NIR applications for emerging vegetal contaminants

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NIR platform – Gembloux - 27 March 2013

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Introduction

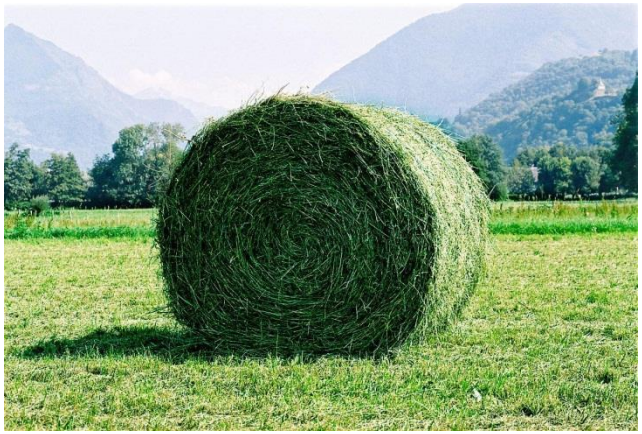
Poisonous plants cause significant losses of livestock every year.

A successful livestock operator must know which poisonous plants occur on a given range or pasture and how they can be controlled or avoided.

Source: University of California Agriculture and Natural Resources, Publication 8398, January 2011

Introduction

The general toxicity of a plant is directly related to the toxin or toxins it contains, the organ or system it affects, and species of animal which ingests it.



The direct digestive process is the most frequent method by which toxins affect animals negatively. However, an additional problem is the tendency of certain toxins to reduce the digestibility of other forages eaten with the toxic plant, resulting in reduced nutritional value for the animal.

Introduction

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;

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ICM > 1997 > IC-478 (5) -- April 21, 1997

Current Newsletter

This website contains archives for the newsletter from 1993-2007. For current news, see Integrated Crop Management News.

Archives 1993-2007

Ergot in 1997

by Gary Munkvold, extension plant pathologist, Departme

Last yea , barley producers in northeast Iowa suffered a serious outbreak of ergot. a
can affect any small grain crop and many grass species. The fungus is called *Claviceps purpurea*,
sclerotia in the seed heads of the plant (see photo). These sclerotia overwinter in or on

ERGOT IN CEREAL CROPS, GRASSES POSES THREAT TO LIVESTOCK

From: US Fed News Service, Including US State News | Date: June 21, 2007

Rye ergot - *Claviceps purpurea*. Ergot is the most frequently mentioned undesirable substance 12 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 samples (member state A) means effectively an occurrence of 0 %. Two member states reported no results (member states IRL and ES). In all other cases (nine labs) ergot appears to be present in low or relevant frequencies, up to 25-50 %. The remark was made that ergot occurrence seems to have increased in recent years. The European

ST. PAUL, MN (
cereal crop worl
fungal disease ha
information and
cause severe

Outbreak of

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 heavy exposure to ergot infested grass and negative findings in a range of other investigations, details of which are given, a diagnosis of ergotism was made.

PMID: 3946070 [PubMed - indexed for MEDLINE]

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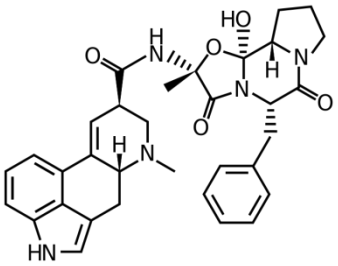
Introduction

- Contamination of food – feed production
- Frequent
- High toxicity for humans – livestock

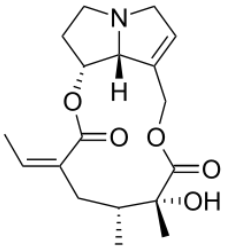
Secondary plant metabolites

1) Alkaloids : e.g. ergotamine



Ergot (*Claviceps purpurea*) in crop production

2) Pyrrolizidin alkaloids : e.g.senecionine



Tansy ragwort (*Senecio jacobaea*) in hay

Detection methods : analytical chemistry
(HPLC, GC-MS, NMR, ELISA, TLC...)

