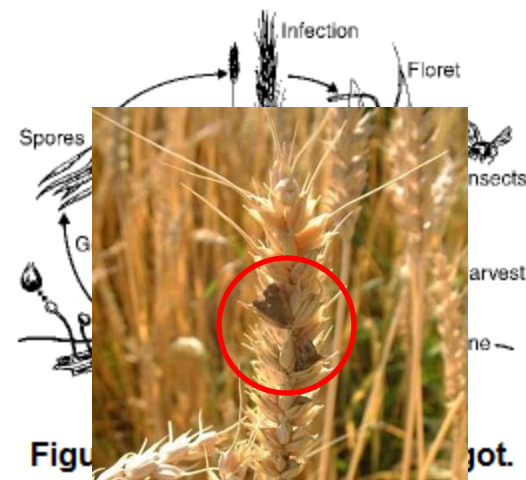
Several agents are responsible of head blight disease in winter wheat



- *F. graminearum* is the major fungi (DON et ZEA)
- *F. avenaceum* can be present (MON/ENNI/FUS C)
- *F. poae* is not much present (NIV)
- No clear relationship between the presence of the fungi and the DON content (2007 et 2008)

What is ergot ?

- Ergot is formed by the fungi *Claviceps purpurea* including ergot alkaloids a class of mycotoxins occurring in grains
- Many hosts: rye, triticale, wheat, durum, barley, oat, sorgho and several grasses
- More information on EFSA



AGROINNOVA



SCIENTIFIC / TECHNICAL REPORT submitted to EFSA

CFP/EFSA/CONTAM/2008/01

Scientific information on mycotoxins and natural plant toxicants

Prepared by Battilani P.^a, Costa L.G.^a, Dossena A.^a, Gullino M.L.^a, Marchelli R.^a, Galaverna G.^a, Pietri A.^a, Dall'Asta C.^a, Giorni P.^a, Spadaro D.^a, Guala A.^a

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Unité Technologies de la Transformation des Produits

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Centre wallon de Recherches agronomiques



Wallonie

Regulation

Regulation for food

(Commission Regulation (EC) No 1881/2006 & 1126/2007)

Maximum levels (in µg/kg or ppb)

		Field				Post-harvest	
		DON	ZEA	FB1+FB2	T2/HT2	OTA	AFB1
Cereals (winter wheat)	Unprocessed	1250	100		?	5	2
	flour	750	75		?	3	2
	bread, cake...	500	50		?	3	2
	babyfood	200	20		?	0,5	0,1
Durum wheat / oats	Unprocessed	1750	100		?	5	2
	flour	750	75		?	3	2
	bread, cake...	500	50		?	3	2
	babyfood	200	20		?	0,5	0,1
Maize	Unprocessed	1750	350	4000	?	5	5
	milling (size < 500 µ)	1250	300	2000	?	3	5
	milling (size > 500 µ)	750	200	1400			
	snack, breakfast	500	100	800/1000	?	3	5
	babyfood	200	20	200	?	0,5	0,1



Difference according

-to the cereal

-To the mycotoxin

-To the product

Regulation (food)

- Foodstuffs not complying with the maximum levels set out
 - shall not be used as food ingredient
 - shall not be mixed with sound foodstuffs
 - Shall not be deliberately detoxified by chemical treatment

Commission recommendation (feed) of 17 August 2006

Maximum levels (in µg/kg or ppb)

Mycotoxin	Cereals	Feedingstuffs	
		general	exception
DON	8000 (maize: 12000)	5000	calves, lambs and kids: 2000
			pigs 900
			piglets and gilts: 100
ZEA	2000 (maize: 3000)		sows and fattening pigs 250
			calves, dairy cattle, sheep, goats 500
			pigs: 50
OTA	250		poultry 100
			pigs, horses, rabbits 5000
			fish 10000
FB1+FB2	60000		poultry, calves < 4 months, lamb 20000
			adult ruminant (> 4 months) 50000

Differences according to the animal species !!!

Ergot : legislation

- The concentration of ergot body in cereals is for **human**, restricted to **500** mg per Kg in foodstuffs

European Commission, regulation (EEC) No 689/92 of 19 March 1992 fixing the procedure and conditions for the taking-over of cereals by intervention agencies, in *Official Journal of the European communities*, L074, 18-22 (1992).



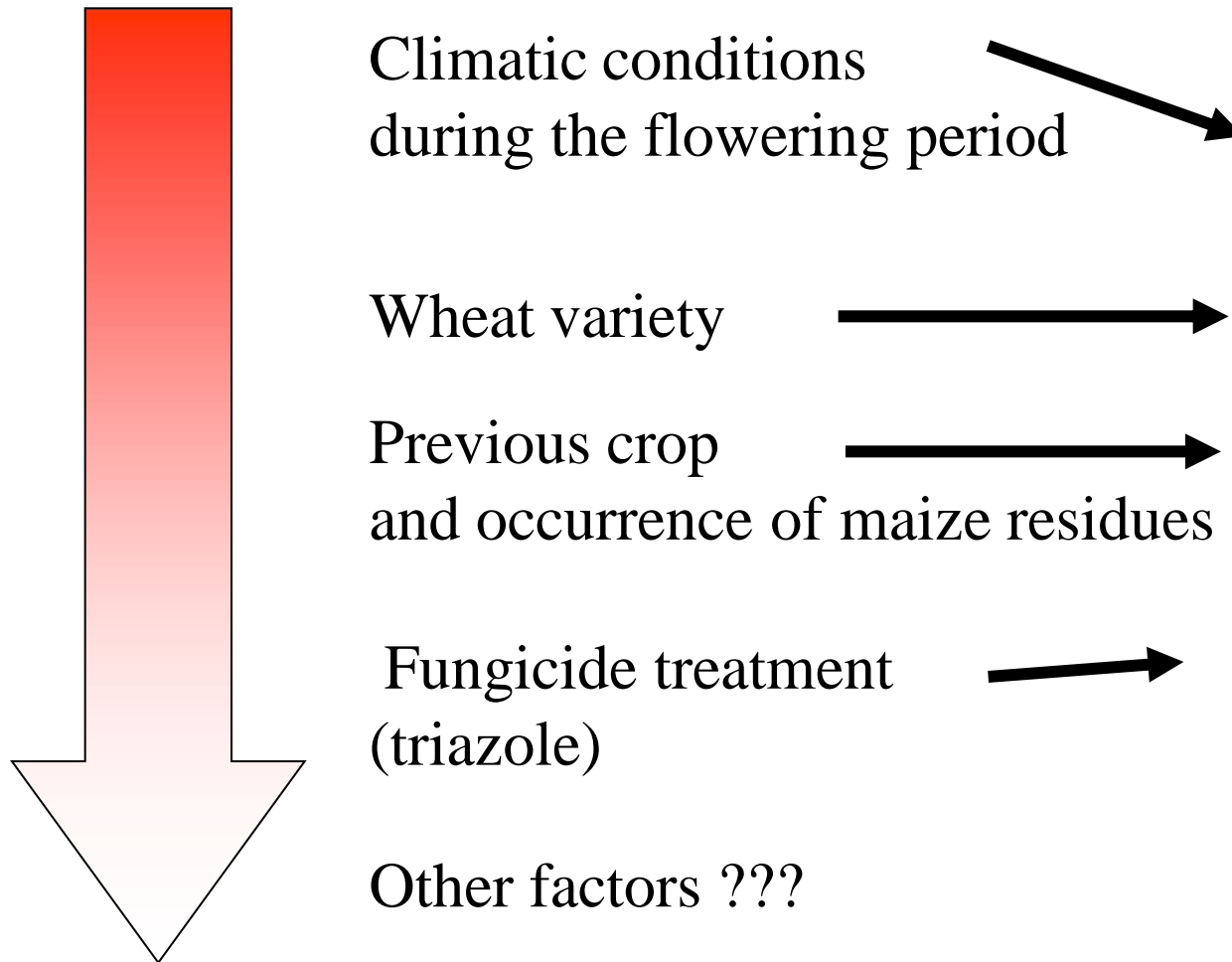
- The concentration of ergot body in cereals is for **animal**, restricted to **1000** mg per Kg in feedingstuffs containing unground cereals

European Commission, directive 2002/32/EC of the European parliament and of the council of 7 May 2002 on undesirable substances in animal feed, in *Official Journal of the European communities*, L140, 10-21 (2003).

Risk of contamination in the field

Risk of contamination in the field : a case study

Head blight in winter wheat – modelling DON content



Strategy to control DON content in wheat

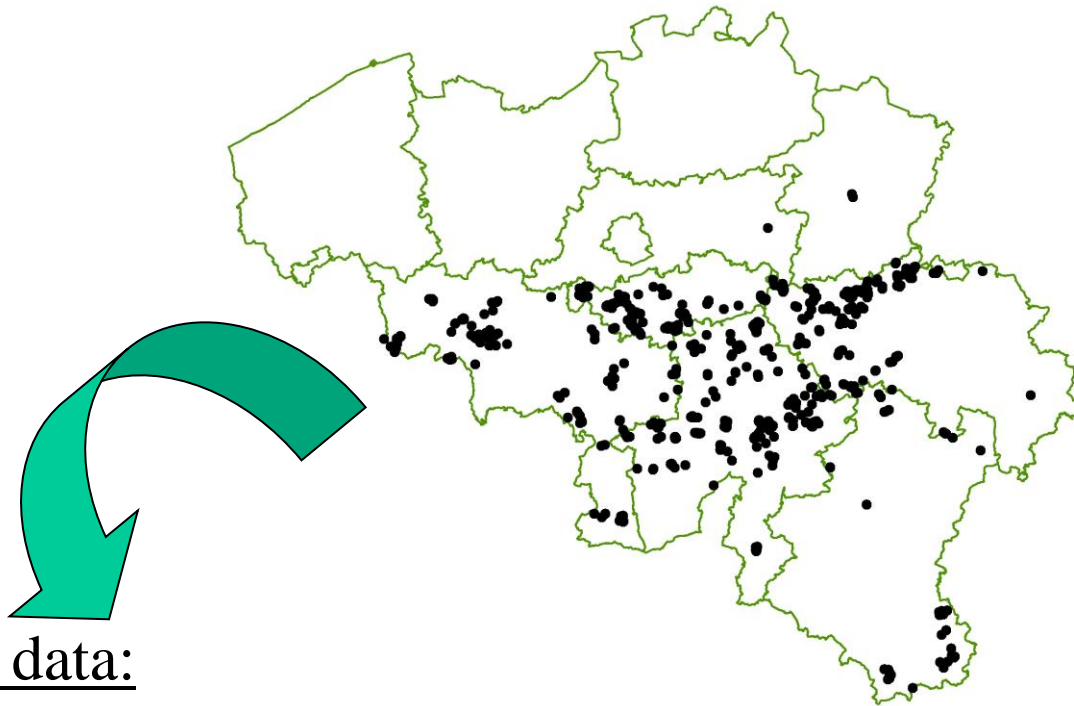
Procedure developed at the CRAW

Step 1 : Risk of DON contamination based
on meteorological data

Step 2: Risk of DON based on cultural practices

- wheat varieties
- Previous crop
- Maize residue

1. Collect of wheat samples in the cereal cropping area (2003-2009, N=690)



Collected data:

- Wheat variety
- Previous crop
- Tillage type
- Geographical coordinates

2. Analysis of DON content

- Grains collected in the field a few days before harvest according to the greatest field diagonal
- ELISA test (LOD=150 ppb)

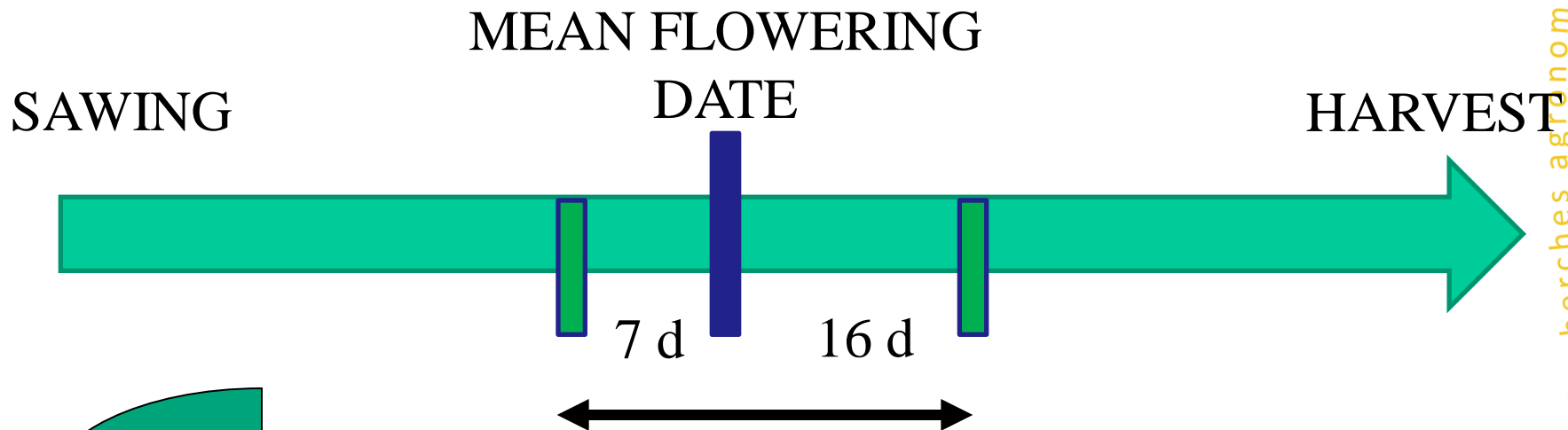
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Samples	67	66	184	112	104	115	67	51	64	90	46
Mean (ppb)	<150	620	270	200	<150	115	1350	826	120	<150	<150
Median (ppb)	<150	400	<150	<150	<150	113	870	450	60	<150	<150
Maximum (ppb)	400	2850	2750	2500	190	680	5610	4790	1310	390	590
% > 150 ppb	8,4	74,7	51	35	8,6	65	100	92	23	1	2
> 1250 ppb (%)	0	18	5	1,8	0	0	36	20	1,6	0	0



