Determination of pyrrolizidine, tropane and ergot alkaloids in honey, feed and cereals; *and detection of ergot contamination in cereals*

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Patrick Mulder, Noan Nivarlet,
Albert Swinkels, Philippe Vermeulen

RAFA Prague, 3 November 2011

SEVENTH FRAMEWORK PROGRAMME

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64°
66°
68°
70°
72°
74°
36°
34°
32°
30°
62°
Amu Darya
Darya-ye Panj
Murghob
Murgab
Tedzhen
Gushgy
Morghab
Harirud
Helmand
Harut
Farah
Khash
Arghandab
Tarnak
Rowd-e Lurah
Pamir
Kunar
Indus
Gowd-e Zereh
Hindu Kush
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Kerki
Kiroya
Termiz
Qurghonteppa (Kurgan-Tyube)
Dusti
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Keleft
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Kunduz
Taluqan
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Fayzabad
Jorm
Eshkashem
Dowlatabad
BALKH
Khanabad
TAKHAR
Farkhar
Baghlan
Shulgarah
Sari Pul
Samangan
Dowshi
Meymaneh
FARYAB
Tokzar
SAMANGAN
BAGHLAN
Qeysar
SARI PUL
Khavak
PANJSHER
Mahmud Raqi
NURISTAN
Nuristan
KUNAR
Gushgy
Towraghondi
BADGHIS
Taybad
Qala-I-Naw
BAMYAN
Charikar
PARWAN
KAPISA
Asadabad
Mehtarlam
LAGHMAN
Dowlat Yar
Chaghcharan
Bamyan
Hirat
Karokh
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Meydan Shahr
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Khost
Qarah Bagh
Bannu
Anar Darreh
Khas Uruzgan
Sharan
URUZGAN
FARAH
PAKTIKA
Farah
Now Zad
Tirin Kot
Kajaki
ZABUL
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Zabol
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Spin Buldak
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NIMROZ
Deh Shu
Chehar Borjak
Quetta
Zahedan
Gilgit
The boundaries and names shown and the designations used on this map do not imply official endorsement or acceptance by the United Nations.
Dotted line represents approximately the Line of Control in Jammu and Kashmir agreed upon by India and Pakistan. The final status of Jammu and Kashmir has not yet been agreed upon by the parties.
0 50 100 150 200 250 km
0 50 100 150 mi
National capital
Provincial capital
Town, village
Airports
International boundary
Provincial boundary
Main road
Secondary road
Railroad

Gulran district

- Approx. 150 km², approx. 110.000 inhabitants
- Remote villages, undulating hills, scanty vegetation, serving as pasture lands

- Inhabitants mostly wheat farmers who may keep sheep and goats
- Diet consists mainly of wheat bread, occasionally meat

Outbreak of liver disease in Gulran district

- More than 270 people affected
- Approx. 50 people died
- WHO alarmed, RIVM consulted
- Hypothesis: PA poisoning due to contamination of grain cereals
- Need for field test identified
- Samples sent to RIVM

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Not all samples well-packed

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Outline of presentation

- Introduction
- Alkaloids and their significance
- Activities in the CON*ff*IDENCE project
- Dipstick methods for various alkaloids
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- Summary and conclusions

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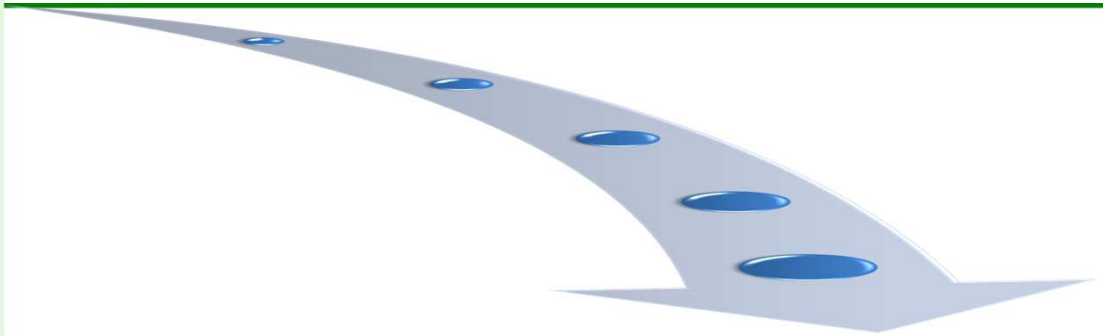
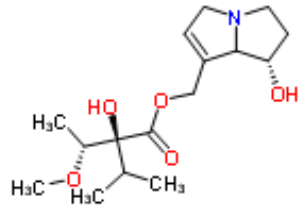
Alkaloids