Status of screening methods

- 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

The amount of ergot fragments in mg/kg (ppm) feedingstuff (original sample) is calculated using the following formula:

$$C = \frac{BC \times 1000}{E} \text{ [mg/kg]}$$

C = amount of component in mg/kg feedingstuff (ppm)

BC = selected fragments of component in the laboratory sample or an aliquot of it [mg]

E = total weight of the laboratory sample or an examined aliquot of the laboratory sample [g]

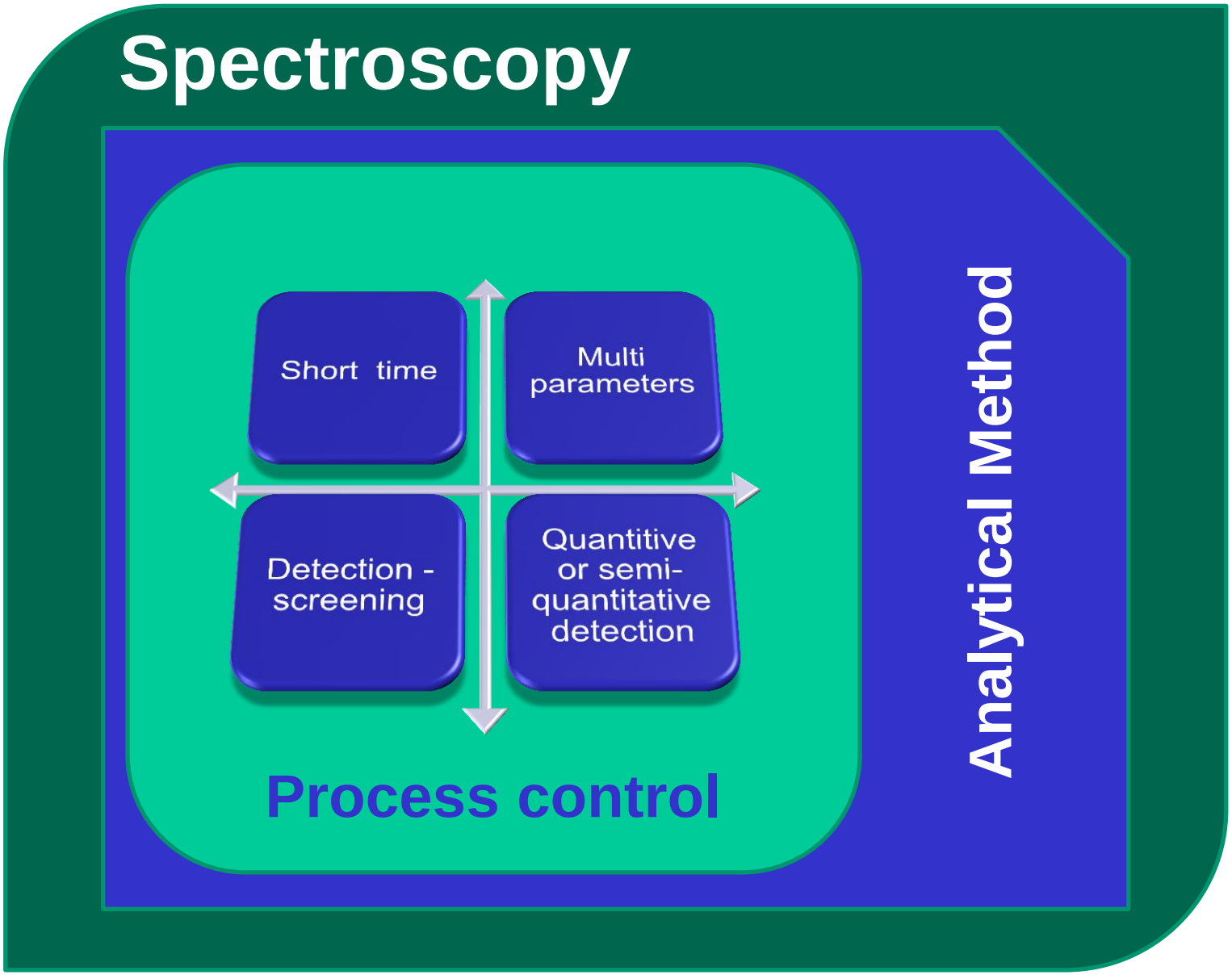
* IAG method

