

New test-kits to detect herbicide effects and resistance in weed plants based on HPTLC-screening



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Overview of presentation:

- **PhytoChemLab**
- **BioTest-kits & ChemiTest-kits**
- **International patent applications**
- **What, why and who?**
- **Claims**
- **Biomarker pattern**
- **Weed plants/herbicides**
- **CAMAG HPTLC-equipment**
- **HWR-Test[®] (BioTest-Kit)**
- **Semi-/field validation**
- **Horizontal/vertical development**
- **HR-Test (BioTest-kit)**
- **Collaborators**
- **Summary**



PhytoChemLab

A Plant Biomarker Laboratory with innovative perspectives

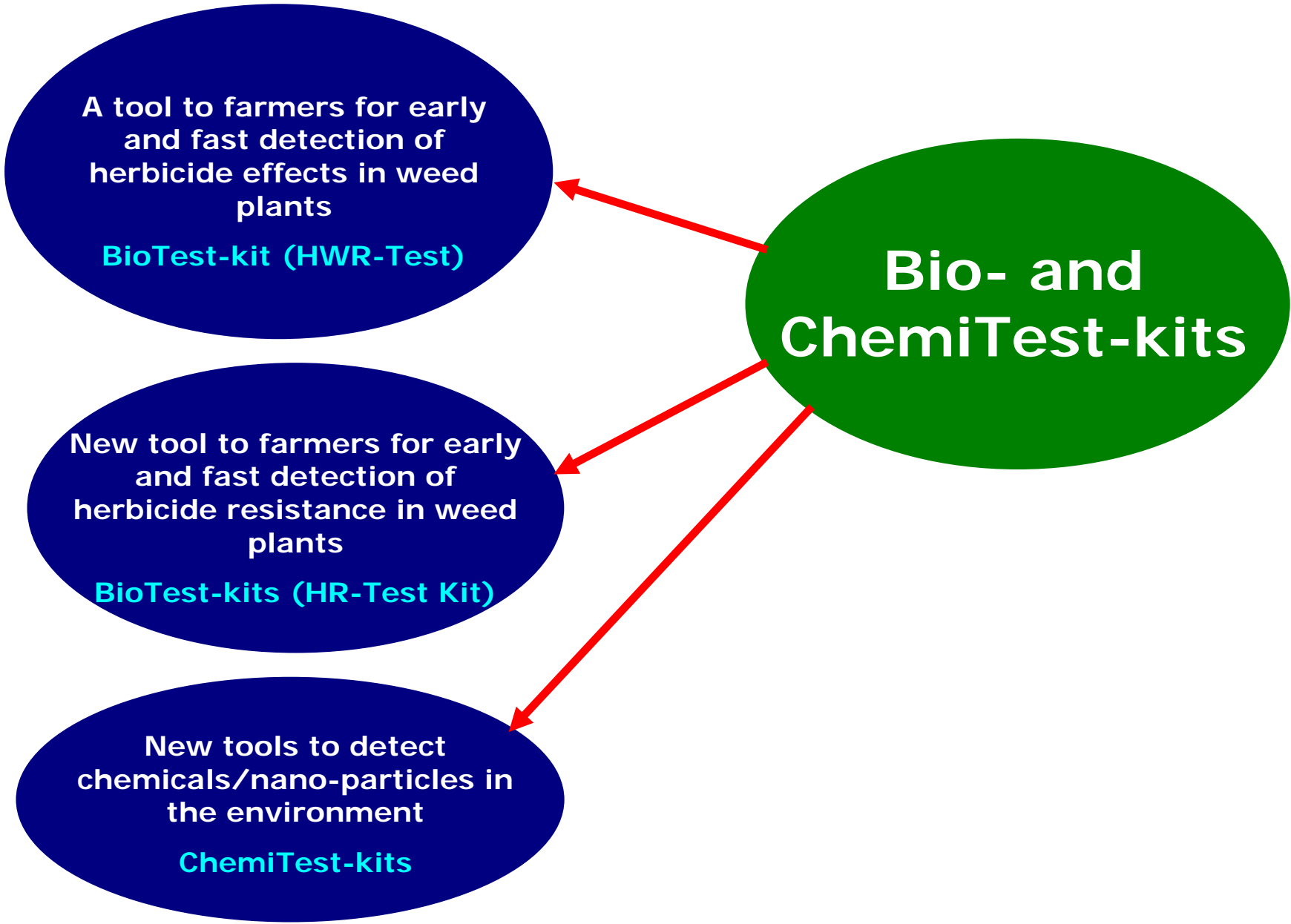


Dr. Helle Weber Ravn (Head of Laboratory)
Lise Lauridsen & Anni Christiansen



- **Research: Biomarkers in biological organisms after exposure to chemicals**
- **Development of simple tools as test-kits to be used in the field for effect studies**
- **Supervision of master and Ph.D-students**
- **International patent applications**
- **Close collaboration with private companies**