- Naturally occurring chemical compounds containing basic nitrogen atoms
- Produced by various organisms, mainly plants
- Often physiologically active and poisonous
- Over 3000 compounds known, including pyrrolizidine, tropane and ergot alkaloids

Pyrrolizidine alkaloids

- Toxins formed in common plants, e.g. *Senecio,* that may contaminate food or feed
- Re-intro of certain species in nature may increase intoxications in wildlife and grazing animals
- Adverse effects in humans and livestock
- Methods of analysis: GC-MS, LC-MS/MS, but not (yet) interlaboratory validated
- Some carry-over from feed to milk observed

Tropane alkaloids

- Plant toxins, formed e.g. by *Solanaceae;* seeds may contaminate plants, e.g. soybean
- Humans: accidental exposure rare
- Animals: pigs very sensitive (*Datura* poisoning)
- Methods of analysis: HPLC, GC, RIA, CE-MS, LC-MS/MS, but not (yet) interlaboratory validated
- Info on carry-over scarce, traces of scopolamine found in eggs, no further data on residues

Ergot alkaloids

- Mycotoxins, formed by *Claviceps purpurea* occurring in grasses, grains, sorghum
- Effects: convulsions, gangrene, hallucinations
- Animal sensitivity: poisoning outbreaks in livestock
- Methods of analysis: LC-FLD and LC-MS/MS, but no rapid field tests available
- Limited data on carry-over do not point at animal products as an important source of exposure

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- LCP Food, Agriculture and Fisheries, and Biotechnology, 2008-2012
- Simple, fast, multi-analyte, multi-class detection
- WP Biotoxins includes sub-package alkaloids
  - determination of alkaloids (PA, TA, EA)
  - determination of ergot
- Intra- and interlaboratory validation studies

