Detection and quantification of ergot in cereals by near infrared hyperspectral imaging

Philippe Vermeulen, Juan Antonio Fernández Pierna, Pierre Dardenne, Vincent Baeten (CRA-W, Belgium)

Hans van Egmond (RIKILT, the Netherlands)

Albert Swinkels (NUTRECO, the Netherlands)

Rotterdam, 8 November 2012

www.conffidence.eu

What is the problem?

- Reemerging of the ergot presence in cereals
  - For the farmer,

    yield reduction (10%)

  - For the feed/food sector,

    high toxicity risk for animal and human

Ergot in 1997

by Gary Munkvold, extension plant pathologist, Department of Plant Pathology

Last year, barley producers in northeast Iowa suffered a serious outbreak of ergot, a fungal disease that can affect any small [grain] and many grass species. The fungus is called *Claviceps purpurea*, and it produces dark scl... ...ent (see photo). These sclerotia overwinter in or on soil and produce s...

ST. PAUL, MN (June 8, 1998) — Sorghum, an extremely important cereal crop worldwide, has developed a serious enemy, ergot. This fungal disease has plant pathologists working intensely to accumulate information and develop strategies to combat the disease which can cause severe crop loss and economic hardship.

Rye ergot - *Claviceps purpurea*. Ergot is the most frequently mentioned undesirable substance. laboratories reported either numbers of evaluated samples, or frequencies of occurrence, or both (Appendix 2, section 2). A frequency of occurrence of < 5 % for two... effectively an occurrence of 0 %. Two mem... all other cases (... remark was ma...

Vet Rec. 1986 Jan 11;118(2):48-9.

An outbreak of ergotism in Ethiopia in 1978 resulted from exposure to ergolines from *C. purpurea* sclerotia. The grain contained up to 0.75% ergot;

Outbreak of bovine abortion attributed to ergot poisoning.

Appleyard WT.

Eleven out of 36 suckler cows, all in late pregnancy, aborted seven to 10 days following introduction to a rye grass pasture heavily infested with ergot. On the basis of known h... exposure to ergot infested grass and negative findings in a range of other in... details of which are given, a diagnosis of ergotism was made.

ERGOT IN CEREAL CROPS, GRASSES POSES THREAT TO LIVESTOCK
From: US Fed News Service, Including US State News | Date: June 21, 2007

PMID: 3946070 [PubMed - indexed for MEDLINE]

What is ergot ?

- Ergot is a sclerotium 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

SCIENTIFIC / TECHNICAL REPORT submitted to EFSA

CFP/EFSA/CONTAM/2008/01

Scientific information on mycotoxins and natural[1] plant toxicants

Prepared by Battilani P.[a], Costa L.G.[b], Dossena A.[c], Gullino M.L.[d], Marchelli R.[c], Galaverna G.[c], Pietri A.[a], Dall'Asta C.[c], Giorni P.[a], Spadaro D.[d], Gualla A.[a]

[a]Università Cattolica del Sacro Cuore, Faculty of Agriculture, Via Emilia Parmense 84, 29100 Piacenza; Italy

[b]University of Parma, Faculty of Medicine, Section of Pharmacology, Via Volturno 39, 43100 Parma; Italy

[c]University of Parma, Department of Organic and Industrial Chemistry, Via Università 12, 43100 Parma, Italy

[d]Centre of Competence for the Innovation in the Agro-environmental Sector (AGROINNOVA), University of Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy,

What about the legislation?

- The concentration of ergot body in cereals is for **animal,** restricted to

  1000 mg per Kg in feedingstuffs containing unground cereals

---

L 140/10 — EN — Official Journal of the European Communities — 30.5.2002

DIRECTIVE 2002/32/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 7 May 2002
on undesirable substances in animal feed

30.5.2002 — EN — Official Journal of the European Communities — L 140/17

Undesirable substances	Products intended for animal feed	Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12%
(1)	(2)	(3)
10. Theobromine	Complete feedingstuffs with the exception of:	300
	— complete feedingstuffs for adult cattle	700
11. Volatile mustard oil	Feed materials with the exception of:	100
	— rapeseed cakes	4 000 (expressed as allyl isothiocyanate)
	Complete feedingstuffs with the exception of:	150 (expressed as allyl isothiocyanate)
	— complete feedingstuffs for cattle, sheep and goats (except young animals)	1 000 (expressed as allyl isothiocyanate)
	— complete feedingstuffs for pigs (except piglets) and poultry	500 (expressed as allyl isothiocyanate)
12. Vinal thiooxazolidone (Vinyloxazolidine thione)	Complete feedingstuffs for poultry with the exception of:	1 000
	— complete feedingstuffs for laying hens	500
13. Rye ergot (Claviceps purpurea)	All feedingstuffs containing unground cereals	1 000
14. Weed seeds and unground and uncrushed fruits containing alkaloids, glucosides or other toxic substances separately or in combination including	All feedingstuffs	3 000
(a) Lolium temulentum L.,		1 000
(b) Lolium remotum Schrank,		1 000
(c) Datura stramonium L.		1 000

Control of ergot contamination?