International patent applications

- International PCT-US patent application:
An assay method and kit for testing biological material for exposure to stress using biomarkers (WO 01/92879 A1 PCT/DK01/00377)
Application date: 30 May 2000
Inventor: Helle Weber Ravn
Rights: National Environmental Research Institute
- International PCT-Patent application:
A method and kit for testing stress effects in living organism (PA 2007 00022)
Application date: 7 January 2007
Inventor: Helle Weber Ravn
Rights: National Environmental Research Institute & The Science and Agricultural Science Faculty, Aarhus University (3:2)
- International PCT-Patent application:
Method and kit for detecting resistance in living organism (PA 2007 00024)
Application date: 7 January 2007; Inventors: Helle Weber Ravn : Per Kudsk Nielsen : Solvejg K. Mathiassen (2:1:1)
Rights: National Environmental Research Institute Institute & The Science and Agricultural Science Faculty, Aarhus University (1:1)



What can we do?

- A cheap, fast and easy tool to control the effect of a given herbicide dose only a week after spraying and before visual signs (**BioTest-kit: HWR-Test[®]**)
- Cheap, fast and easy tools to detect herbicide resistance in weed plants short time after spraying (**BioTest-kit: HR-Test**)



Who can use the tools?

- **Farmers**
- **Consultants**

Why do we want that?

- The farmer will save time and money if no further spraying is needed (e.g. rain shortly after spraying) (HWR-Test[®])
- The farmer can optimize the use of e.g. reduced doses of herbicides, protection of the Environment (HWR-Test[®])
- The farmer can detect herbicide resistance and use other pesticides (HR-Test)



What is the claims to test-kits?

- **Reliability**
- **Easy to use**
- **Fast results**
- **Cheap to use**
- **Low detection limit (ChemiTest-kits)**



Principle

BioTest-Kits:

- to use the early biochemical responses in plants short time after herbicide spraying
- to use the differences in the plants chemical composition and concentration (biomarkers) in relation to the wanted effect

ChemiTest-Kits:

- to detect the concentration of chemicals/nanoparticles in different biological media and the environment



Biomarkers in BioTest-Kits

Biomarkers (invisible)



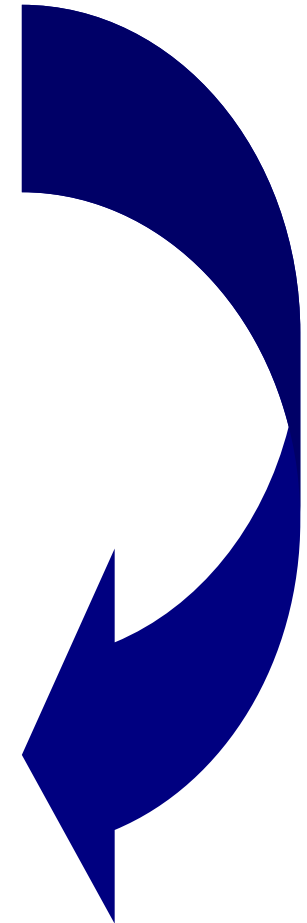
Biochemical changes



Physiological changes



**Visual effects
(morphological changes)**



BioTest-kit (HWR-Test) (effect): Weed plants & herbicides

Species

- Loose silky bent (*Ápera spica-vénti*)
- Perennial rye grass (*Lolium perénne*)
- Annual meadow grass (*Poa ánnua*)