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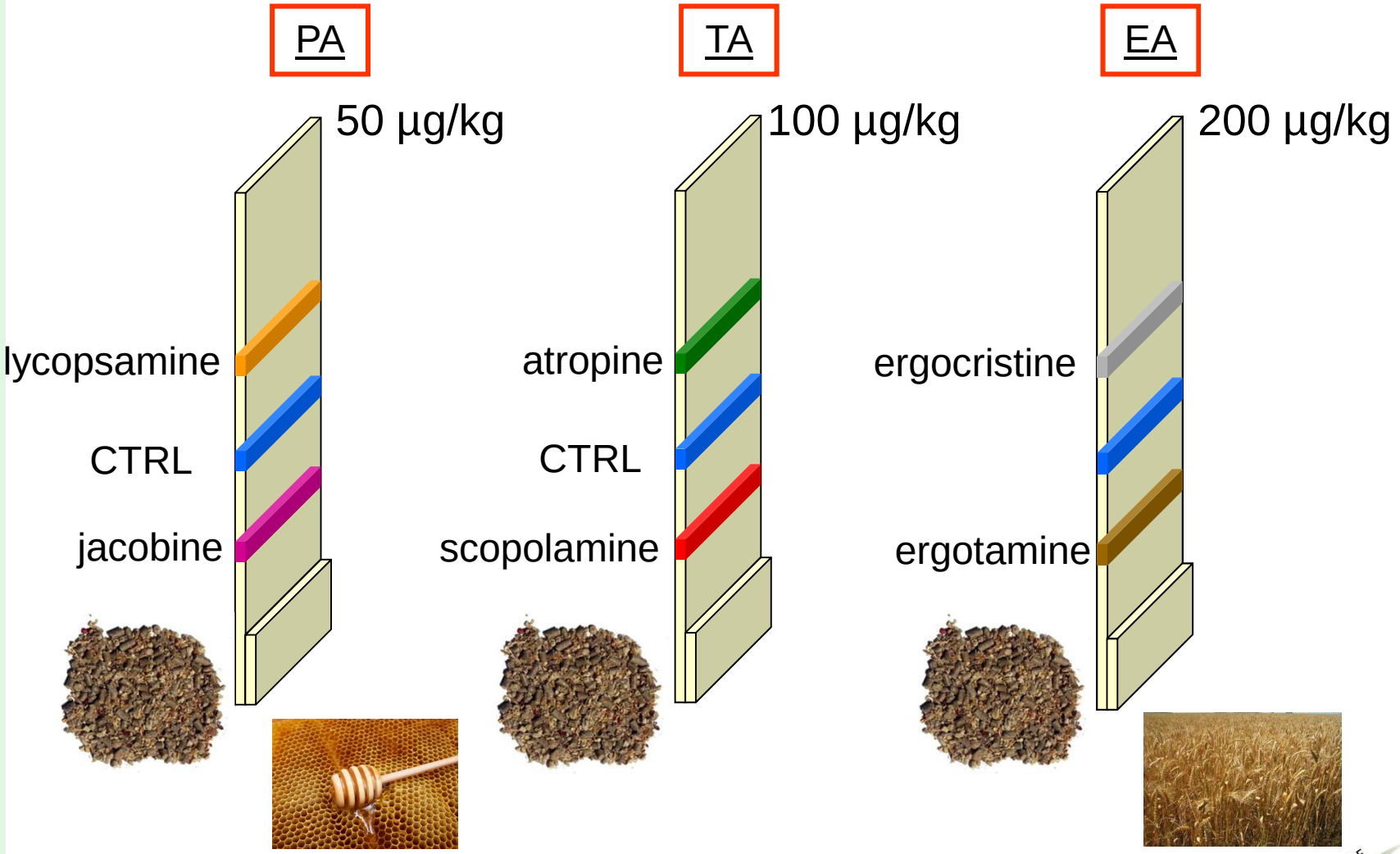
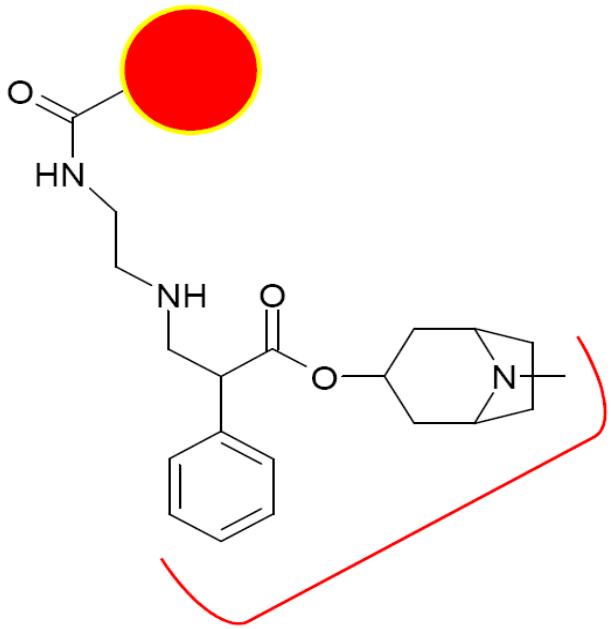
Multiplex dipsticks for PA, TA and EA

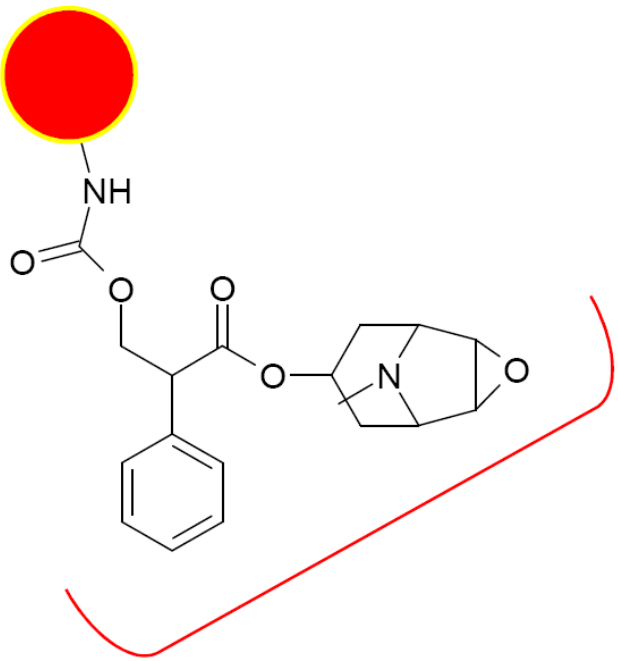


Conjugates for tropane alkaloids

- Target alkaloids: atropine and scopolamine
- BTG / BSA Immunogens
- OVA Competitor