Status of screening methods

Light microscopy, some disadvantages

- Time
- At lab only
- Human skills
- Sample
- Hidden morphological features

Need of rapid methods



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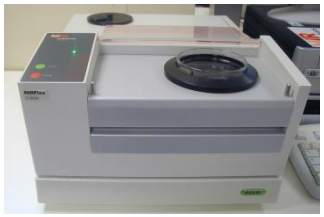
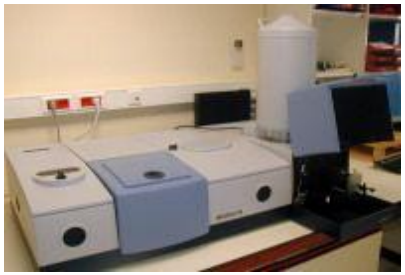
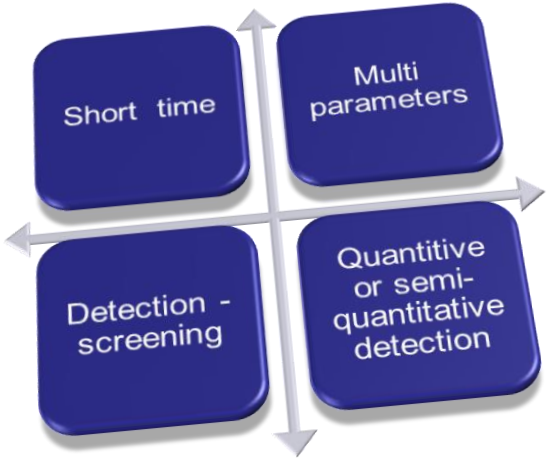
Vibrational Spectroscopy

Near infrared spectroscopy



Raman and mid-infrared spectroscopy





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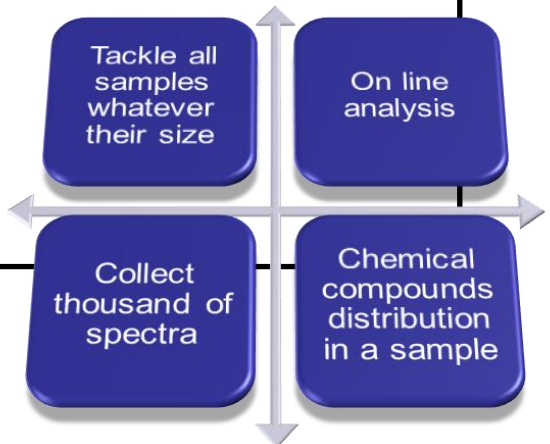
Vibrational Spectroscopy