3 years out of 11 with risk of DON:
2002, 2007 and 2008

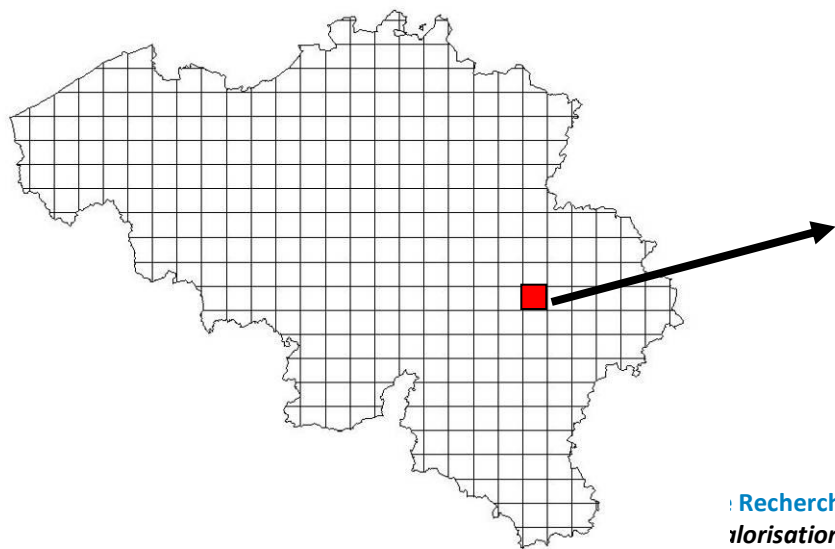
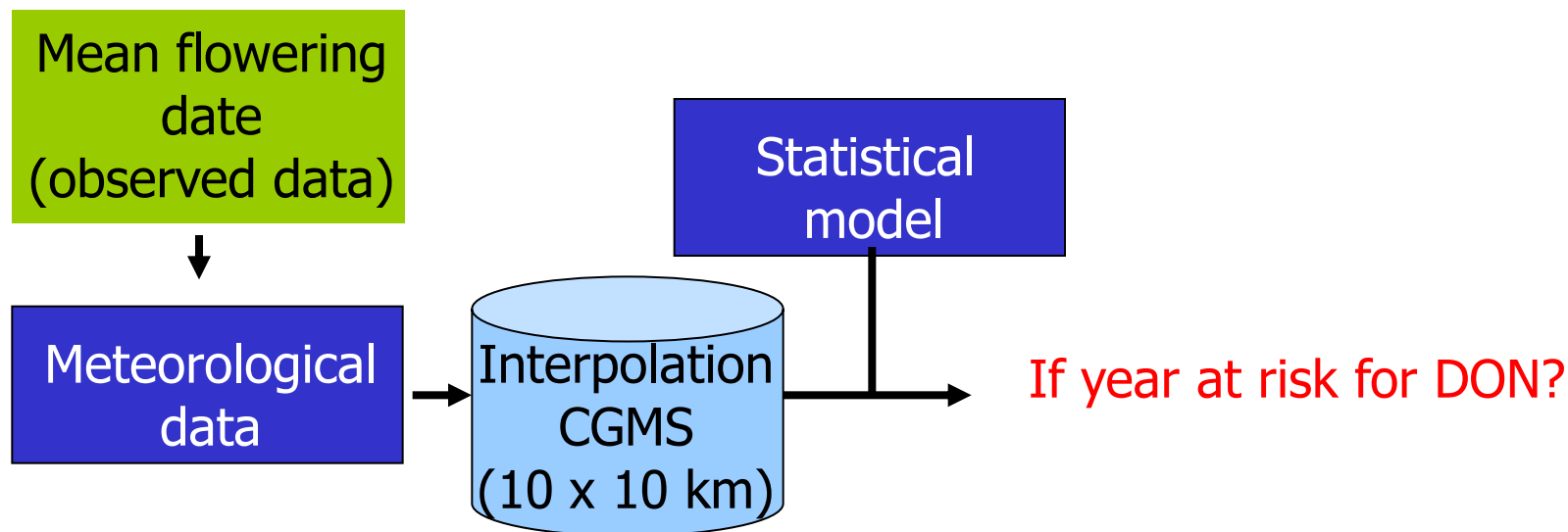
3. Definition of the mean flowering date

(from data provided by the farmers participating to the survey)



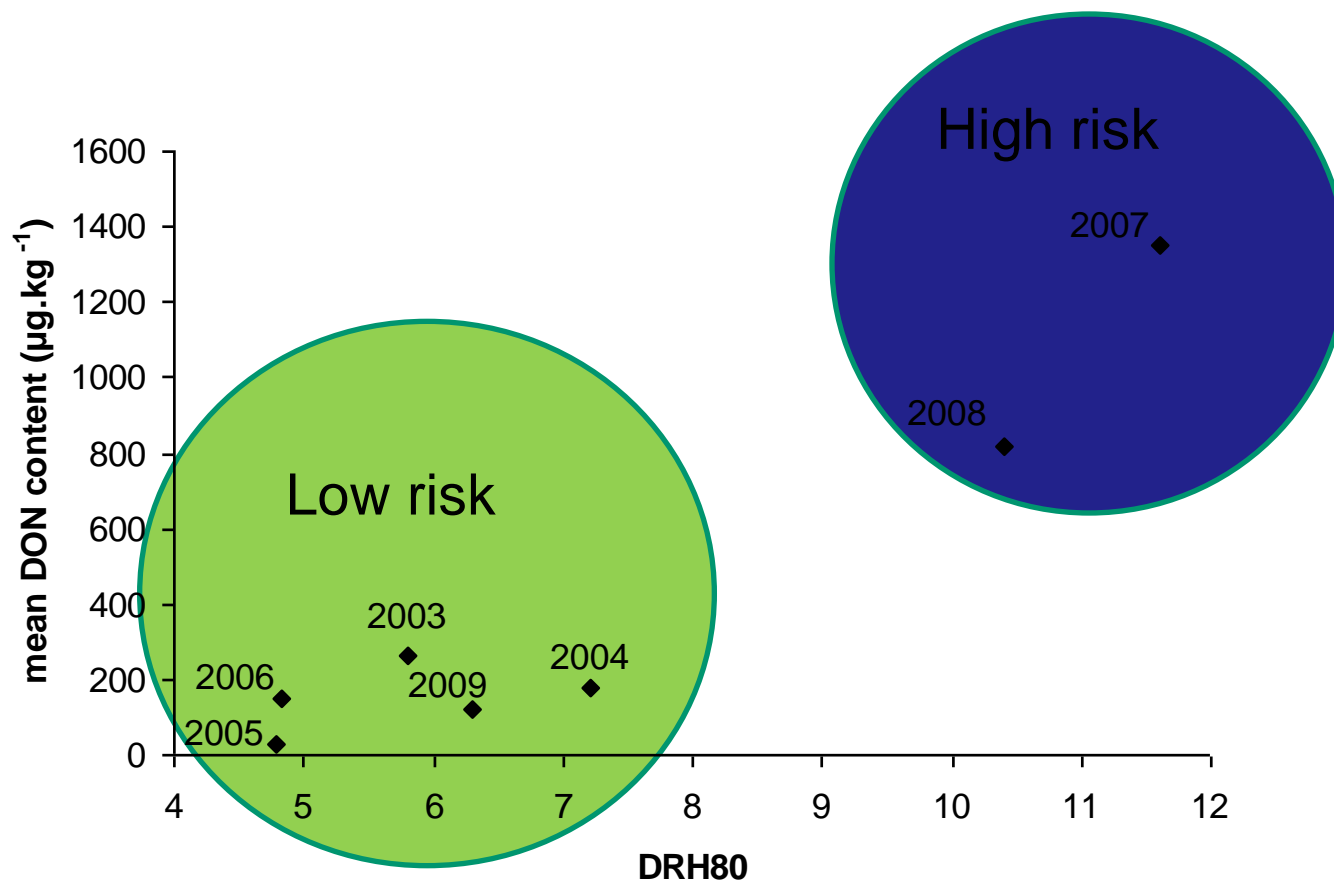
Collect of meteorological data for a time period of 24 days around the flowering date

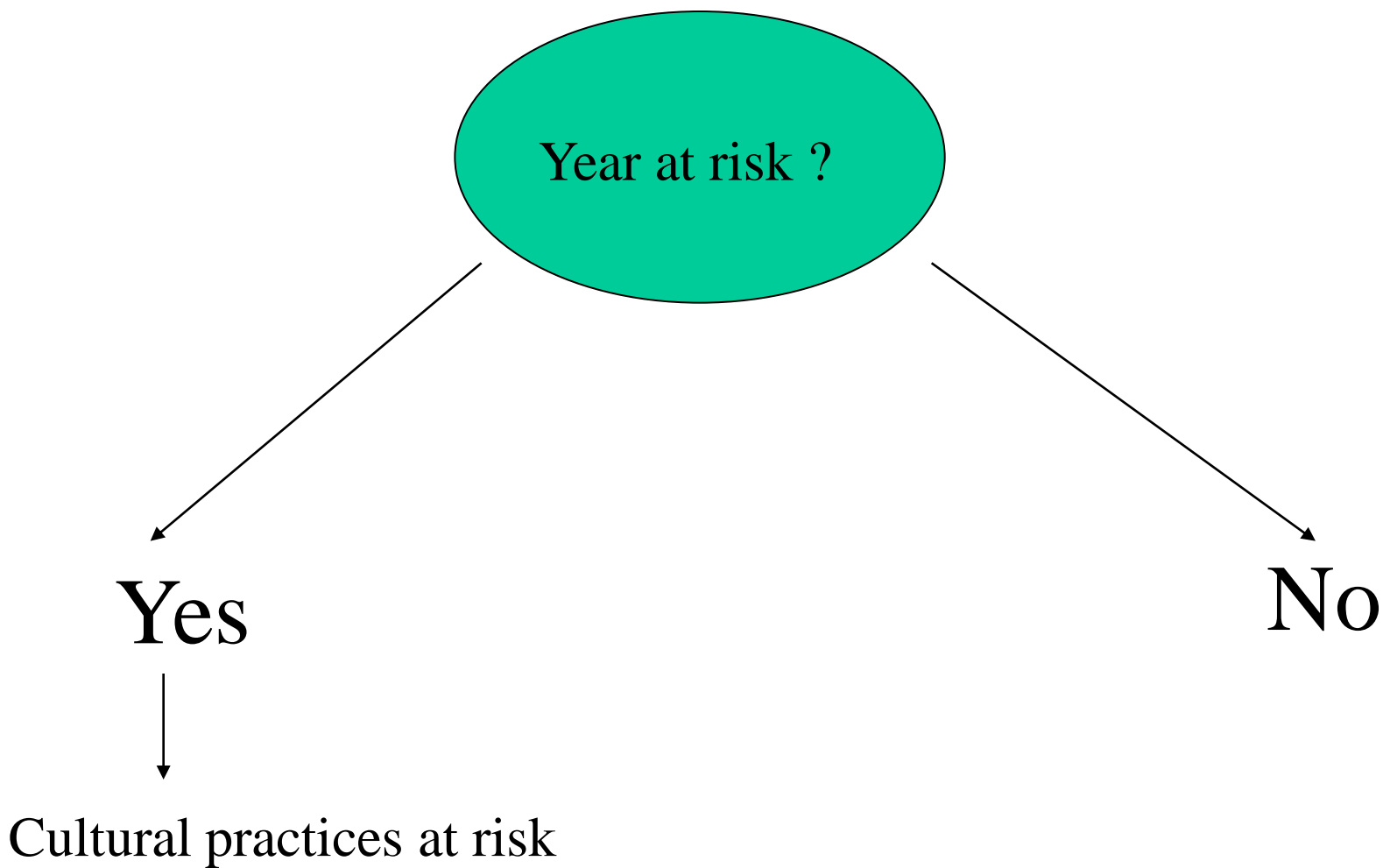
4. Type of meteorological data and interpolation



- Average daily temperature (°C)
- Average daily relative humidity (%)
- Average daily total rainfall (mm)
- Number of days with RH > 80%
- Number of days with rainfall
- Evapotranspiration

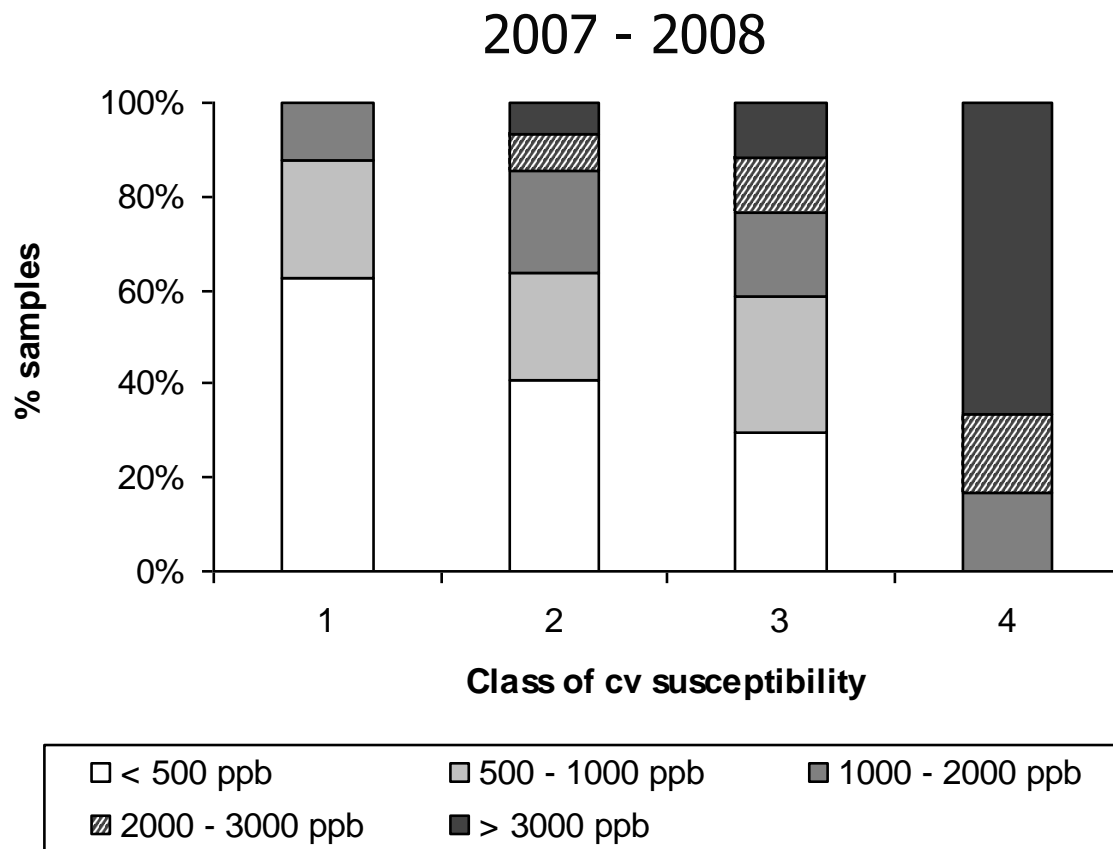
Relationship between **DON content** and the number of days with RH>80% (long period) around the mean flowering date over a 7 year period (2003-2009)





Influence of the wheat variety

Varieties are classified into 4 groups based on visual observation of FHB
(1= resistant; 4= very susceptible)



In year at risk, 100% of the fields with type 4 cultivar had a DON content > 1000 ppb

Influence of the previous crop / maize residues

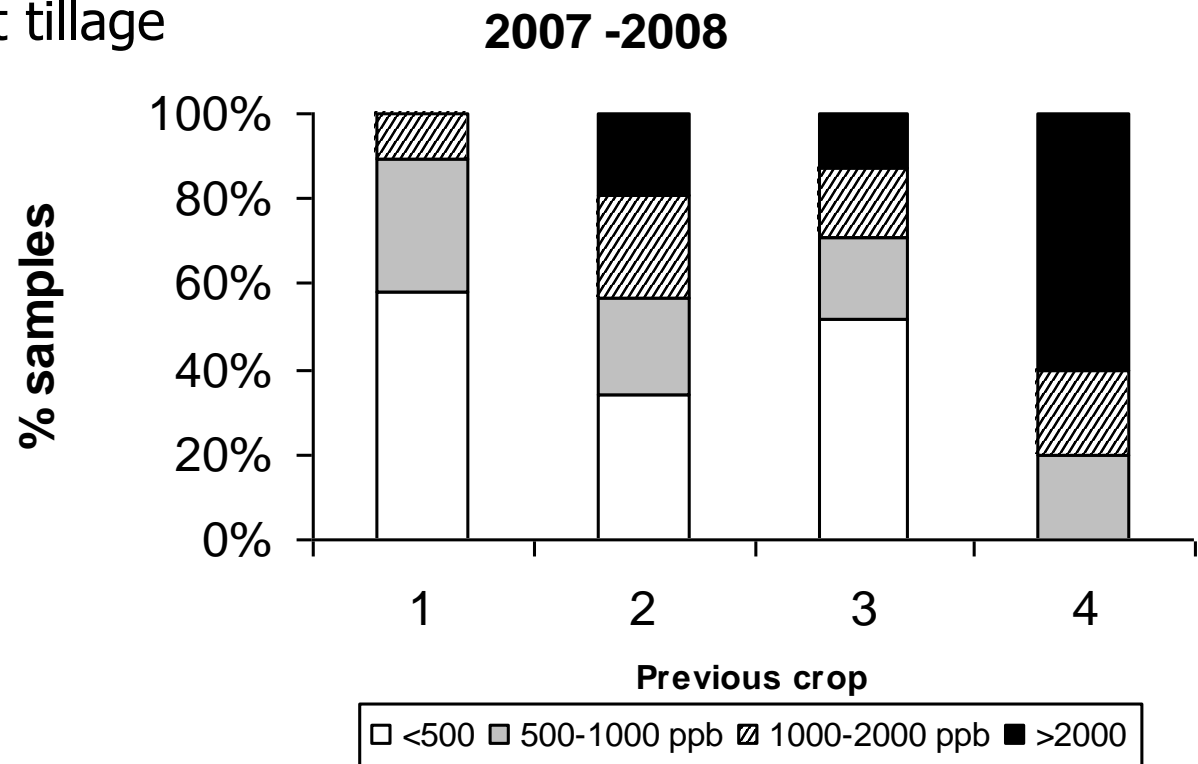
Samples are classified into 4 groups according to the previous crop

1= rape, flax, pea;

2 = small grain cereals, sugar beet, potato

3= maize for silage with tillage

4= maize for silage without tillage



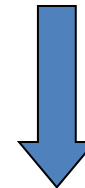
In year at risk, > 80% of the fields had a DON content > 1000 ppb !