- **In the field**:
  - Crop rotation
  - Varietal resistance

- **In the grain industry**: detection of ergot bodies
  - Modern cleaning machinery
  - Microscopy method (IAG method)
  - Imaging system: CONffIDENCE

- **In the mills**: detection of alkaloids
  - Methods of analysis: LC-FLD and LC-MS/MS

Status of the analytical aspects?

- The existing microscopy method provides an elegant early warning tool for ergot contamination but is time-consuming

Method for the Determination of Ergot (*Claviceps purpurea* Tul.) in Animal Feedingstuff, IAG-Method A4

International Association of Feedingstuff Analysis

Section Feedingstuff Microscopy

New project CONffIDENCE

Increasing of ergot occurrence

and no rapid method available

involved

new project CONffIDENCE

- One of the objectives :

Ergot detection by NIR Hyperspectral imaging

- T.4a.7 : Development of imaging method, validation and comparison with existing method
- T.4a.8 : Transfer of the imaging method to the feed sector (NUTRECO)

What is hyperspectral imaging?

Spatial information

NIR imaging instrument

Frequency information (i.e. wavelengths)

Intensity information (i.e. absorbance)

Calibration process
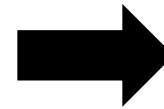
Data-base
Ergot – Cereals spectra

Reference values → Mathematical model
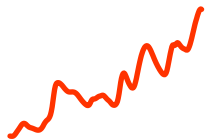
Unknown spectrum + Mathematical model → Predicted values

NIR line scan camera: instrument

NIR camera

Light source

SWIR ImSpector N25E spectral camera
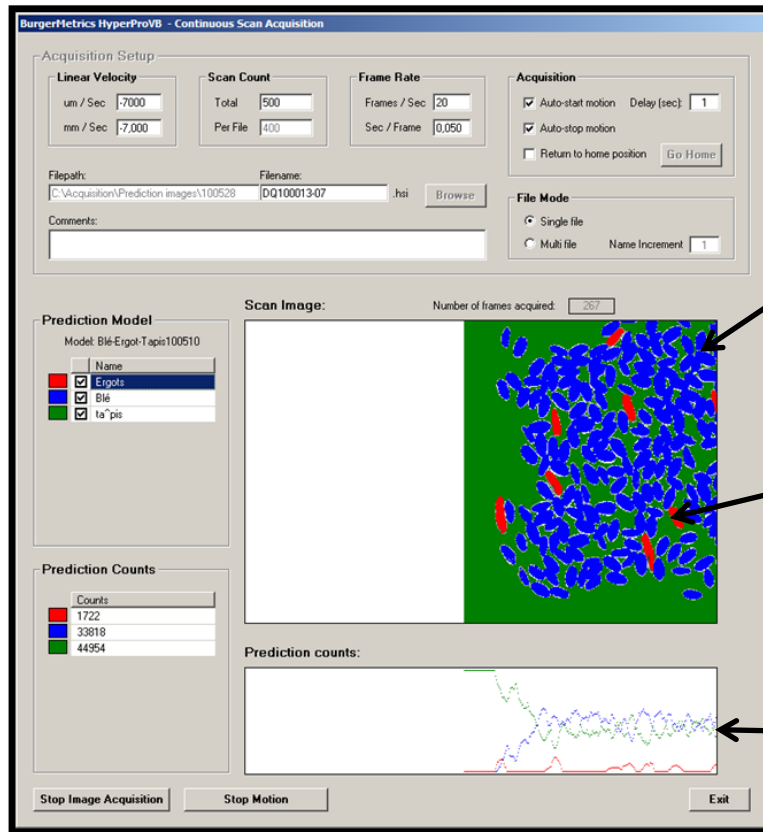(Specim Ltd)