Hyperspectral Imaging



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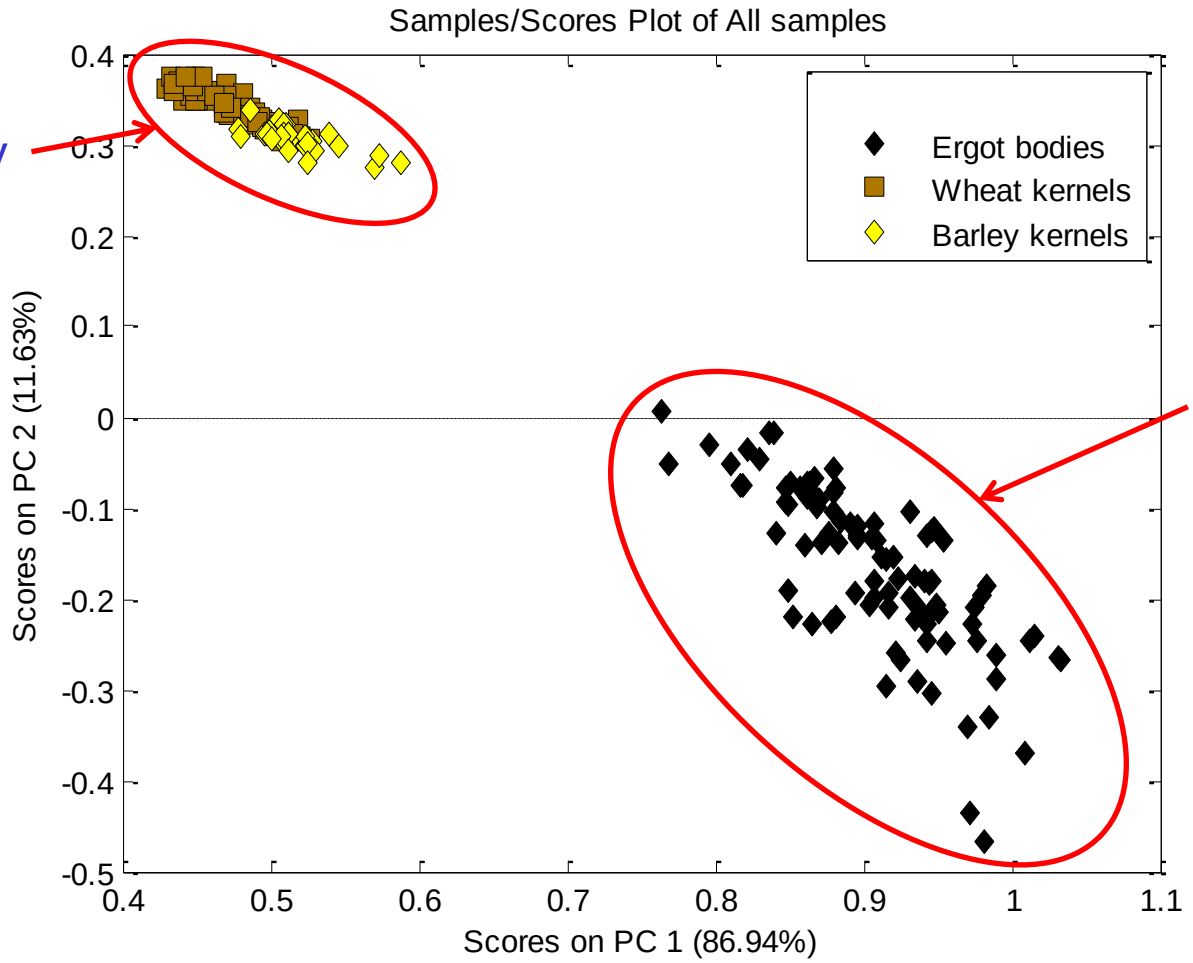
Ergot detection

NIR Hyperspectral Imaging

PCA discrimination ergot bodies – wheat – barley kernels

Wheat and barley



Ergot bodies

Ergot detection

Food Additives and Contaminants
Vol. 29, No. 2, February 2012, 232–240

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

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(*Received 1 August 2011; final version received 25 September 2011*)

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

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Ergot detection

Light microscopy	NIR hyperspectral imaging
High skilled personal	Low skilled personal
45 min / 250g	Few minutes / 250 g
Reduced samples	Large samples (sampling more representative)
Dedicated to ergot	Multiple contaminants

Senecio sp. detection

- The existing microscopy method* provides an elegant early warning tool for Senecio sp. contamination but is difficult, based strictly on morphological features…

Method for the Determination of poisonous plants in roughage, IAG-Method A8

International Association of Feedingstuff Analysis
Section Feedingstuff Microscopy

- *Senecio jacobaea*
- *Senecio vulgaris*
- *Senecio fuschii*
- *Hypericum perforatum*
- *Tanacetum vulgare*

* IAG method

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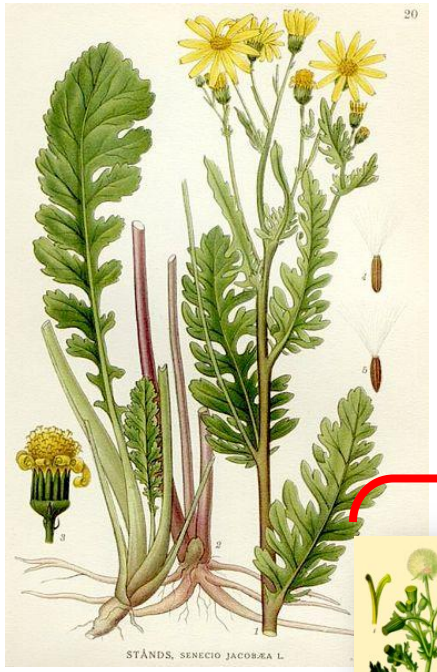
Senecio sp. detection