Decision support system

- Evaluation of the type of year
number of days with RH >80 % above 10
- DON threshold at 900 ppb (= 1250 ppb – Ue)
- Risk factors :
wheat cultivars of type 3 **or** type 4 wheat **or**
previous crop of type 4

		Observed		Total
		+	-	
Expected	+	31	15	46
	-	20	52	72
Total		51	67	118

Correct: $(31+52)/118=70.3\%$
False positive: $15/67=22.3\%$
False negative: $20/51=39.2\%$



Other criteria ?
(fungicides, ...)

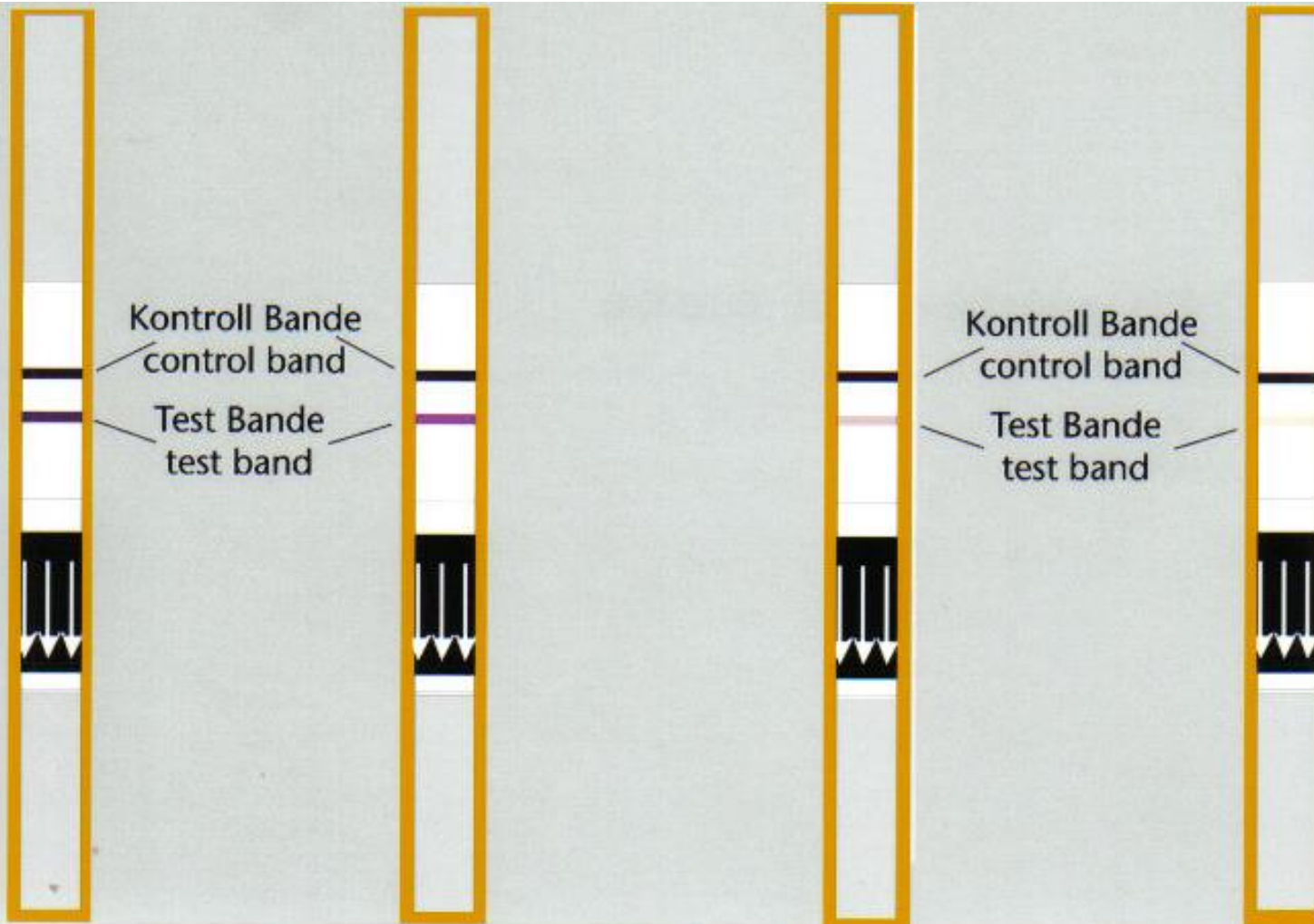
Analytical methods

Detection of mycotoxins – LFD

Test négatif
DON < 1000 ppb

Rhone Biopharm

Test positif
DON > 1000 ppb



Detection of mycotoxins – LFD

Neogen : Reveal DON



15€/test

1800€



$0 \leq \text{DON} < 0.5$

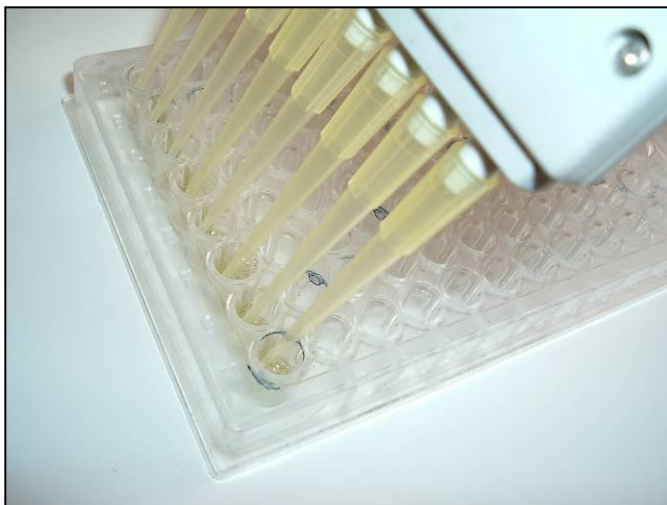
$0.5 \leq \text{DON} < 1.0$

$1.0 \leq \text{DON} < 2.0$

$\text{DON} > 2.0$



Detection of mycotoxins – ELISA tests



Competitive ELISA test

Validation according to the standard AFNOR NF-03-110 at the CRAW

- rapid and easy to use
- applicable in the milling company
- semi-quantitative (only for screening)
- risk of false positive (cross reaction)
- one mycotoxin each time

Analytical performances:

LOD:	150 ppb
LOQ:	360 ppb
Ue:	36 %

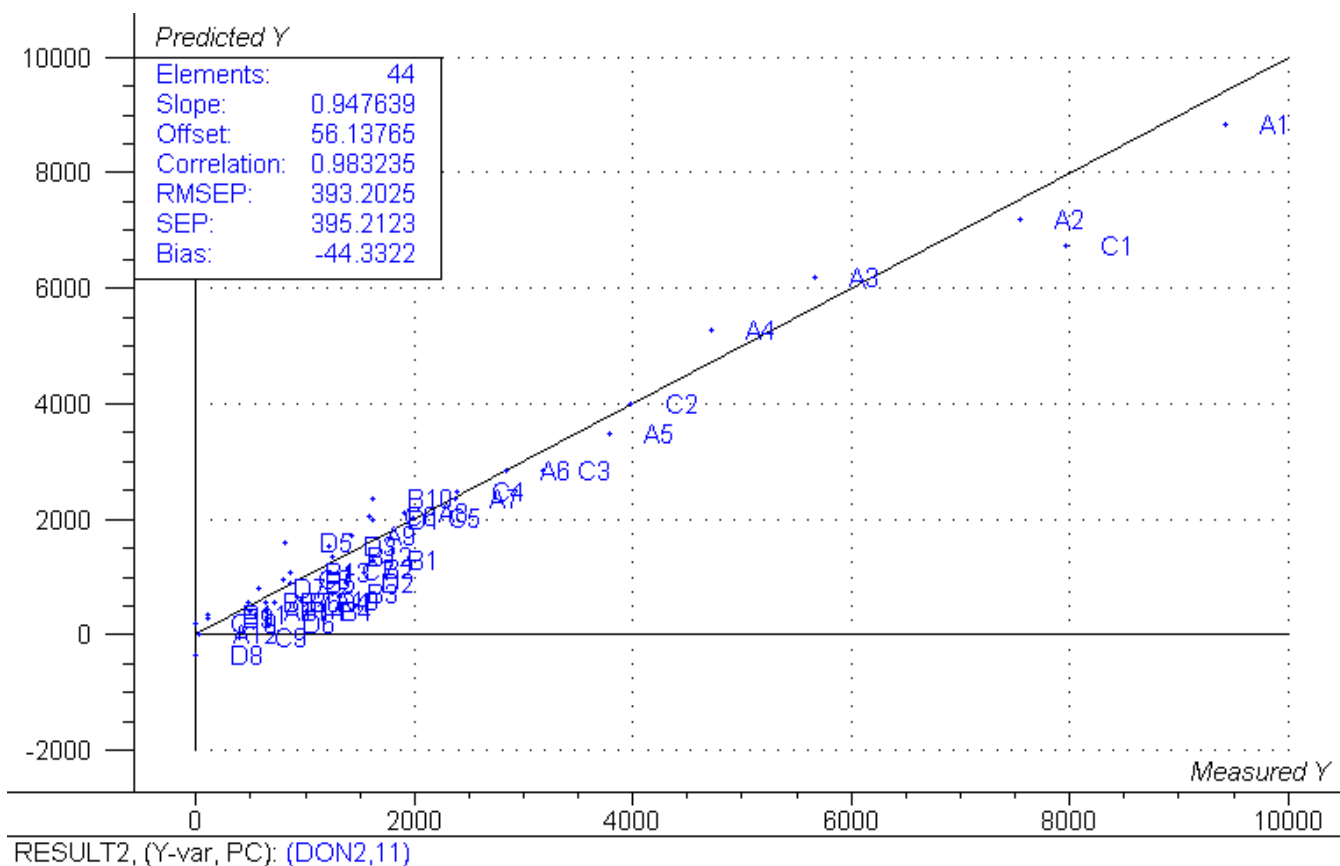
Test strips Vs Elisa



ELISA	Reveal
0.05	DON < 0.5
0.08	DON < 0.5
0.10	0.5 ≤ DON < 1.0
0.11	0.5 ≤ DON < 1.0
0.12	DON < 0.5
0.13	DON < 0.5
0.56	0.5 ≤ DON < 1.0
0.62	0.5 ≤ DON < 1.0
0.82	0.5 ≤ DON < 1.0
0.97	0.5 ≤ DON < 1.0
1.00	1.0 ≤ DON < 2.0
1.04	0.5 ≤ DON < 1.0
1.22	1.0 ≤ DON < 2.0
1.31	1.0 ≤ DON < 2.0
2.45	DON ≥ 2.0
2.69	DON ≥ 2.0
3.27	DON ≥ 2.0
3.57	DON ≥ 2.0
4.18	DON ≥ 2.0



Near infrared Transmittance : Foss Infratec 1241



Hans Pettersson¹⁾ and Lena Åberg²⁾

Near Infrared Spectroscopy for Determination of Mycotoxins in Cereals

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Multimycotoxins – UPLC/MS/MS