Conveyor belt (Burgermetrics)
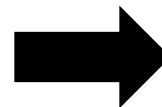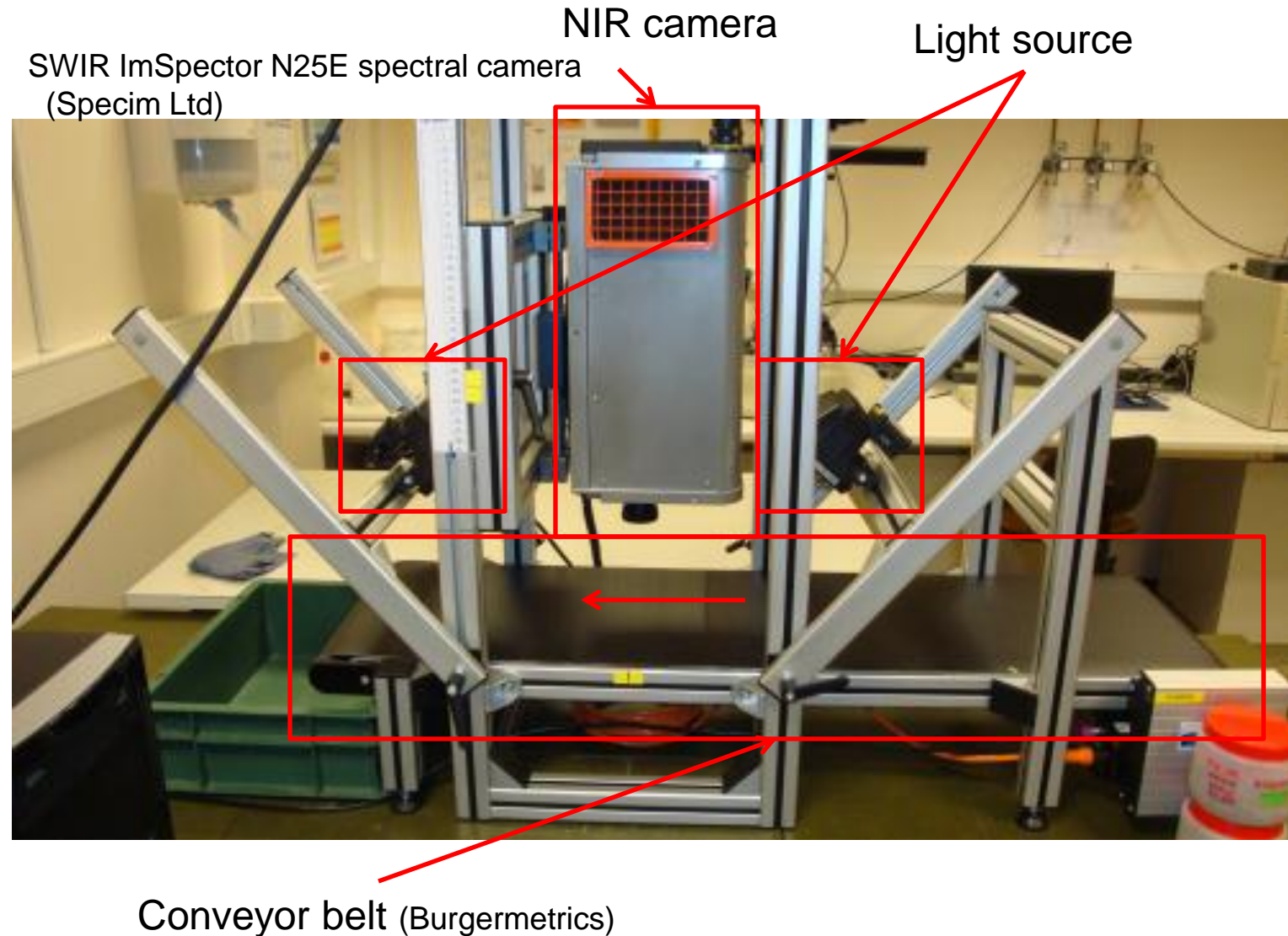
NIR line scan camera: features