- Major concern is Tansy ragwort (*S. jacobaea*)



Livestock (horses, cattle)

• Common Groundsel *(S. vulgaris)*	PAs
• *S. fuschii*	PAs
• St Johns' wort *(Hypericum perforatum)*	hypericin
• Tansy *(Tanacetum vulgare)*	thujone

(from Wikipedia, 2012)

Senecio sp. detection

....but toxicity affects also humans

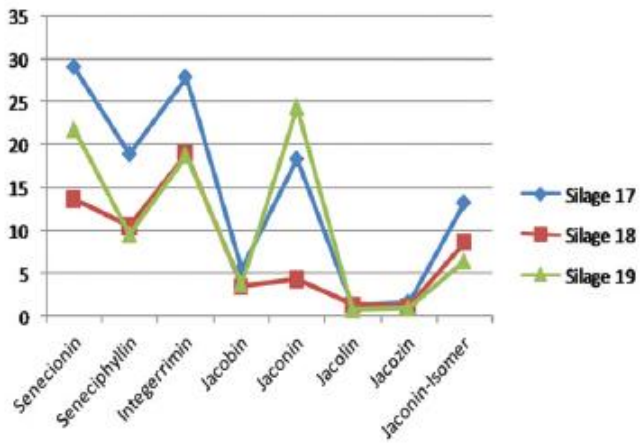


Figure 7. PAs (µg g^{-1}) in silage from *Jacobaea vulgaris*, syn. *Senecio jacobaea*.

Figure 8. Salad sample (left *Senecio vulgaris*; right rucola).

(from Wiedenfeld, 2011)

Germany, august 2009

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Senecio sp. detection

Light microscopy, some disadvantages

Morphological features not always visible !

- Dried material
- Clearing of material
- Small fragment are missing features
- Markers destroyed in silage !

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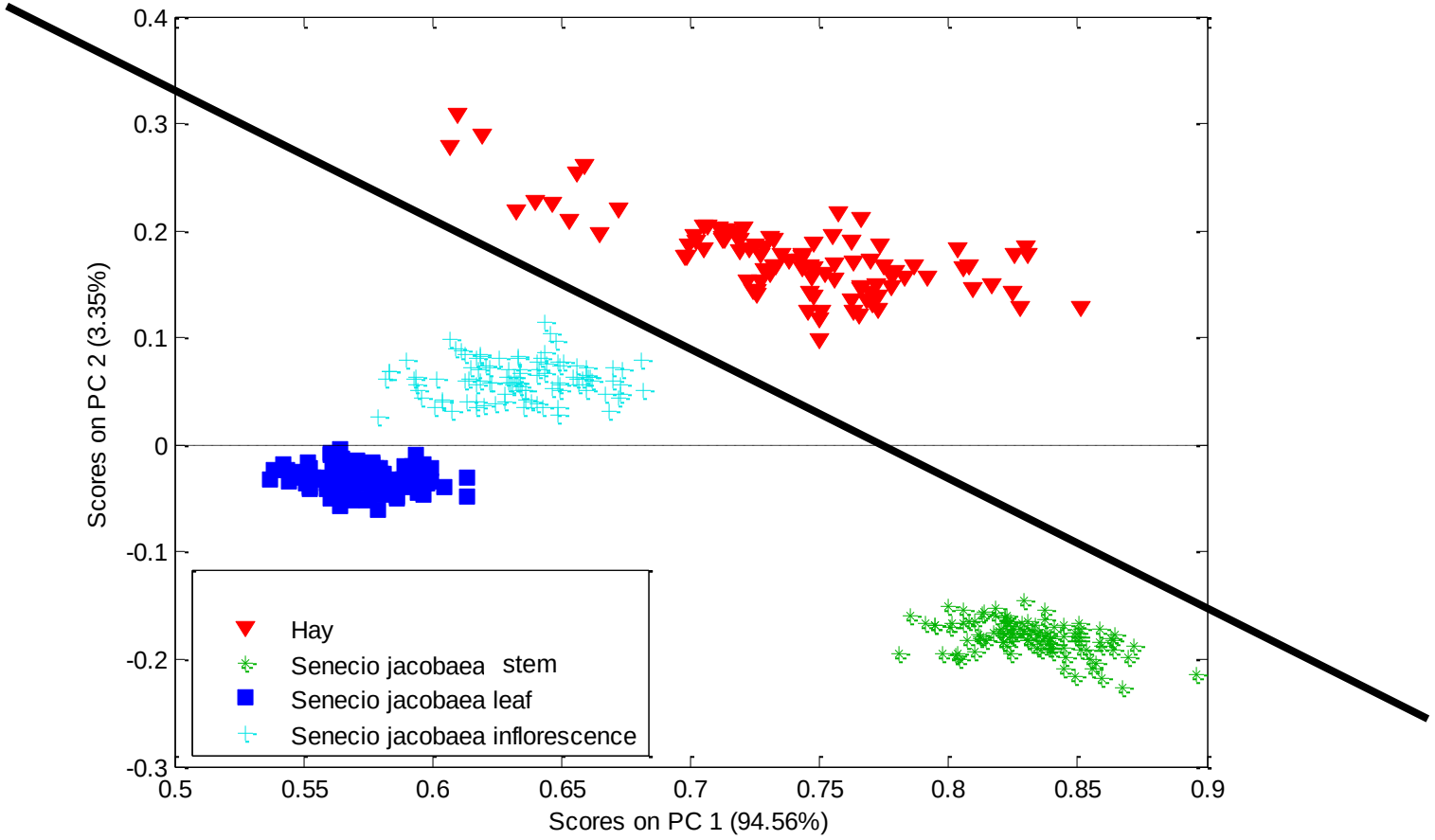
Senecio sp. detection