atropine



scopolamine

TA: AB production & characterisation

by ELISA

atropine-DSC-BTG/BSA	no immune response
atropine-CDI-BTG/BSA	-best IC_{50}: 0.2 ng/ml atropine in buffer -CR < 1% with scopolamine, no CR towards several pyrrolizidine and ergot alkaloids
scopolamine-CDI-BTG/BSA	no immune response
scopolamine-AA-BTG/BSA	no immune response
scopolamine-CDI-jeffamine-BSA	no immune response
scopolamine-CBDI-BTG/BSA	-best IC_{50}: 3.6 ng/ml scopolamine in buffer -similar CR with atropine, no CR towards several pyrrolizidine and ergot alkaloids

one Ab used to develop the dipstick for TA

Simple and quick extraction

4g of ground sample

+ 40ml MeOH/H_2O/form.acid (60:40:0.4)

↓

Blending 2 minutes

Dilution 5 times in buffer (EA)

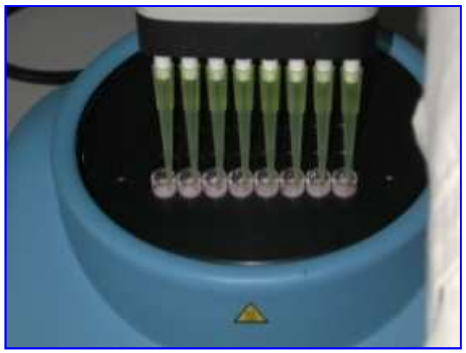
or

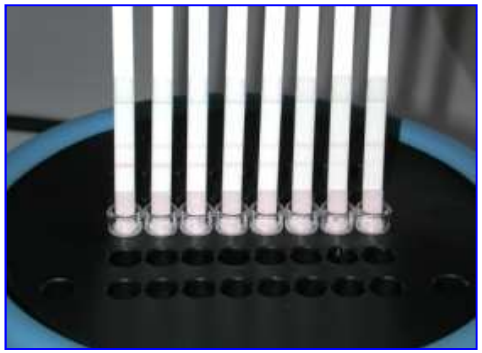
Dilution 10 times in buffer (TA)