- ## NIR camera setup

push-broom (line) **scan**
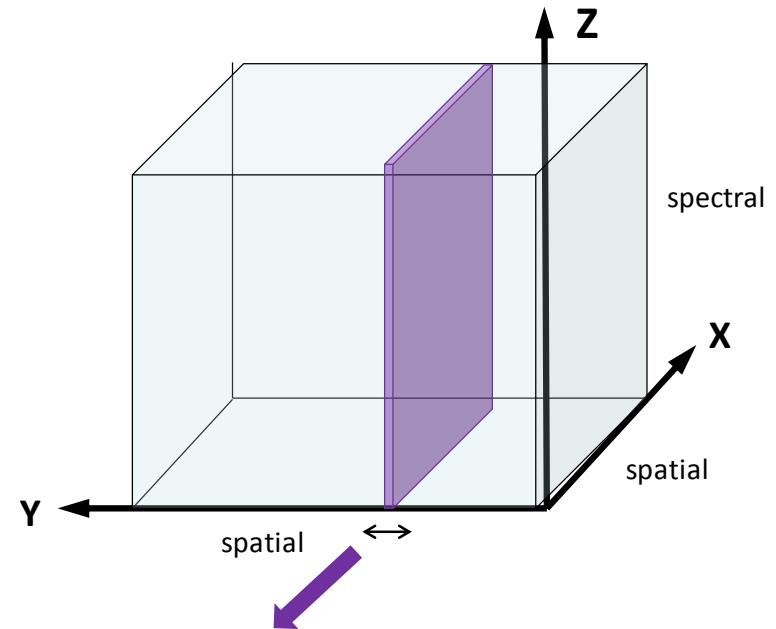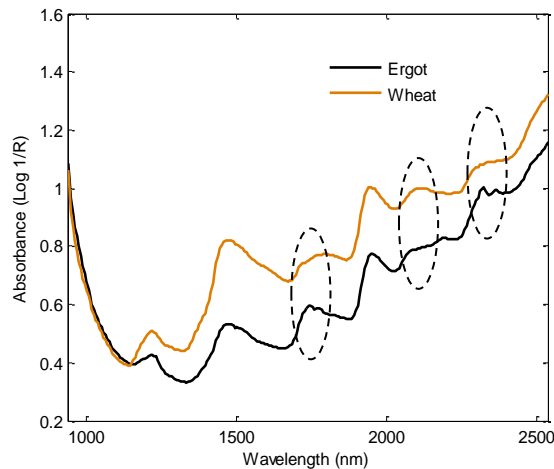
- Wavelength range: 1000-2500 nm by step of 6 nm
- 1 line = 320 pixels = 320 spectra
- Analysed surface = continuous
- Time of acquisition = 50 millisec/pixel line

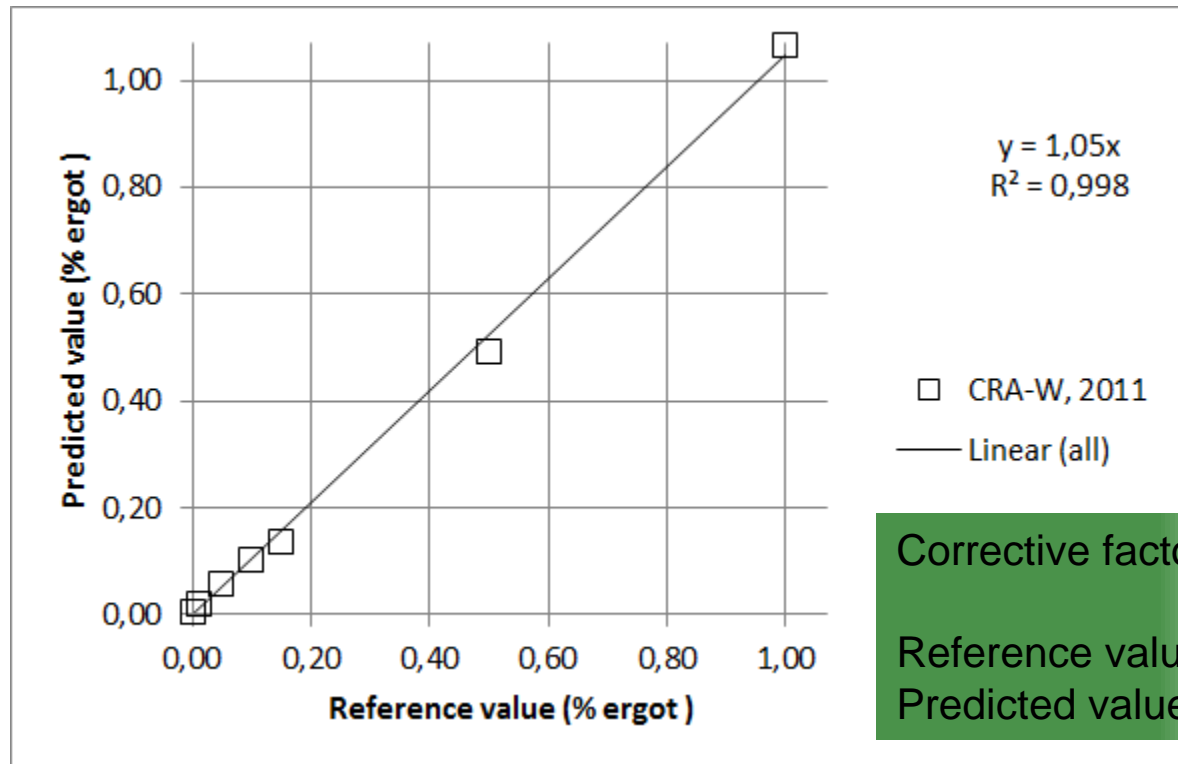# Ergot detection in wheat by NIR imaging

Wheat kernels

Ergot Bodies

The number of pixels counted for each class of the model is also provided.