S. jacobaea vs Hay

Principal Component Analysis (PCA)



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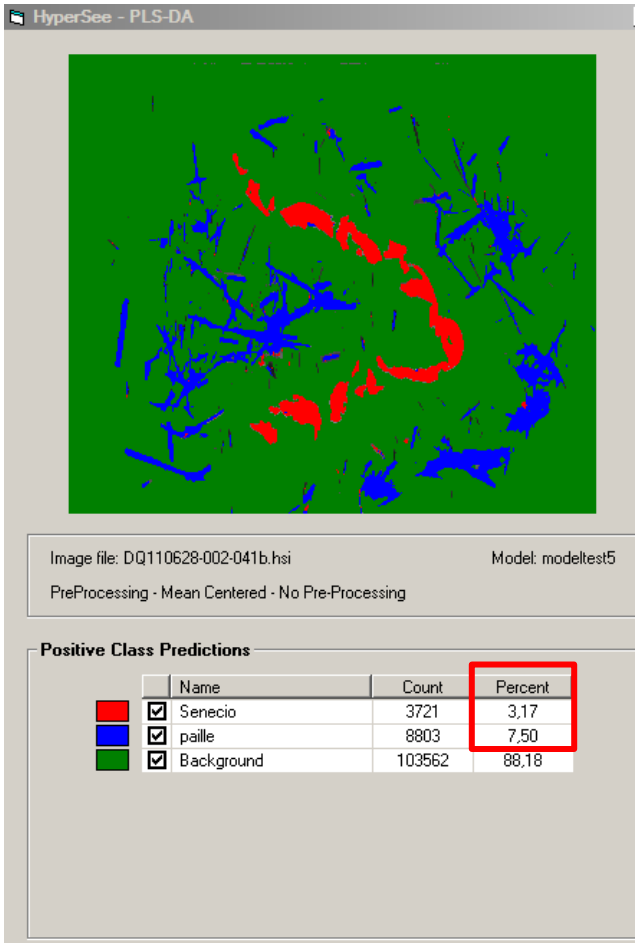
Senecio sp. detection