↓

Run the dipstick test

Dipstick procedure and reading

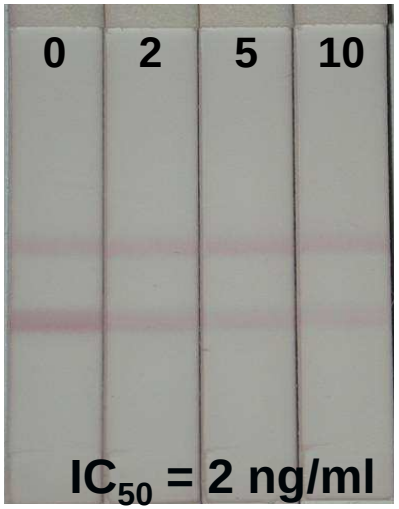




Multiplex dipsticks in practice

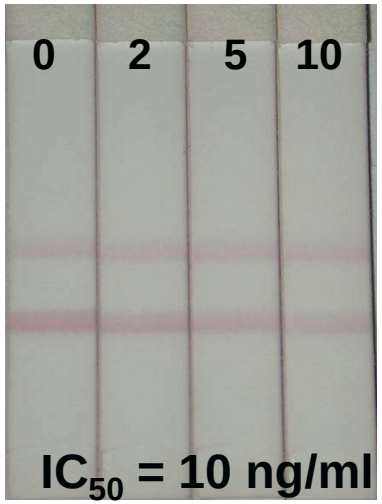
Spiked feed extracts

Atropine



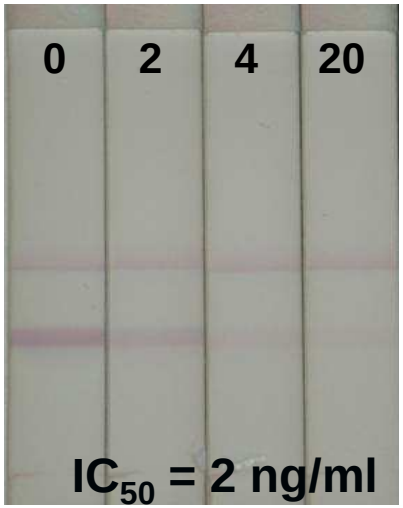
ng/ml

Scopolamine



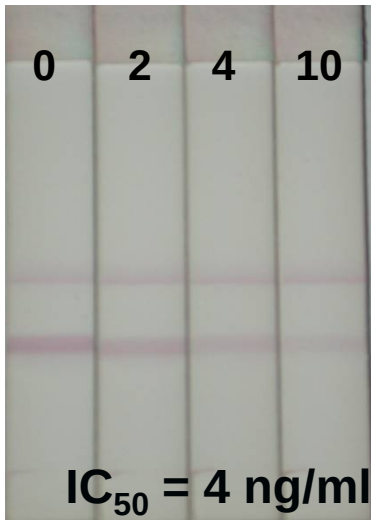
ng/ml

Ergotamine



ng/ml

Ergocristine



ng/ml

Further details: see poster Noan Nivarlet *et al*

Next steps

- Cross-laboratory testing of method for TA
- Amendment of plans:
  - to include ergometrine in addition to ergotamine and ergocristine in the EA dipstick method
  - to change format for PA into multiplex ELISA, and to include heliotrine & monocrotaline in addition to jacobine and lycopsamine
- Small-scale interlab. testing of various methods for the alkaloids to derive performance characteristics