Analytical parameters used and on-line prediction results of the PLSDA (Partial Least Squares Discriminant Analysis) model

Results of ergot bodies detection

On set of 7 laboratory samples with 0 0,01 0,05 0,1 0,15 0,5 and 1%
of ergot in cleaned wheat

$y = 1,05x$
$R^2 = 0,998$

□ CRA-W, 2011
— Linear (all)

Corrective factor weight/area

Reference value: % weight
Predicted value: % area * 0,8

Peer reviewed Journal article

Online detection and quantification of ergot bodies in cereals using near infrared hyperspectral imaging

Ph. Vermeulen[a]*, J.A. Fernández Pierna[a], H.P. van Egmond[b], P. Dardenne[a] and V. Baeten[a]

[a]*Food and Feed Quality Unit (U15), Valorisation of Agricultural Products Department (D4), Walloon Agricultural Research Centre (CRA-W), Henseval Building, 24 Chaussée de Namur, 5030 Gembloux, Belgium;* [b]*Cluster Natural Toxins and Pesticides, RIKILT-Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE Wageningen, The Netherlands*
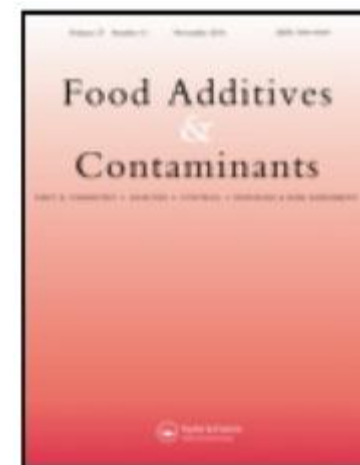
The occurrence of ergot bodies (sclerotia of *Claviceps purpurea*) in cereals presents a high toxicity risk for animals and humans due to the alkaloid content. To reduce this risk, the European Commission fixed an ergot concentration limit of 0.1% in all feedstuffs containing unground cereals, and a limit of 0.05% in 'intervention' cereals destined for humans. This study sought to develop a procedure based on near infrared hyperspectral imaging and multivariate image analysis to detect and quantify ergot contamination in cereals. Hyperspectral images were collected using an NIR hyperspectral line scan combined with a conveyor belt. All images consisted of lines of 320 pixels that were acquired at 209 wavelength channels (1100–2400 nm). To test the procedure, several wheat samples with different levels of ergot contamination were prepared. The results showed a correlation higher than 0.99 between the predicted values obtained using chemometric tools such as partial least squares discriminant analysis or support vector machine and the reference values. For a wheat sample with a level of ergot contamination as low as 0.01 %, it was possible to identify groups of pixels detected as ergot to conclude that the sample was contaminated. In addition, no false positives were obtained with non-contaminated samples. The limit of detection was found to be 145 mg/kg and the limit of quantification 341 mg/kg. The reproducibility tests of the measurements performed over several weeks showed that the results were always within the limits allowed. Additional studies were done to optimise the parameters in terms of number of samples analysed per unit of time or conveyor belt speed. It was shown that ergot can be detected using a speed of 1–100 mm/s and that a sample of 250 g can be analysed in 1 min.

Keywords: ergot; contaminant; alkaloid; cereal; feed; food; NIR hyperspectral imaging; multivariate imaging analysis