Investissement 400.000 €

30 €/ éch réactif

150€/éch prix coûtant

Acquity™
Ultra Performance LC



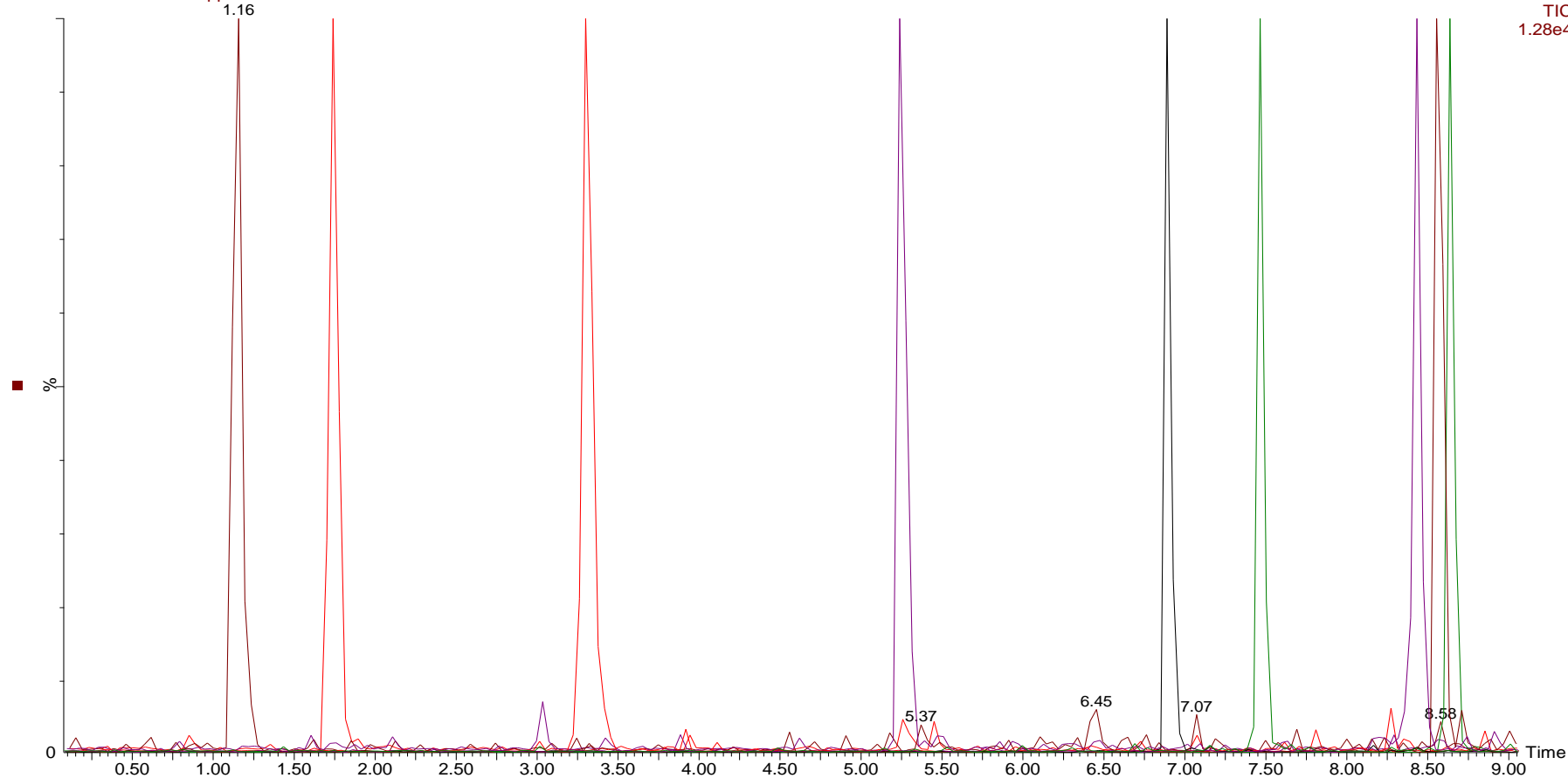
Quatro Premier XE

10-Sep-2007

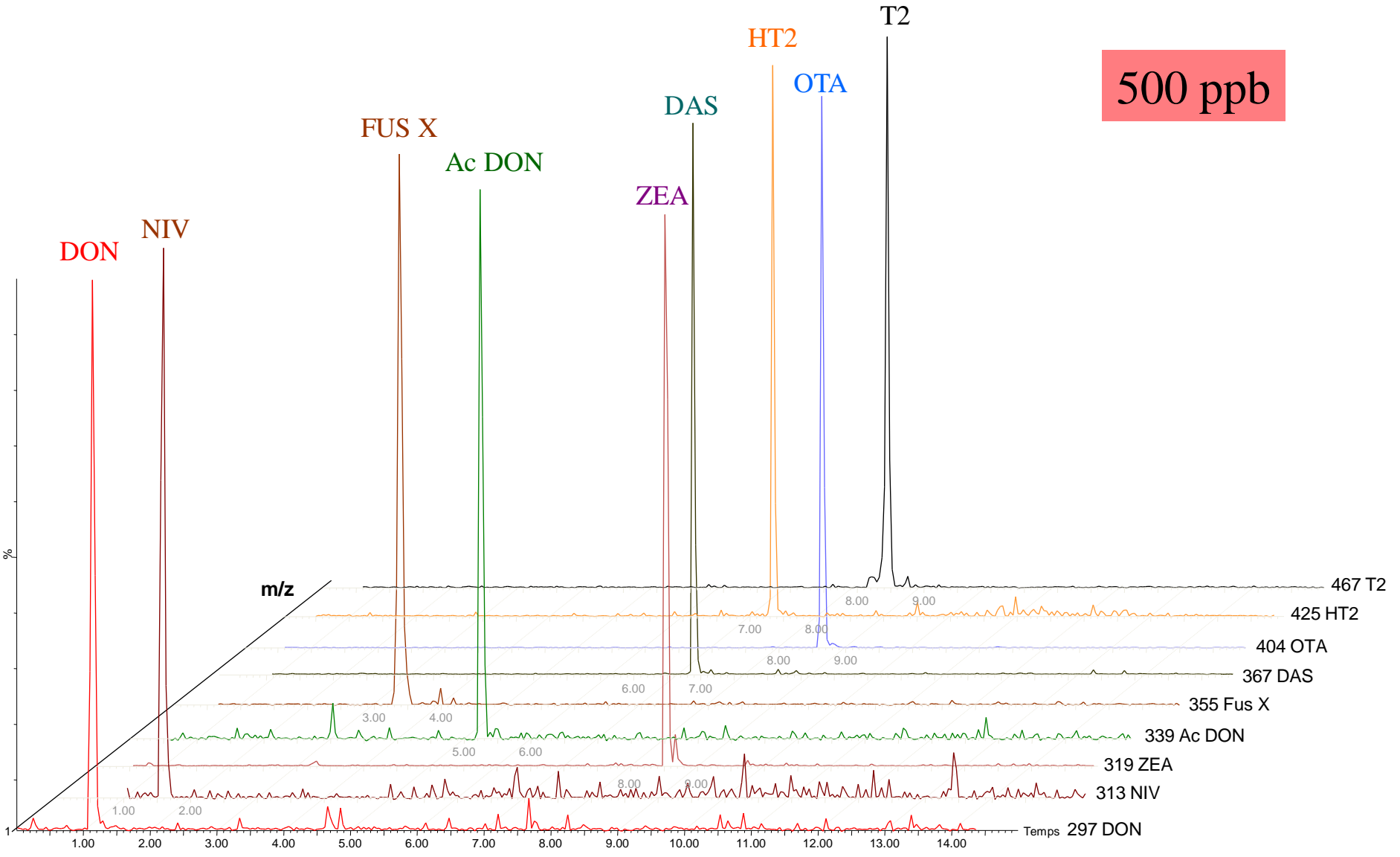
2:21

Tricho Mix 4 + OTA 500 ppb 20070910 07 00

14:33:21



500 ppb



What is hyperspectral imaging?

Spatial information



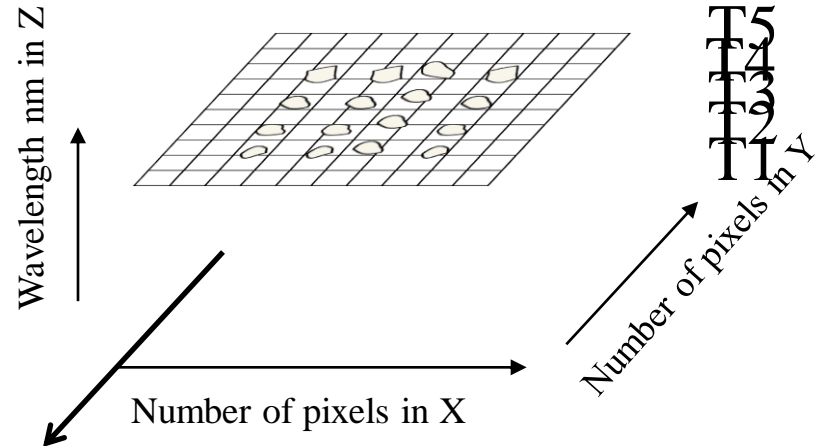
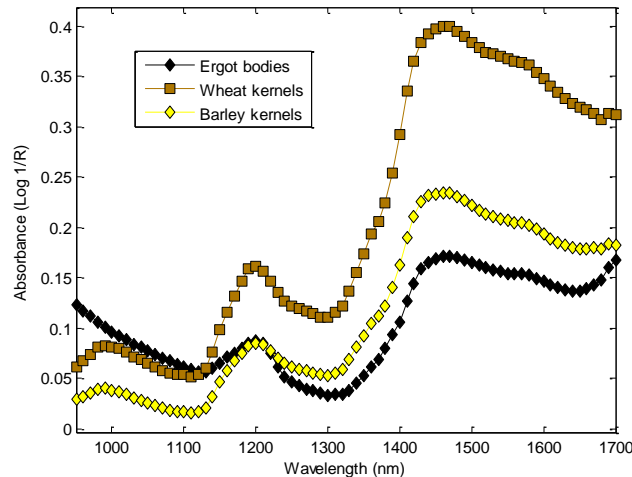
NIR imaging
instrument

**Frequency information
(i.e. wavelengths)**

**Intensity information
(i.e. absorbance)**

NIR plane scan camera: features

- NIR camera setup

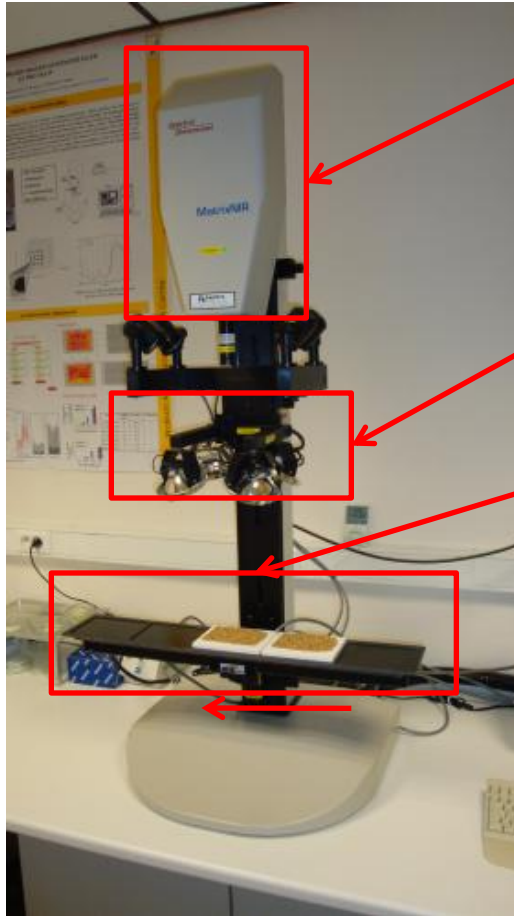


Cereal kernels and ergot bodies

- 1 image = 240 x 320 pixels = 76 800 spectra
- Analysed surface = 76800 pixels = +/- 5cm²
- Wavelength range: 900-1700nm by step of 10 nm
- Time of acquisition = 5 min/image

NIR plane scan camera: instrument

High setup

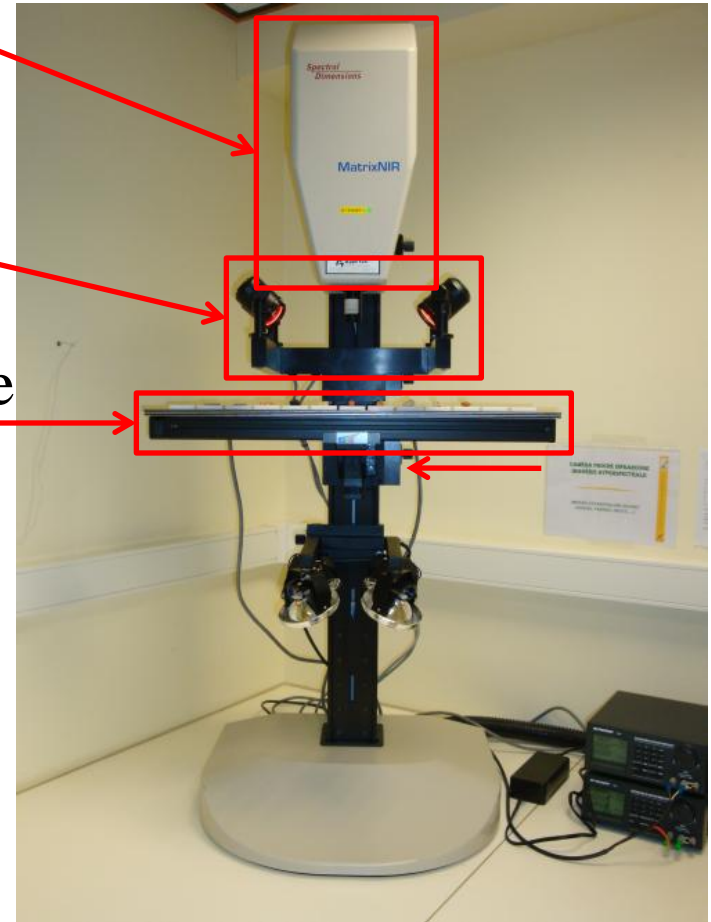


NIR camera

Light source

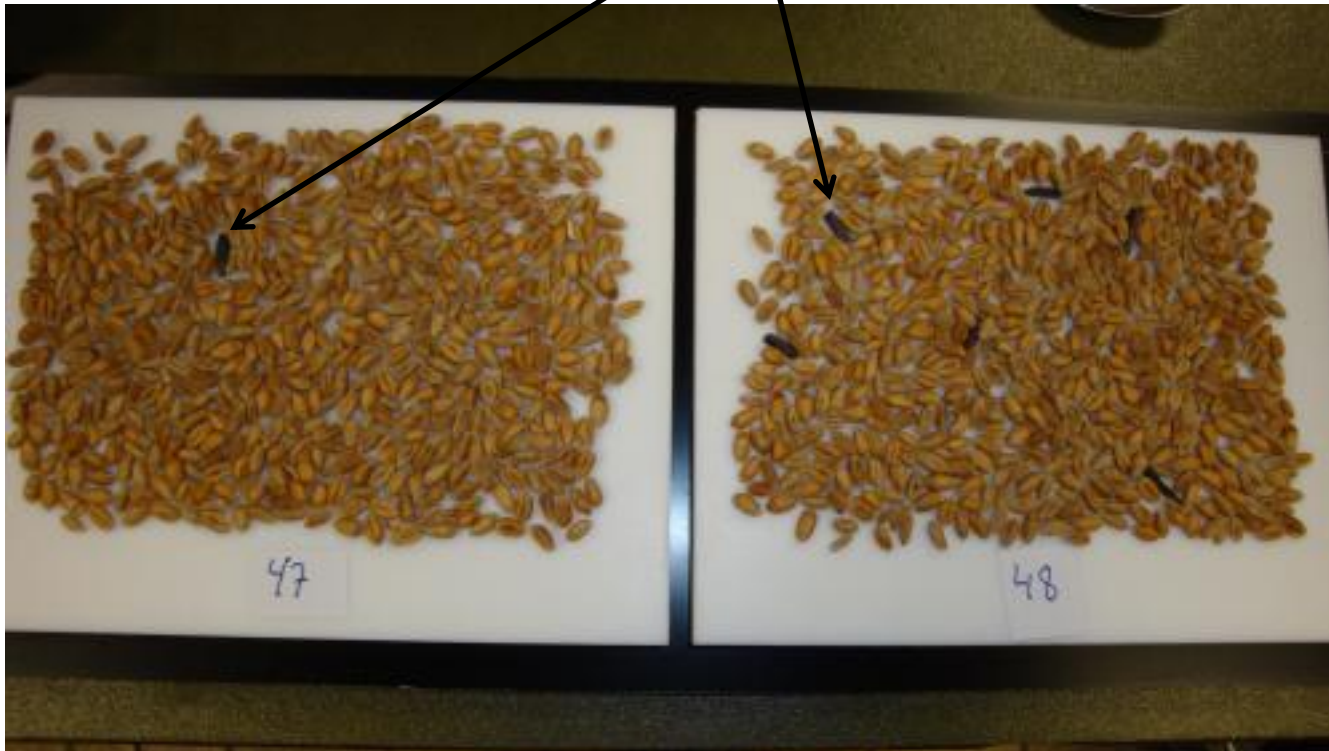
Samples plate

Low setup



NIR plane scan camera: analysis

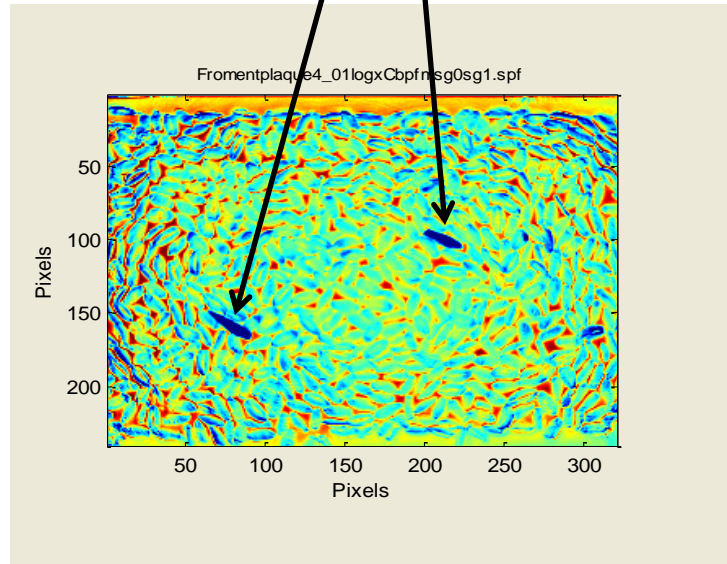
Ergot bodies



Ergot detection in wheat by NIR imaging

In low setup

Ergots



Wheat sample including 2 ergot bodies

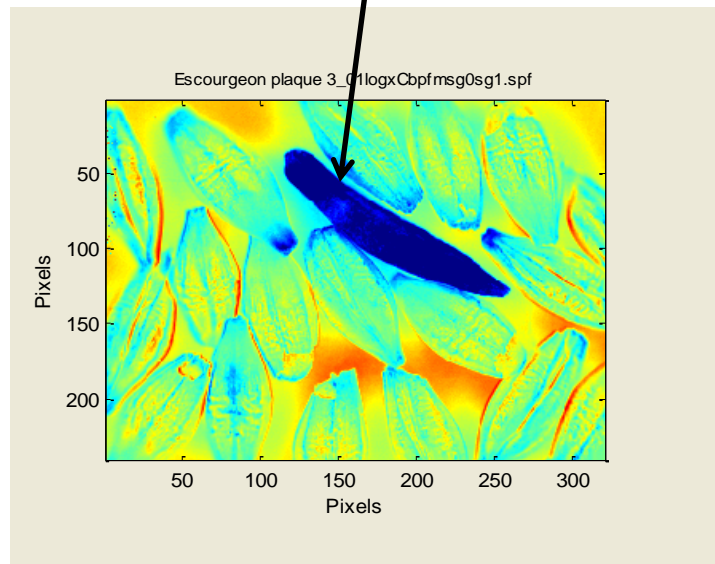
Ergot detection in barley by NIR imaging