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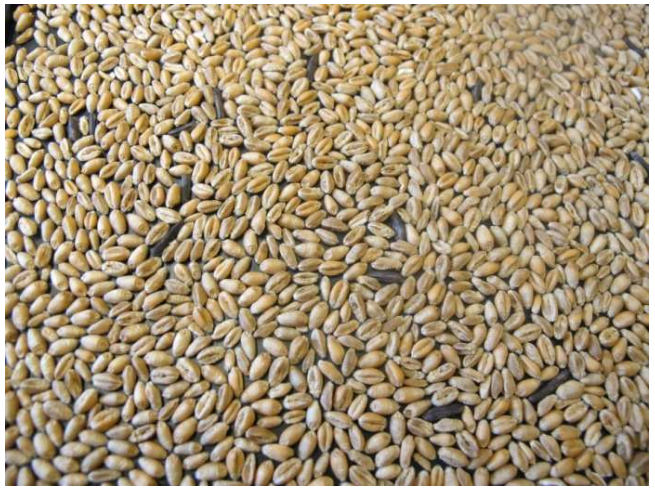
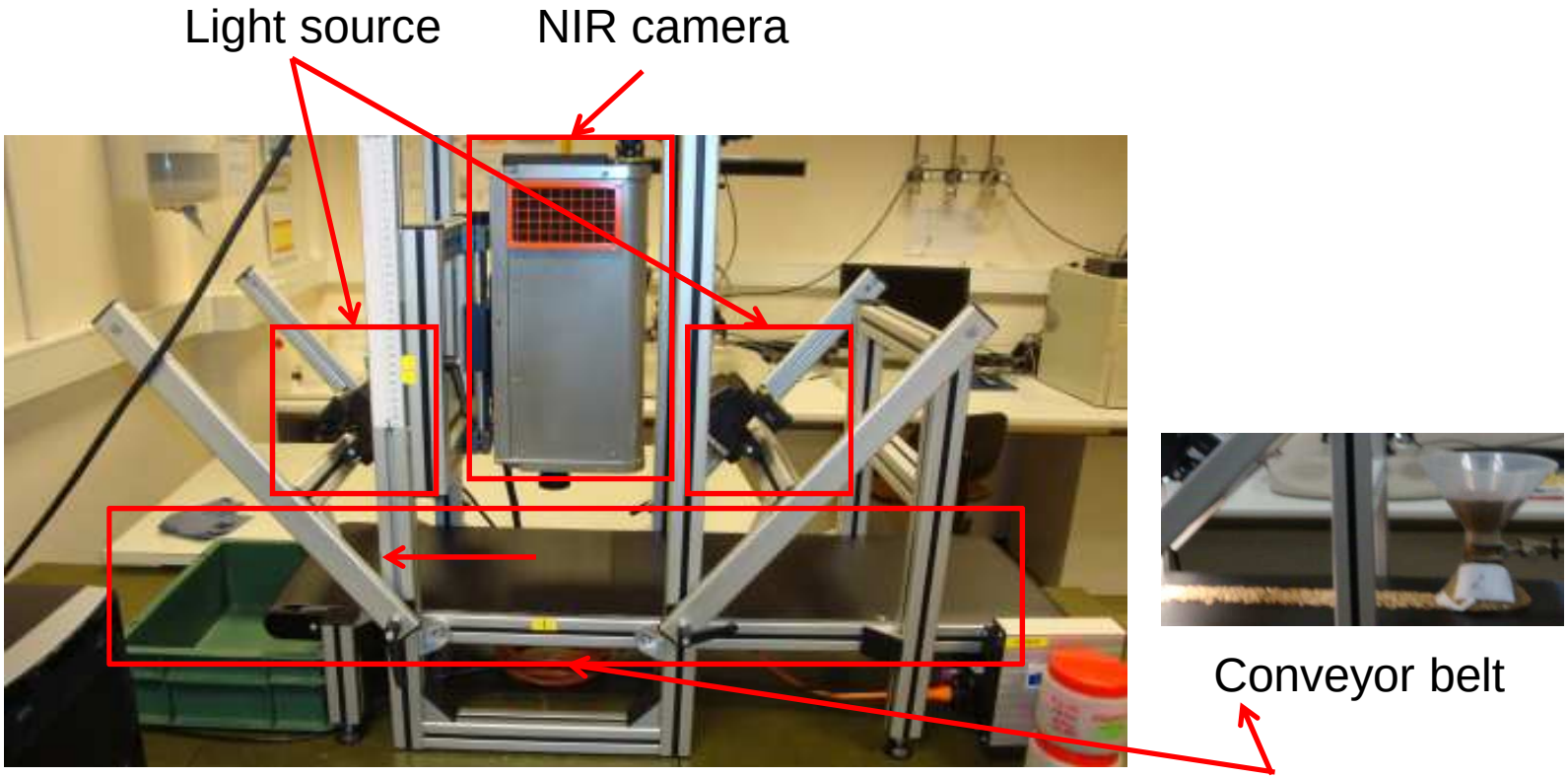
Ergot limits in grain cereals in the EU



- EU Directive 2002/32/EC on undesirable substances in animal feed: 0.1% ergot in all feedingstuffs containing unground feed
- Commission Regulation 742/2010 on buying-in and selling of agricultural products under public intervention: 0.05% ergot in wheat

NIR hyperspectral imaging method to detect ergot



NIR line scan or push-broom imaging system

Demo by Vermeulen *et al* at Nutreco (2011)

Next steps

- Publication of method *"On-line detection and quantification of ergot bodies in cereals by near infrared hyperspectral imaging"* in Food Additives and Contaminants (accepted)
- Ongoing: testing line-scan set-up on grain samples from harvest 2011, and comparing results with those of classical microscopy method
- Presentation of full results at international conferences

Further details: see video and poster
Philippe Vermeulen *et al*

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Summary and conclusions

- Alkaloids: an area of growing concern
- CON*ff*IDENCE: FP7 project with attention for e.g. rapid methodology to detect alkaloids and ergot
- Dipstick methods developed for TA and EA, multiplex ELISA will be explored for PA
- Methods will undergo small scale interlab. testing
- NIR hyperspectral imaging method developed for ergot determination
- Visit posters and video for details and discussion with specialists, at CONffIDENCE session

Open Day CONffIDENCE

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Open Day at RAFA 2011

3 November 2011

Stella Hall: 13:00 – 16:00

Posters (23)

Demonstrations (8)

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