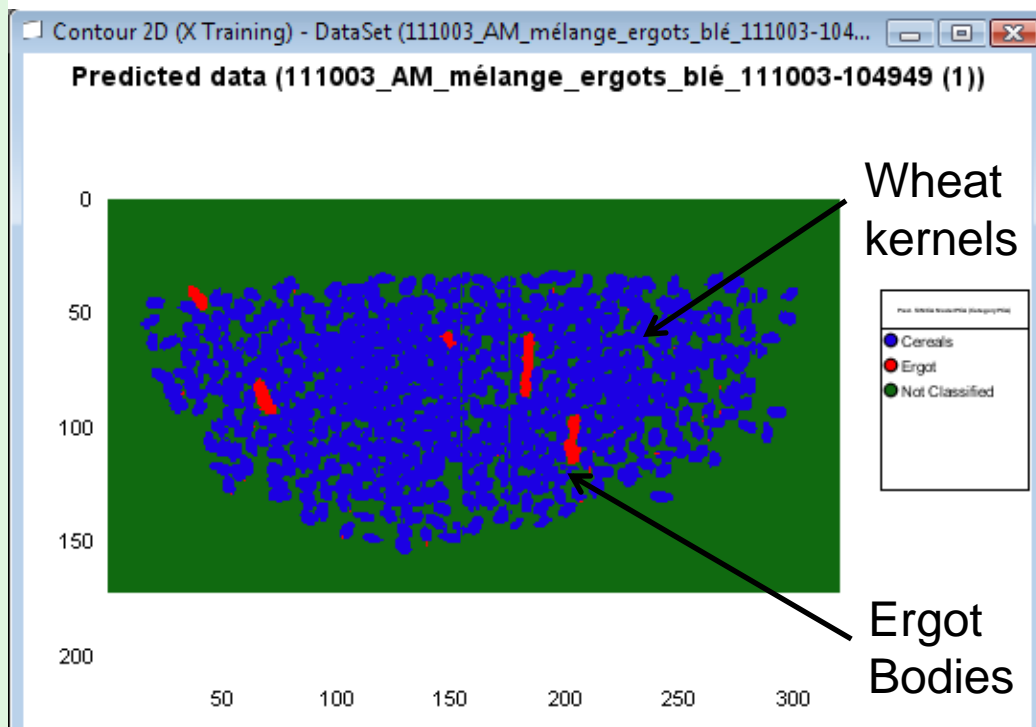# NIR line scan camera: instrument in demonstration at NUTRECO

NIR camera
SWIR
ImSpector
N25E
Spectra
Camera
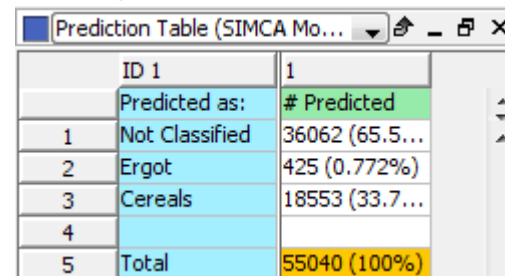(Specim Ltd)

Light source

Tray

Ergot detection in wheat by NIR imaging

Prediction results of the SIMCA (Soft Independent Method of Class Analogy) model

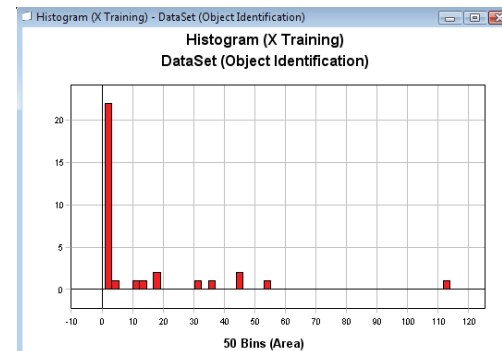The number of pixels counted for each class of the model and … (**object quantification**)

	ID 1	1
	Predicted as:	# Predicted
1	Not Classified	36062 (65.5…)
2	Ergot	425 (0.772%)
3	Cereals	18553 (33.7…)
4		
5	Total	55040 (100%)

… the distribution of groups of pixels detected as ergot are also provided (**object identification**)

Results of ergot bodies detection

Set of 7 samples (2009-2010)
wheat, rye
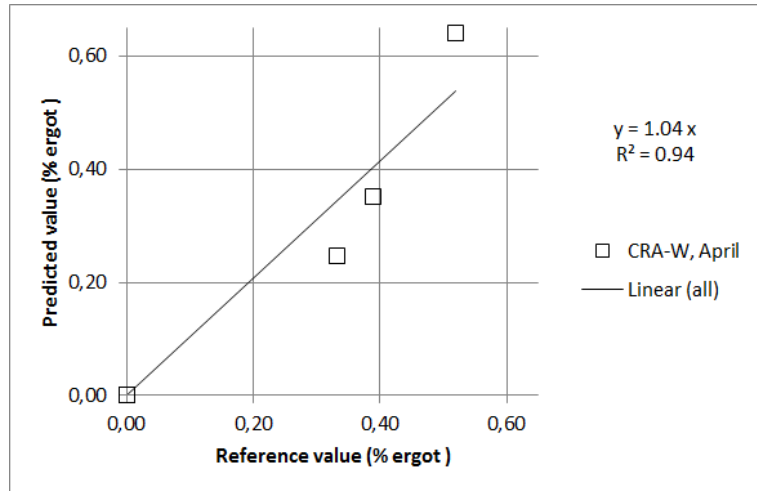
Set of 6 samples (2011)
rye, triticale, oat

BurgerMetrics Instrument (Pilot imaging system)