In high setup

Ergot body

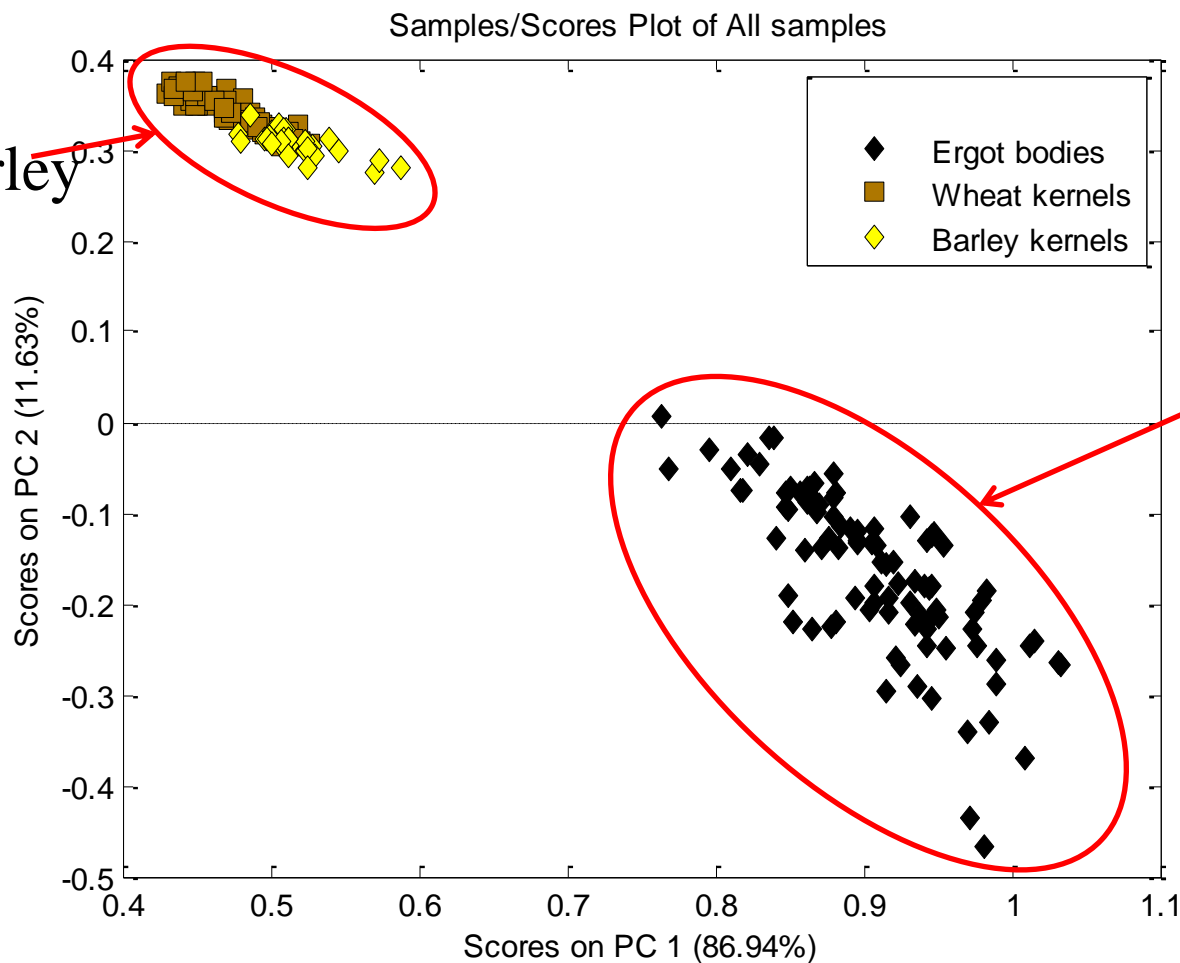


Barley sample including an ergot body



Discrimination by PCA

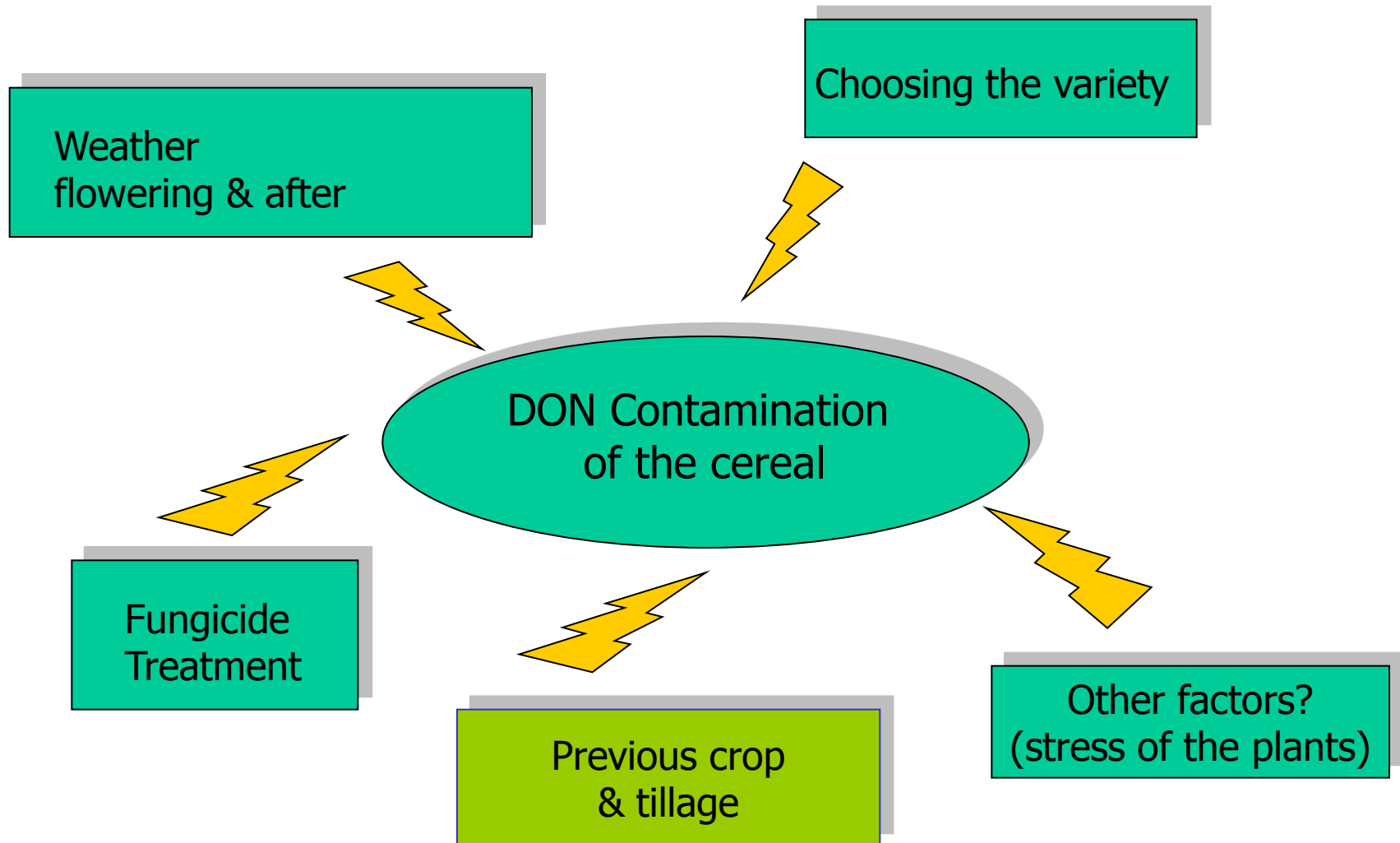
between ergot bodies and wheat/barley kernels



Reducing mycotoxins through the chain

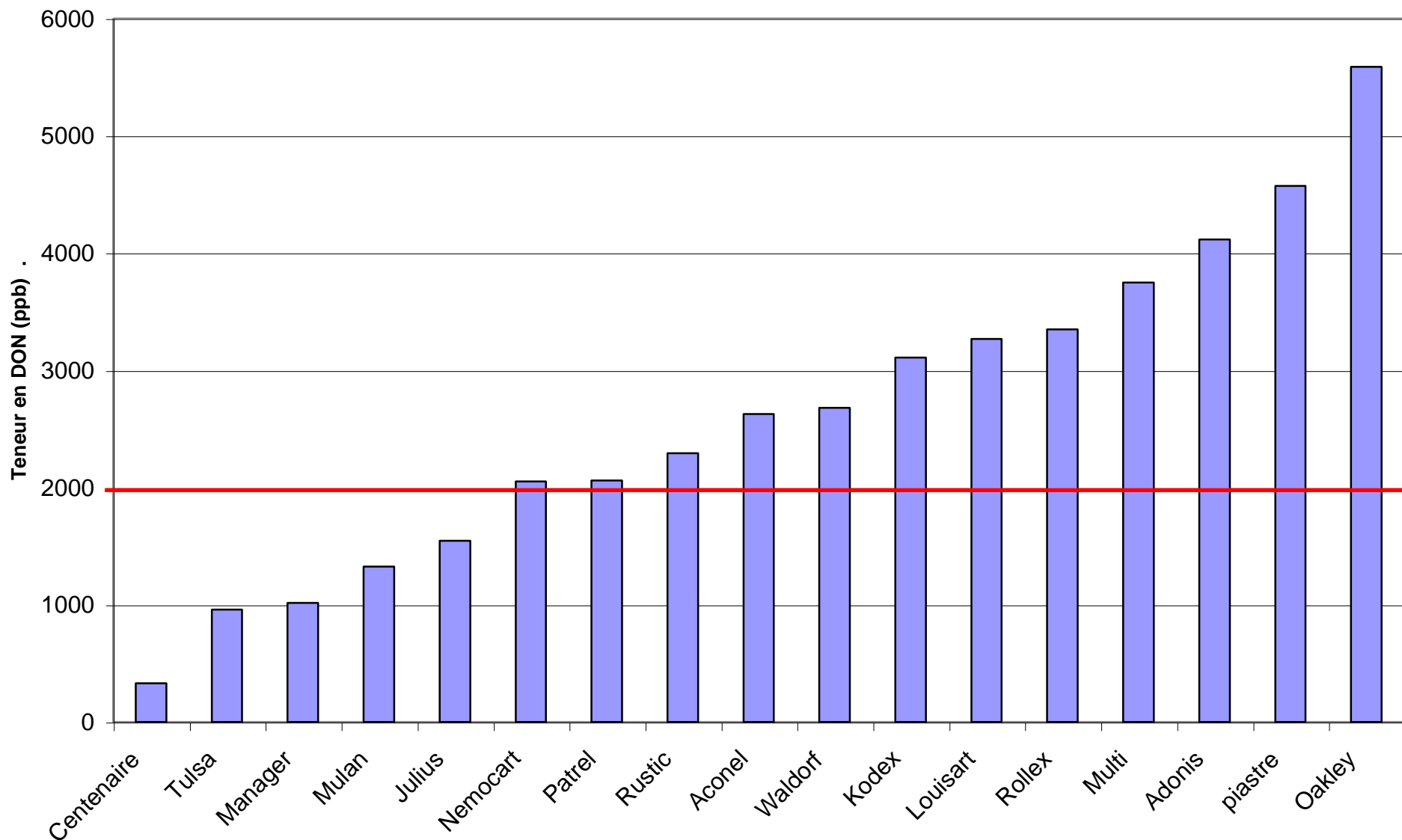
Reducing the mycotoxin contents through the chain