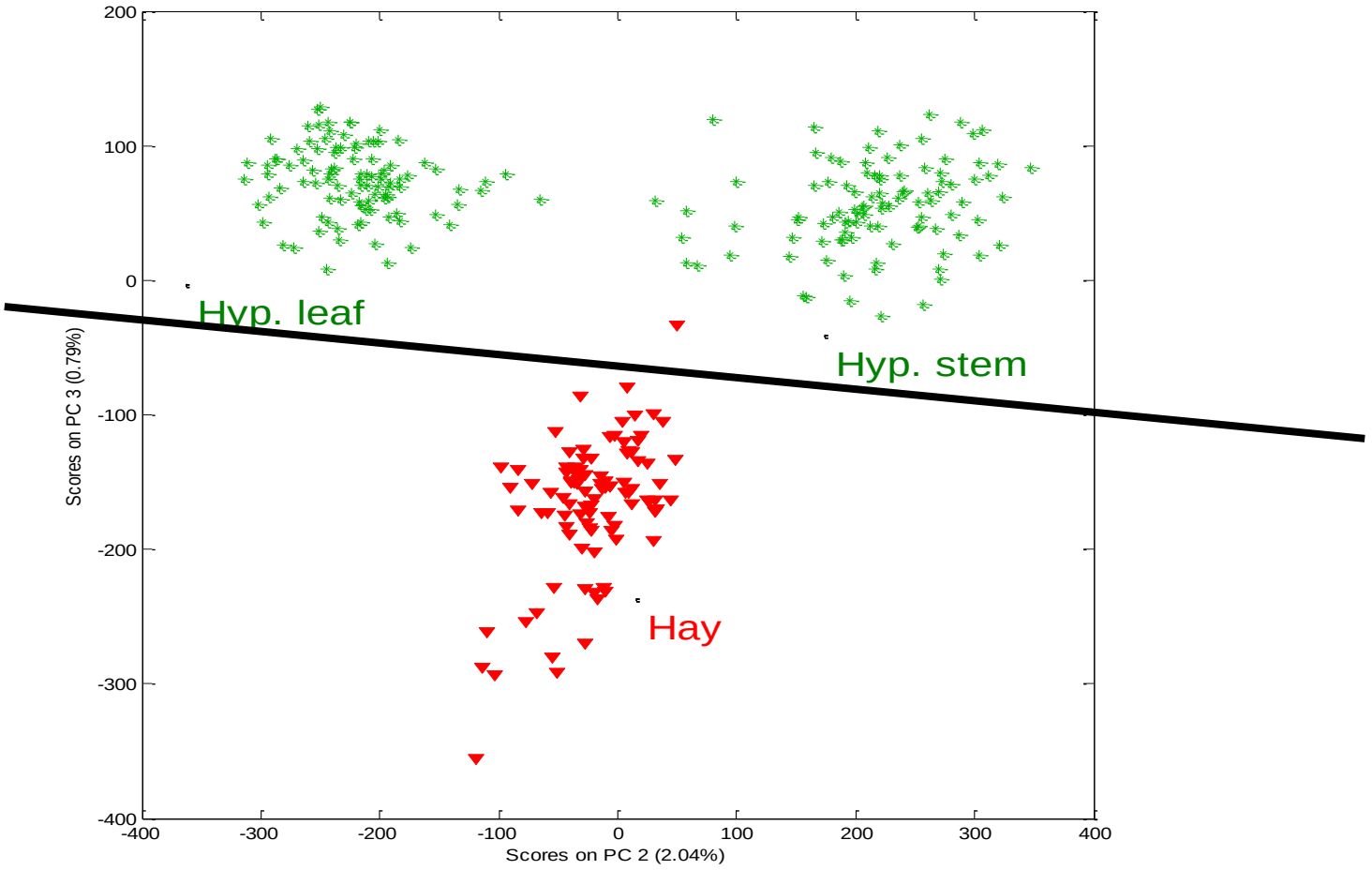
- Discriminant equation (mathematical model)