Corrective factor weight/area

Reference value: % weight
Predicted value: % area * 0,8

SisuChema Instrument (Commercial imaging system)
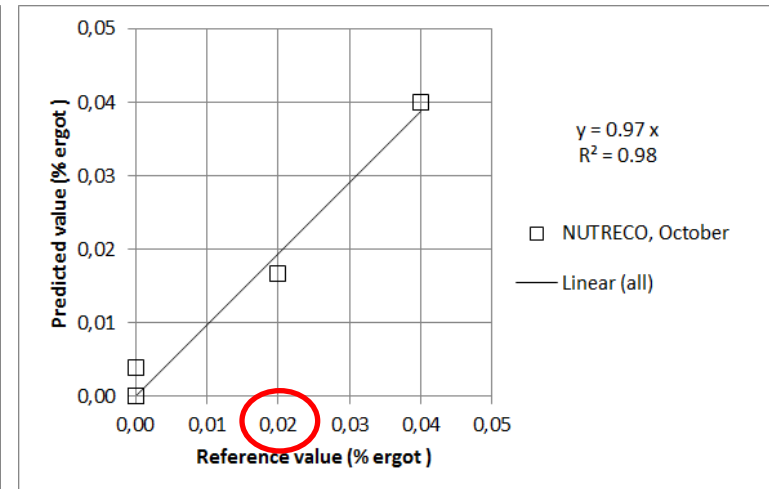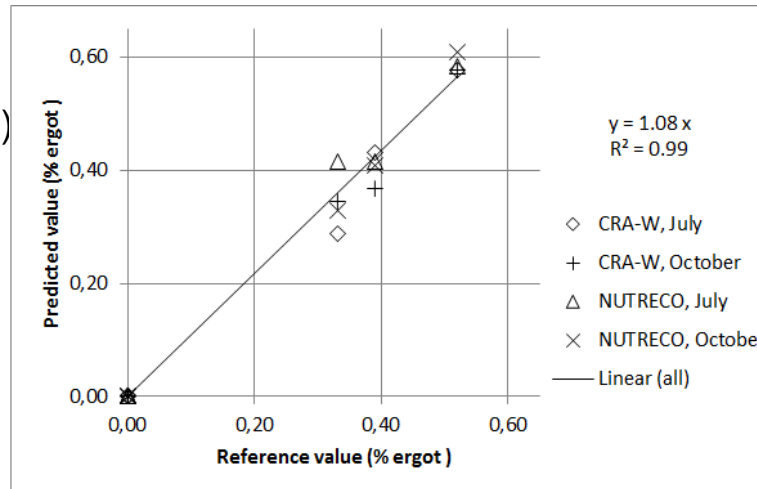
Validation and transferability study of a method based on near infrared hyperspectral imaging for the detection and quantification of ergot bodies in cereals.

Ph. Vermeulen[a*], J.A. Fernández Pierna[a], H. P. van Egmond[b], J. Zegers[c], P. Dardenne[a] and V. Baeten[a]

[a] Food and Feed Quality Unit (U15), Valorisation of Agricultural products Department (D4), Walloon Agricultural Research Centre (CRA-W), Henseval Building, 24 Chaussée de Namur, 5030 Gembloux, Belgium

[b] Cluster Natural Toxins and Pesticides, RIKILT - Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE Wageningen, The Netherlands

[c] Masterlab Analytical Services, NUTRECO, Veerstraat 38, P.O. Box 1, 5830 MA Boxmeer, The Netherlands