1. At the producer level : risk factors



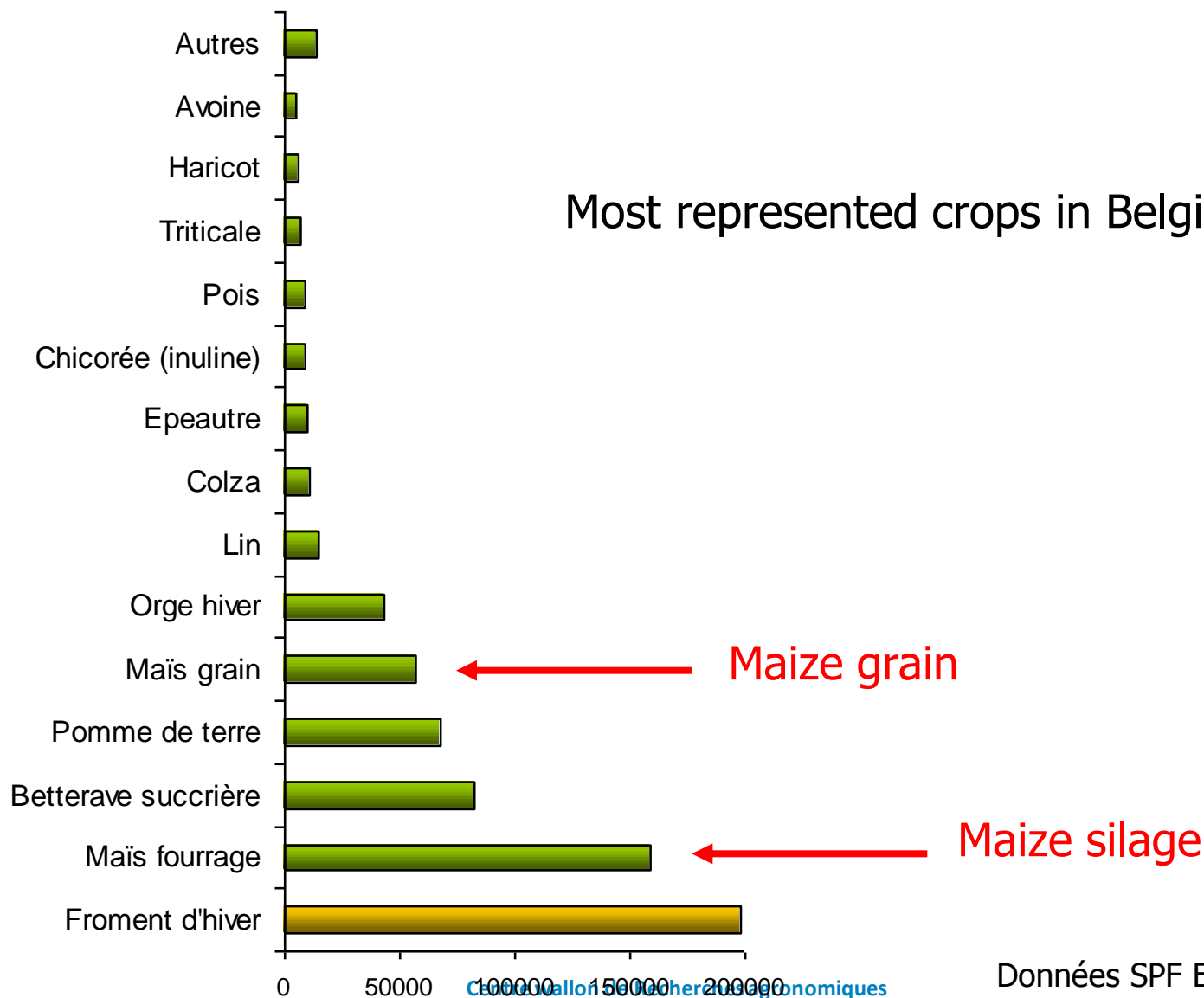
The risk is linked to variety

Infected by maize residues

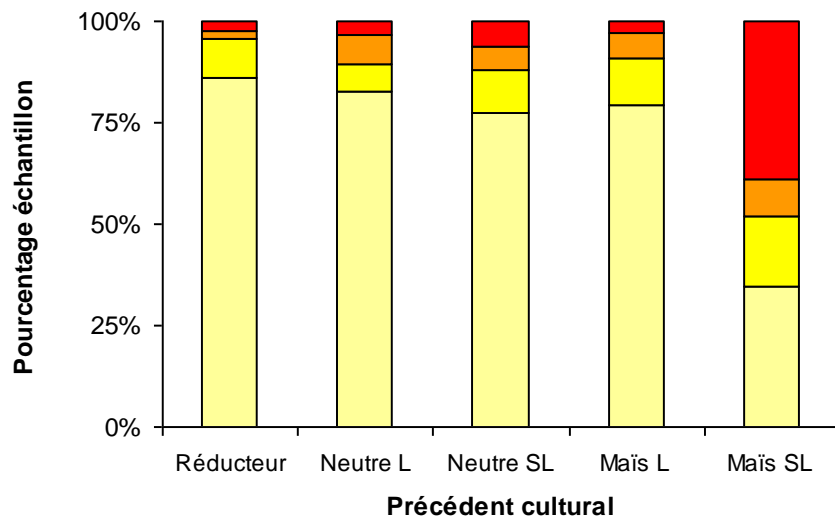


The risk is linked to the previous crop

Most represented crops in Belgium ?



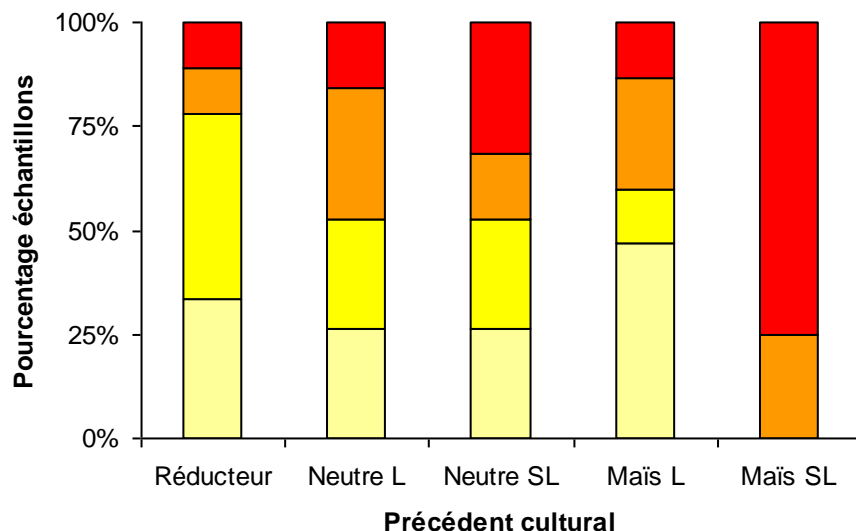
The risk is linked to the previous crop and tillage



Data between
2003-2007 (N=559)

>1000 ppb

- Reduction (rapeseed/flax/pea): 5%
- Neutral (sugar beat, wheat, Potatoes / Maize L (with tillage) : 15 %
- Maize SL (without tillage) : 50%



Data 2007
(N=69)

>1000 ppb

- Redcution (rapeseed/flax/pea): 20%
- Neutral (sugar beat, wheat, Potatoes / Maize L (with tillage) : 45 %
- Maize SL (without tillage) : 100%

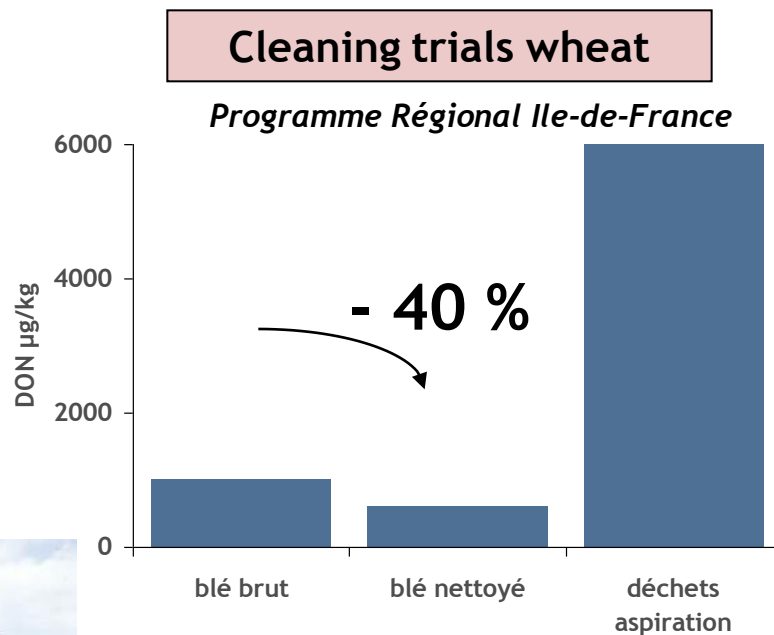
Effet of the cleaning on the DON content in wheat

At the harvest or at the grain trader: Separation of the sound grains



Sound grains
kept

Shriveld
Grains
Disgarded



Small grains
infected

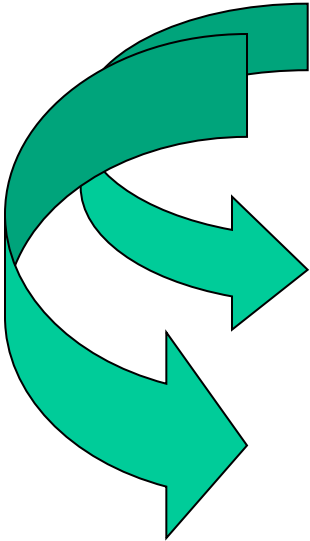
Bruno BARRIER-GUILLOT
ARVALIS - Institut du végétal - France

Reducing the mycotoxin contents through the chain

2. Grain traders

To try to avoid the mixture of sound and contaminated lots

Constraint : ~ 200 lots / day, harvest in 3 to 5 days !!!



Suitable analytical methods

Pre-harvest analysis

Methods at the grain traders



Pre harvest analysis

Pre harvest data communicated to the chain actors

Low risk

(2001, 2003,
2004, 2005
et 2006)

Nothing to do*

Medium Risk

(2002 & 2008)

Analysis before
entering the silo

Rapid test

(Food)**

- *previous crop maize
no tillage

High risk

2007

Analysis before
entering the silo

Rapid test

(Food & Feed)

- previous crop maize no tillage
- Varieties Sensitive

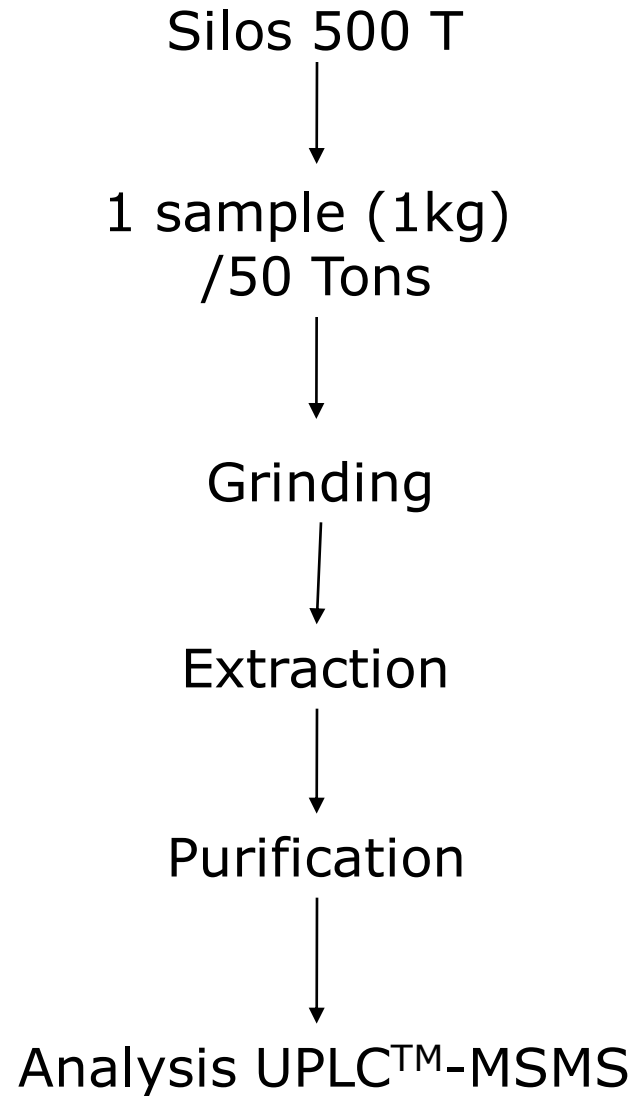
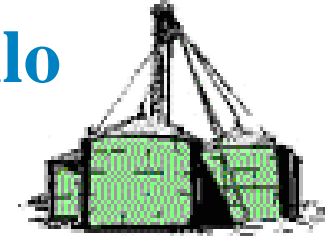
HPLC outlet of the silo

Exception :

babyfood* (200 ppb)

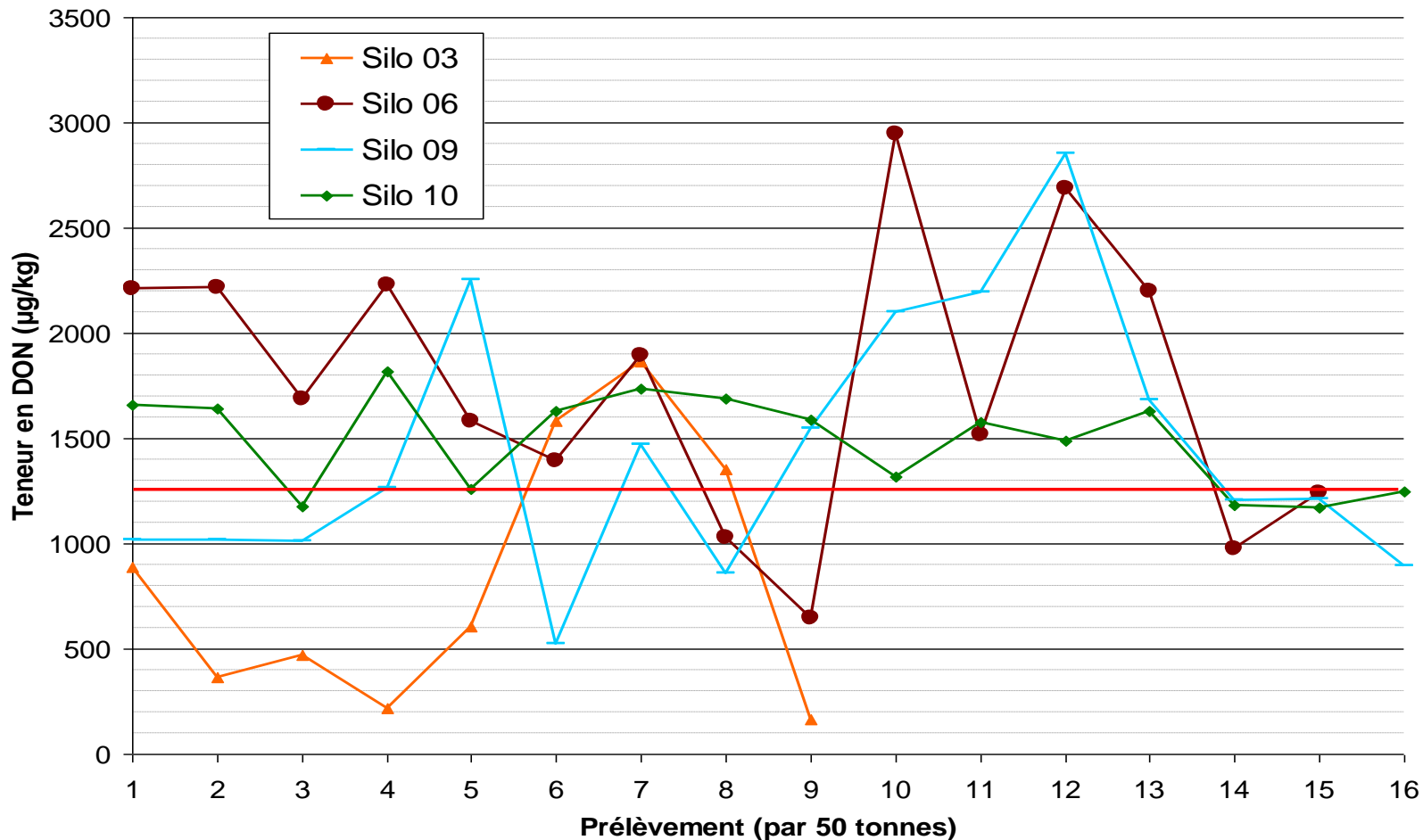
aliment pour porcs** (900 ppb)