% of pixels

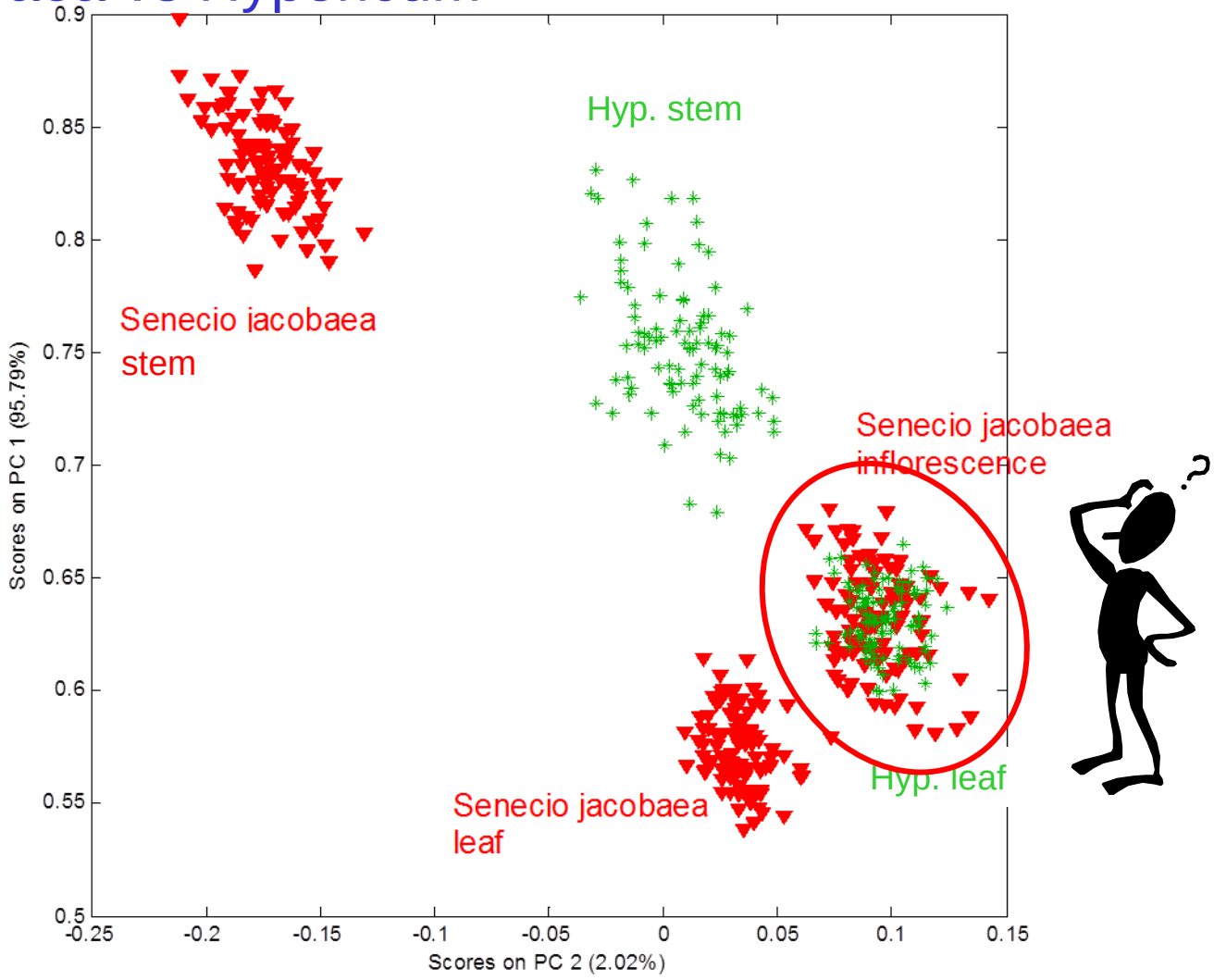
What about other species ?

Hypericum vs Hay



What about other species ?

S. jacobaea vs Hypericum



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Conclusions

NIR hyperspectral imaging allows

- Automatic detection (time, large samples)
- Disclosure independent from morphological markers
- Non destructive → other analytical methods
- Possibility for species detection

Proposes valuable alternatives for screening (industry) of

Ergot 

Plant contaminants (at large) 

Conclusions

Plant contaminants (at large)

NIR hyperspectral imaging shows

- Promising first experiments
- Spectral differences according to organs

  Chemical accumulation of PA / secondary plant metabolite ?
- Tuning of model

Experiments on other PAs containing species

Crotalaria spp., Lolium sp.,

…Boraginaceae, Asteraceae, Fabaceae !

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Acknowledgements

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THANK YOU FOR YOUR ATTENTION

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