Under reviewing

Further developments

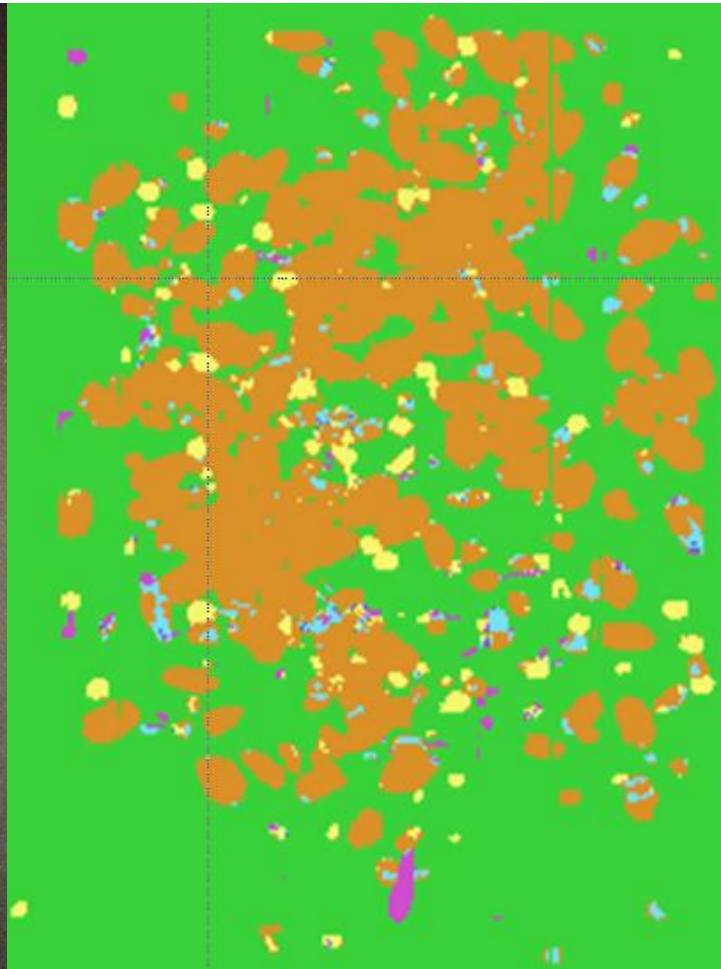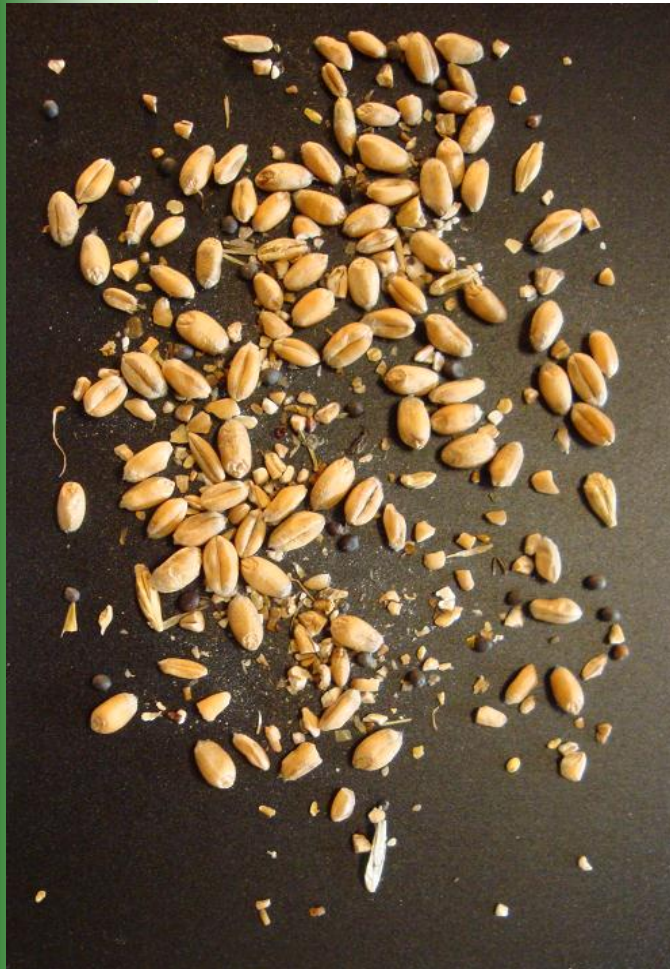- Multicontaminants detection: ergot, datura, …

Ergot in black oat

Mixture of wheat, black oat
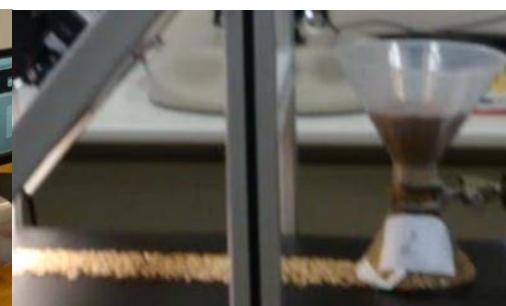Rape seed, ergot and datura

Video

Further developments

- Impurities quantification: straw, weed seeds, broken grains, germinated seeds

- 🟢 Background
- 🔵 Germinated seeds
- 🔵 Broken wheat
- 🟡 Weed seeds
- 🟠 Wheat kernels
- 🟣 Straw, husks

Benefits of the method for a feed Company

Classical microscopy	NIR hyperspectral imaging
High skilled personal	Low skilled personal
45 min / 250g	A few minute / 250 g
Reduced samples	Large samples (sampling more representative)
Dedicated to ergot	Multiple contaminants

Thank you for your attention

More information

Contacts:

Ph. Vermeulen - V. Baeten (CRA-W)

vermeulen@cra.wallonie.be

baeten@cra.wallonie.be

You are welcome to
CONffIDENCE cluster workshop
During the lunch break
8 November 2012
12H30 – 14H00

www.conffidence.eu