Analysis at the outlet of the silo



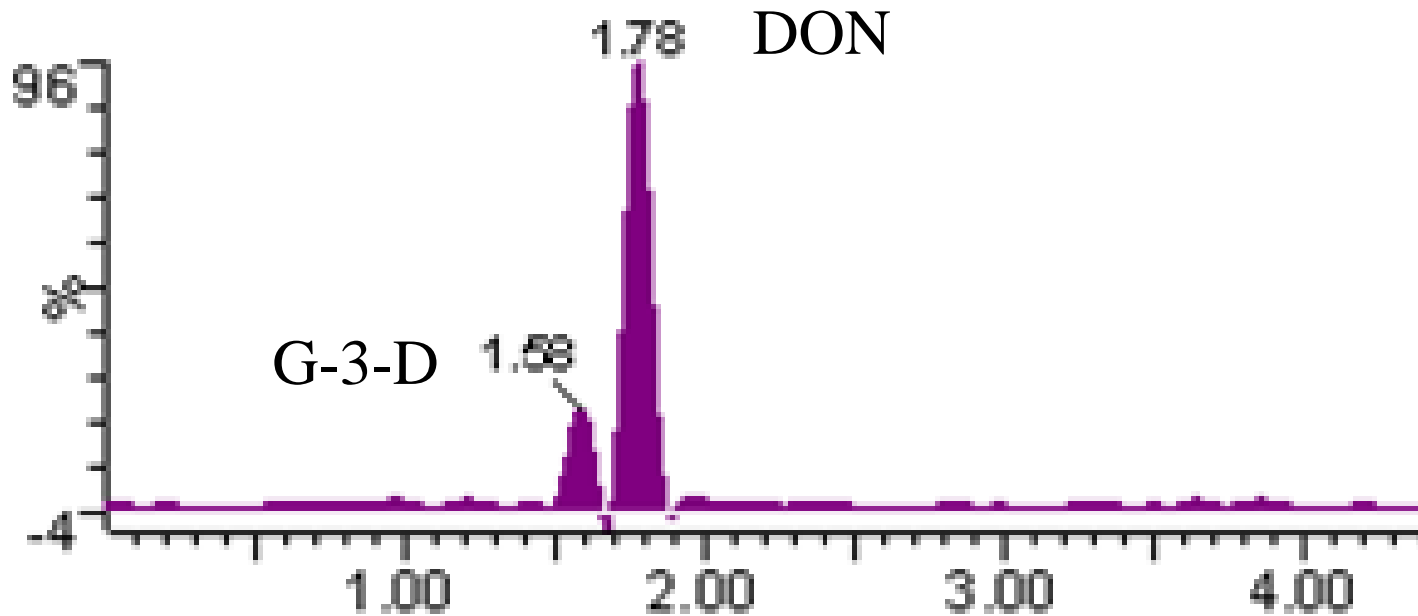
Analysis at the outlet of the silo

DON content ppb



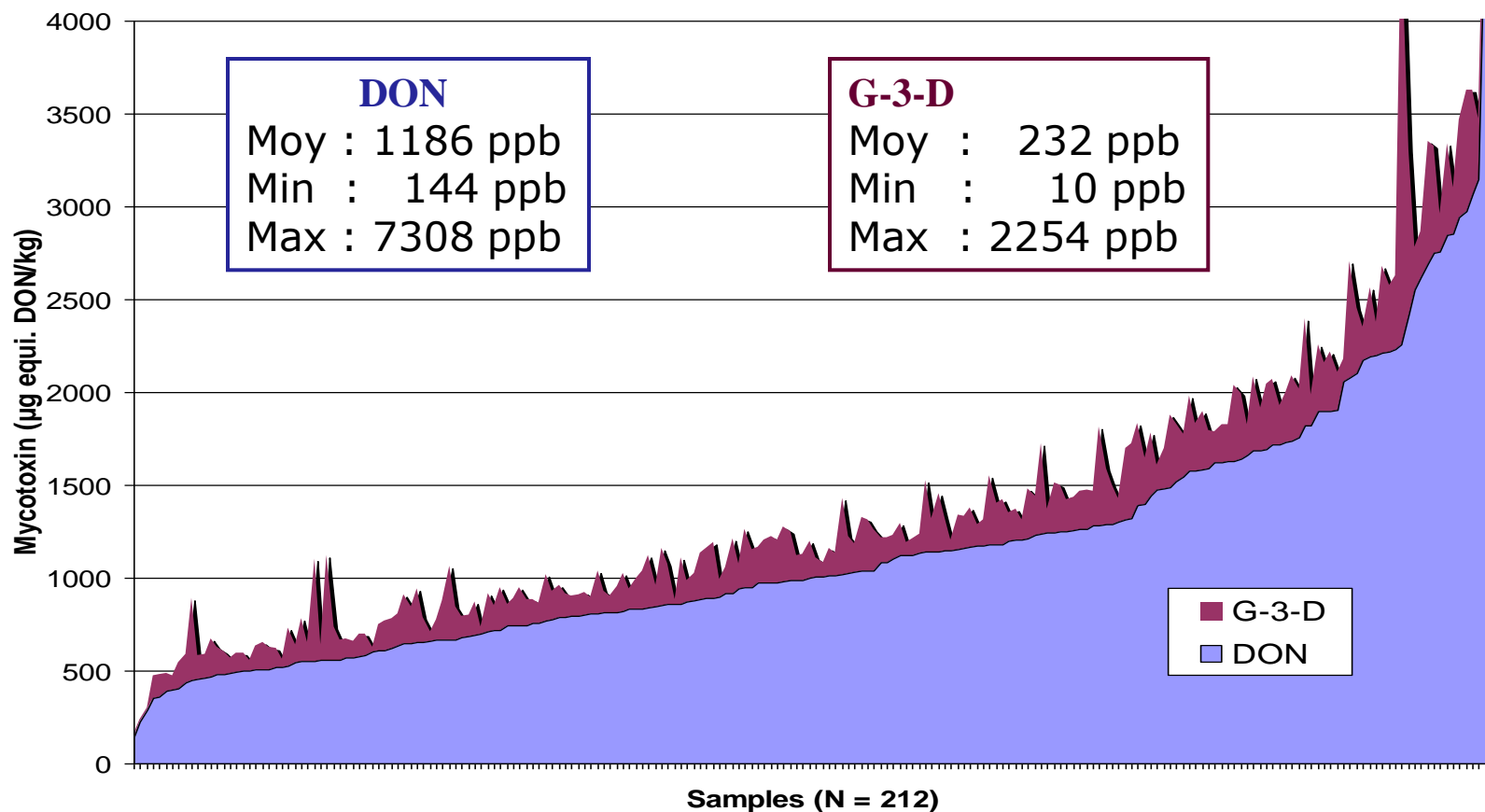
145 deliveries out of 212 (68%) with a DON content < 1250 ppb

Hidden mycotoxins : DON-3-glycoside



- ➡ Beside the DON, another compound : DON-3-G
- ➡ Same transition as DON $297 > 249$ et $297 > 231$
- ➡ DON-3-G may release DON

Outlet of the silo : DON + DON-3-glycoside (ppb)



➡ 116 deliveries out of 212 (55%) DON + DON-3-G < 1250 ppb

Cleaning and sieving the grains



- Removing the dust
- Removing small grains
- Effect of pre-milling process

Masayo Kushihiro

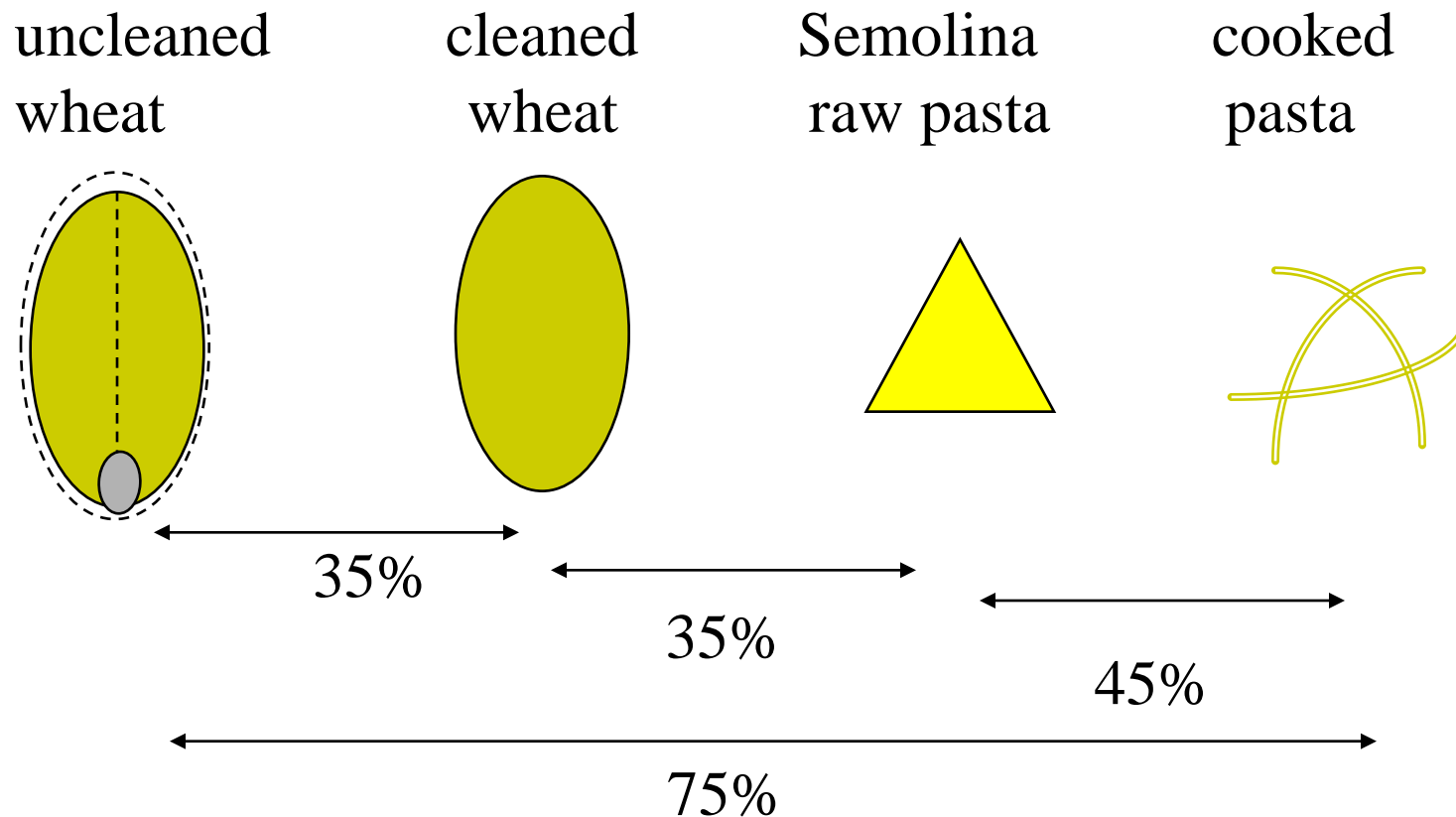
Effects of Milling and Cooking Processes on the Deoxynivalenol Content in Wheat

Int. J. Mol. Sci. 2008, 9, 2127-2145; DOI: 10.3390/ijms9112127

Table 2. Effects of pre-milling processes on the removal of DON.

Wheat	Mycotoxin	Treatment	Effect	Ref.
Hard Red Spring (Canada)	DON (7.1 mg/kg)	cleaning (Carter dockage tester)	DON in cleaned wheat was 4.6 mg/kg, while DON in dockage was 16.7 mg/kg	[25]
Soft White Winter (Canada)	DON	cleaning (Carter dockage tester)	cleaning did not reduce DON level	[26]
Soft wheat (USA)	DON	cleaning (combination of screening and air flow)	DON content was decreased by 16% and screening had 4.7 fold higher DON contents than cleaned wheat	[27]
wheat (USA)	DON (7.9-9.6 mg/kg)	cleaning	The percent reduction found in the cleaned wheat ranged from 6 to 19%.	[28]
Hard Red Winter (USA)	DON (0.64-5.1 mg/kg)	cleaning (Superior cylinder separators) followed by washing (Smico wheat washer)	normal, single cleaning obtained the cleaning efficiency ranging from 48% to 86% depending on DON concentration.	[29]
Canadian Western Red Spring, variety Sinton (Canada)	DON (12.5 mg/kg)	scouring	scouring reduced DON level by 22%	[30]
Canadian Western Amber Durum, variety Coulter (Canada)	DON (9.6 mg/kg)	scouring	without scouring did not affect DON level	[30]
Canadian Eastern White Winter (Canada)	DON	cleaning (Carter C-989 dockage tester) followed by fractionating (SY 300 gravity separator, Spiroll Kipp Kelly Inc.)	DON is highest in the least dense fractions	[31]
wheat naturally contaminated with DON and ZON	DON (2.4 mg/kg)	density segregation by soaking in water and 30% sucrose	removing wheat buoyant in water and then 30% sucrose decreased the DON present by 96%, and reduced the DON content by 96%	[33]
wheat naturally contaminated with DON and ZON	DON (0.6 mg/kg)	density segregation by soaking in water and 30% sucrose	removing wheat buoyant in water and then 30% sucrose decreased the DON present by 68%, and reduced the DON content by 67%	[33]

DON drop from the field to the pasta



Treatment with flash light



Décontamination et détoxification de
surface des produits agricoles par
impulsions lumineuses à haute densité
d'énergie

- *Procédé breveté*
- *Partenariat CTCPA/Montena Technology Suisse*

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Courtesy : CTCPA

Treatment with flash light



PROTOTYPE MONTENA



Courtesy : CTCPA

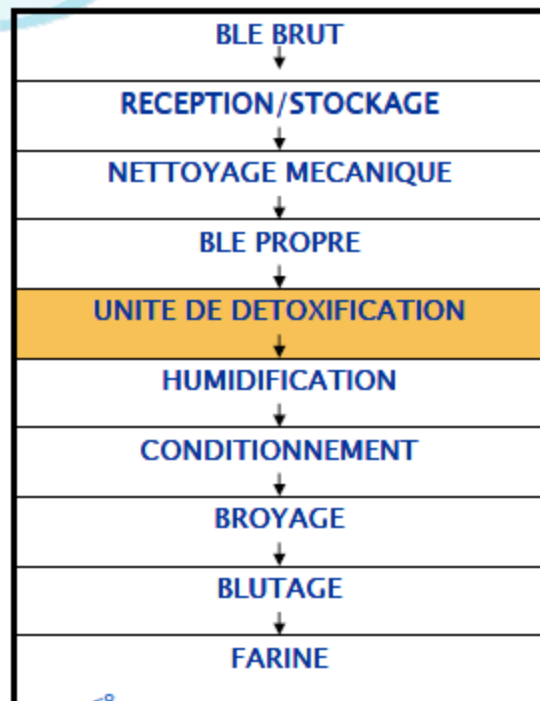
Treatment with flash light

DETOXIFICATION DE MYCOTOXINES (DON&ZEA) SUR BLE

ctcpa

Résultats sur blé en ppb

Diagramme Blé



AQ053 b

Triccho.B	Témoins	T1	T2	T3	Réduc. %
Nivalenol	200	150	85	70	65
DON	5900	4900	3100	1885	68,1
DOM-1 (Dé-epoxy DON)	20	20	15	10	/
Fusarenone X	<10	<10	<10	<10	/
15-O-acetyl-deoxynivalenol	65	50	45	40	/
3-acetyldeoxynivalenol (3 ac DON)	25	25	20	20	/
ZEA et métabolites					
Zéaralénone	180	130	75	55	69,4
Zéaralanol-alpha	<10	<10	<10	<10	/
Zéaralanol-béta	<10	<10	<10	<10	/
Zéaralénol-alpha	<10	<10	<10	<10	/
Zéaralénol-béta	<10	<10	<10	<10	/

Courtesy : CTCPA

XENON

Instantaneous Sterilization with Pulsed UV Light

Workshop

Emerging Food Processing Technologies

USDA, CSREES – Washington State University

26 - 27 May 2005

Louis Panico, CEO

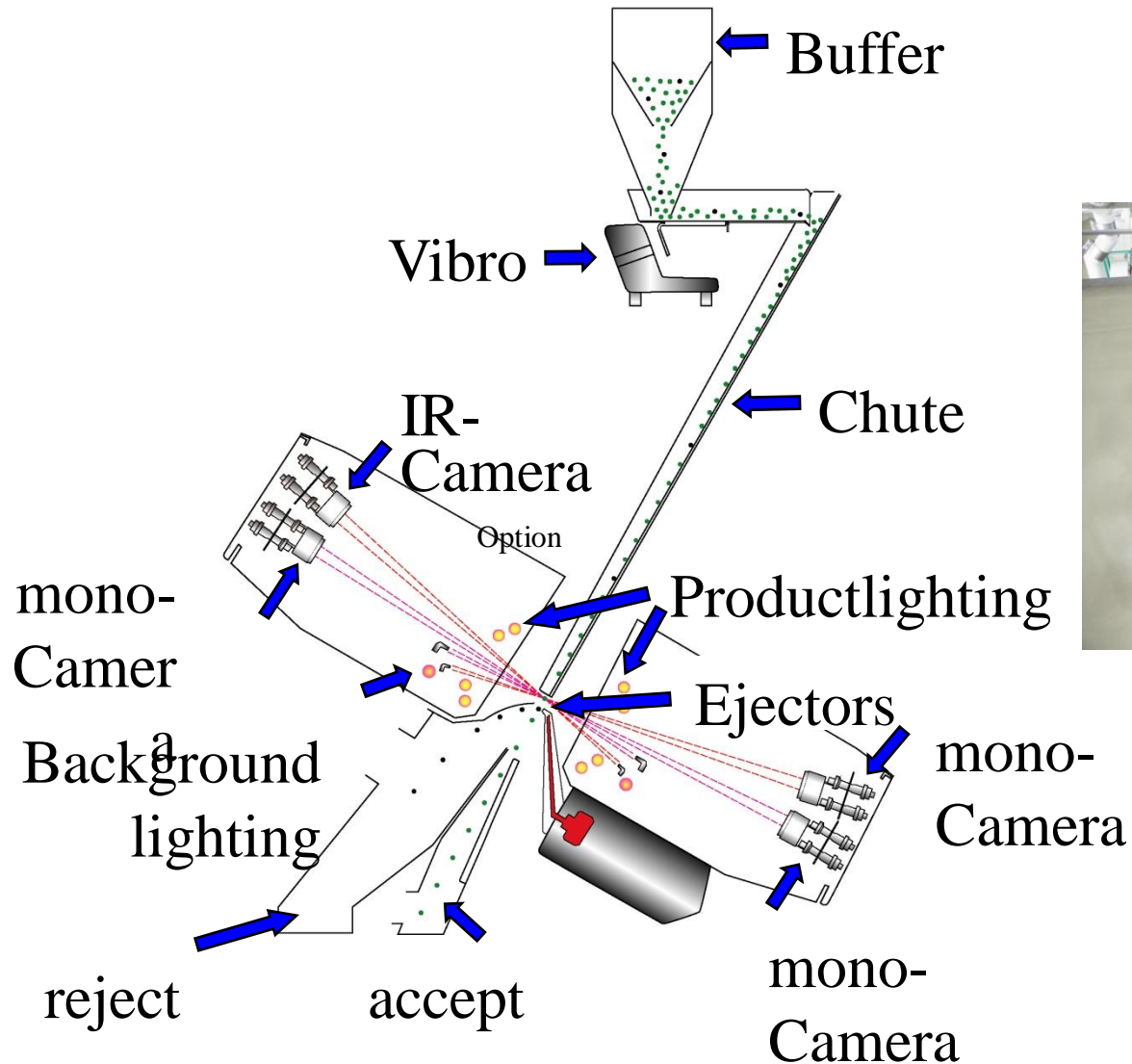
The optical sorter removes contaminations

- Discolored germs
- Seeds
- Fungus damage (Mycotoxines)
- Ergot



Courtesy : Bühler

Design and features of the optical sorter



Courtesy : Buhler

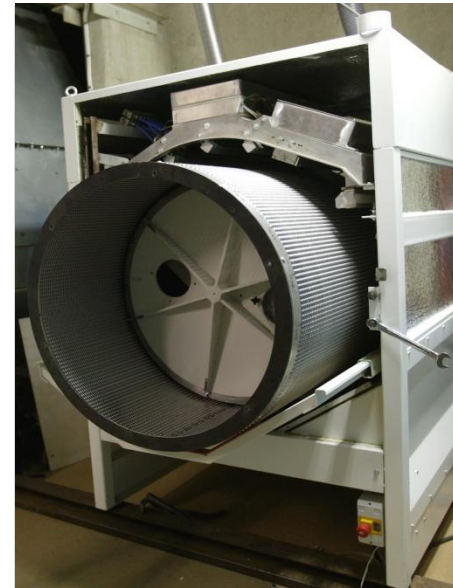
Sorting the grains

Using NIT – Near Infrared Transmittance



IQ Seed Sorter 1002

- 1000+ kernels/min
- 2-3 kg/h
- 6 quality fractions



TriQ 30

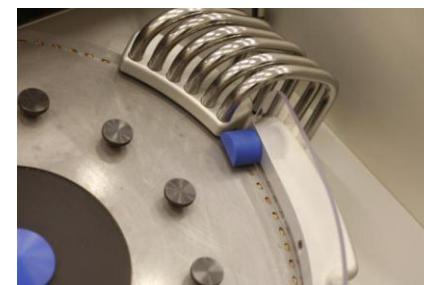
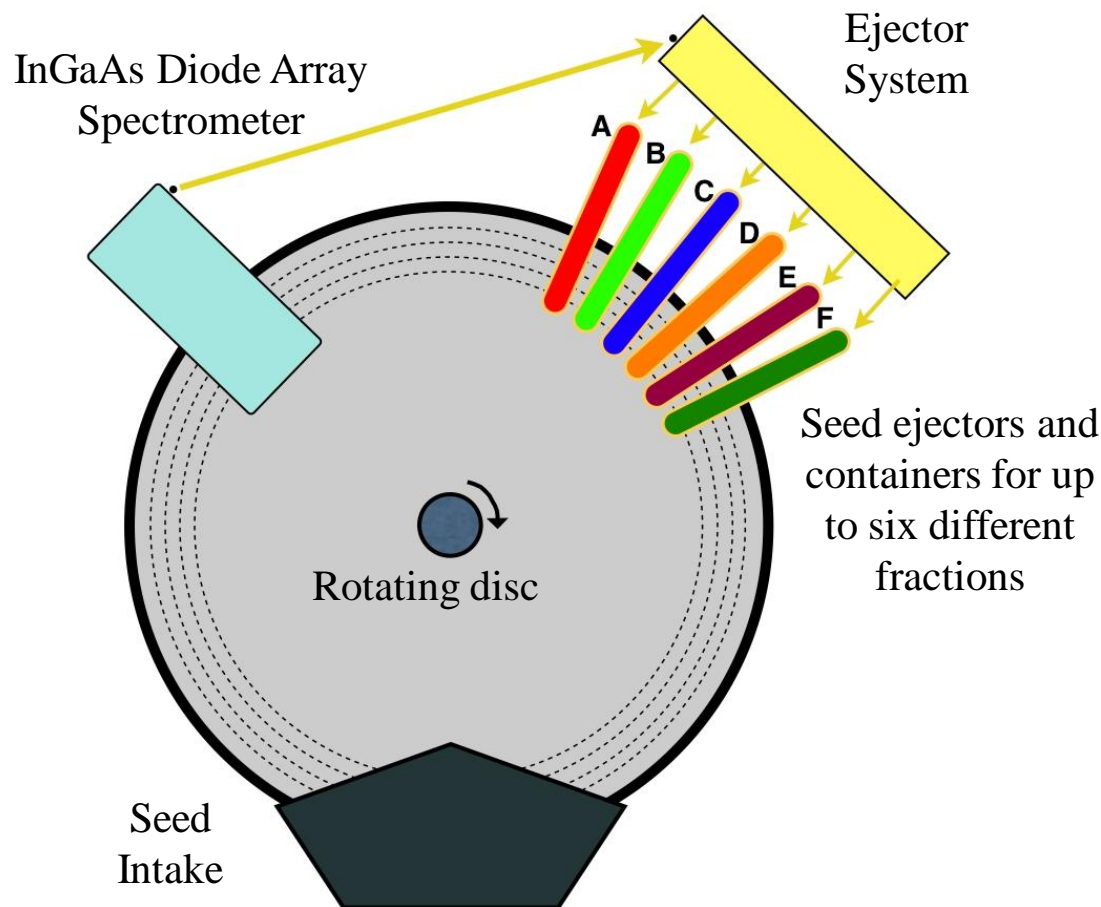
- 1 800 000 kernels/min
- 3 tonnes/h
- 3 quality fractions

Courtesy

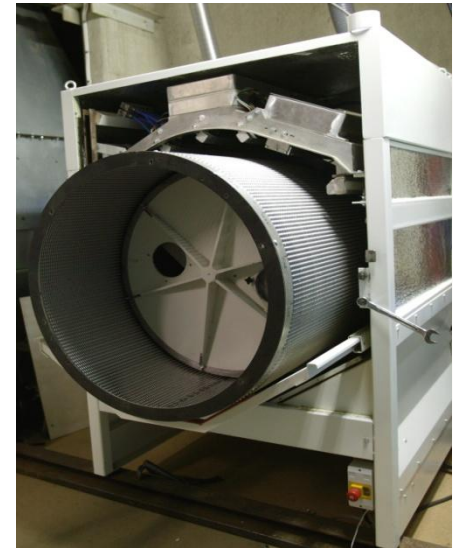
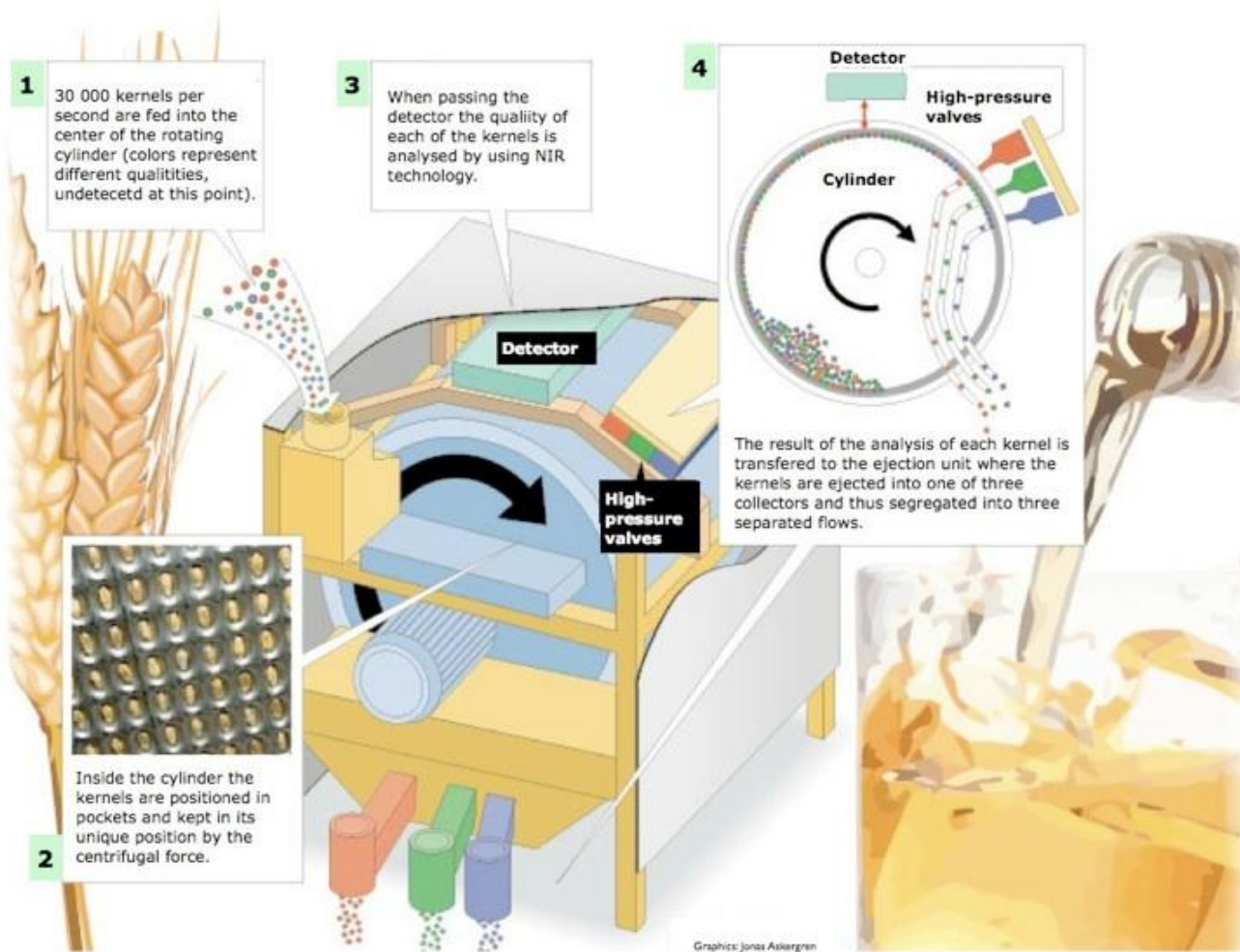


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The functionality of the IQ sorter



TriQ 30 – 3 tonnes/hour and module



Courtesy



Fusarium infected wheat

Sample	Unsorted	Fraction 1	Fraction 2	Fraction 3
1 DON Yield	1390 ppb	9600 ppb 3.1%	720 ppb 64.4%	290 ppb 32.5%
2 DON Yield	1470 ppb	2710 ppb 1.9%	335 ppb 47.5%	120 ppb 50.5%
3 Don Yield	870 ppb	4040 ppb 4.5%	510 ppb 21.7%	< 100 ppb 73.7%

Fractionation, milling



Masayo Kushiro

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Table 3. DON distribution and reduction after milling.

Wheat	Mycotoxin	Treatment	Effect	Ref.
Hard Red Spring (Canada)	DON (4.6 mg/kg)	milling (Allis-Chalmers laboratory mill)	DON in 75% yield straight-grade flour was 4.1mg/kg, while 4.6, 6.9, 8.0 ppm in bran, shorts, and feed flour (red dog), respectively	[25]
Soft White Winter (Canada)	DON (0.42-0.62 mg/kg)	milling (industrially milled and pilot-milled)	milling led to a fractionation of DON, with increased levels in the outer kernel (bran, 0.98 mg/kg, for example) portions and decreased levels in the inner flour portions (break flour, 0.28 mg/kg)	[26]
soft Wheat, variety Pike, Hart, Stadler, Oasis, Pioneer, Mcnair, Arthur (USA)	DON (0.03-3.35 mg/kg)	dry milling (Miag Multomat mill)	DON was found in all fractions, which included straight-grade flour, four break flours, six reduction flours, break and reduction shorts, red dog, and bran. Mean DON concentration in straight-grade flour was about 90% of that in cleaned wheat.	[27]
wheat (USA)	DON (7.9-9.6 mg/kg)	milling (Bühler automatic laboratory mill Model MLU-202)	DON was found throughout all the milling fractions. The greatest (21 mg/kg) concentration of DON was found in the bran, and the smallest (1 mg/kg) was found in the break flour.	[28]
Hard Red Winter (USA)	DON (0.64-5.1 mg/kg)	milling (Miag Multomat Mill)	all mill fractions contained DON with lower concentration in flours and higher concentration in offals compared to cleaned wheat, DON concentration of straight-grade flour was 75% of cleaned wheat	[29]
Canadian Western Red Spring, variety Sinton (Canada)	DON (12.5 mg/kg)	milling (Bühler laboratory mill Model MLU-202)	mould counts highest in bran, 29% of DON was retained in flour	[30]
Canadian Western Amber Durum, variety Coulter (Canada)	DON (9.6 mg/kg)	milling (Bühler laboratory mill Model MLU-202)	mould counts highest in shorts, 52% of DON was retained in semolina	[30]

Conclusions

- ➡ **Fusariotoxins are largely dependent on the weather conditions**
- ➡ **Have to take into account several mycotoxins**
Regarding feed the regulations will be more severe (species)
- ➡ **Take the mycotoxin sensitivity**
for the inscription of the varieties
- ➡ **Act early in the chain**
- ➡ **Requirements for harmonisation of the methods**
- ➡ **Hidden mycotoxins?**

The mycotoxins iceberg