Herbicides

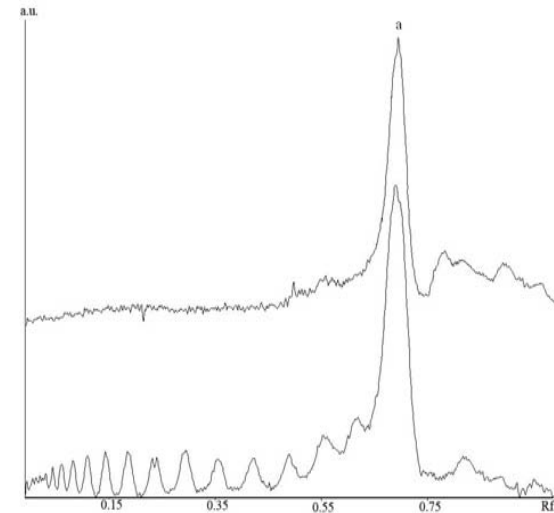
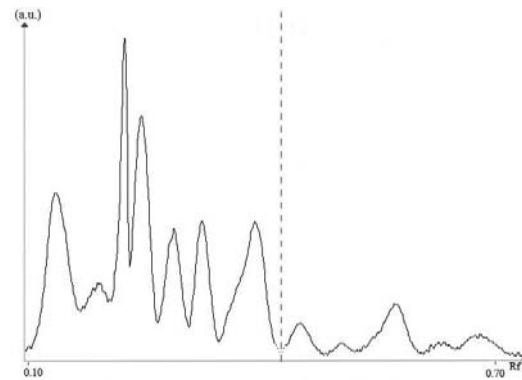
- Iodosulfuron (Hussar OD, Bayer CropScience)
- Mesosulfuron + iodosulfuron (Atlantis WG, Bayer CropScience)



Biomarkers



Two different groups of compounds



Control/Herbicide exposed plant extracts



Equipment for method development



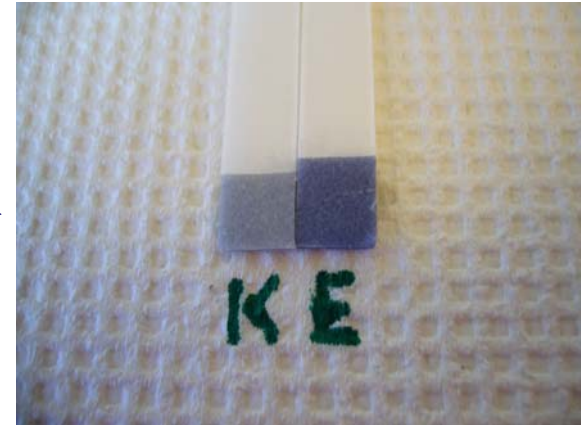
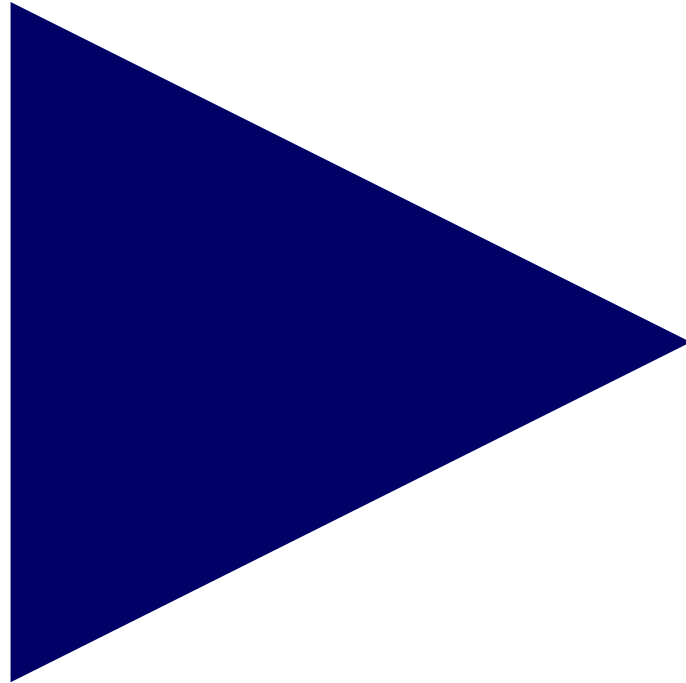
Camag Applicator, Video-Store & Scanner

(Supported by the National Research Council, Denmark)



BioTest-kit development

Plant Biomarkers - Dr. Helle Weber Ravn



HWR-Test[®] S

Herbicide Weed Response Test
to Sulfonylurea-herbicides

www.HWR-Test.com

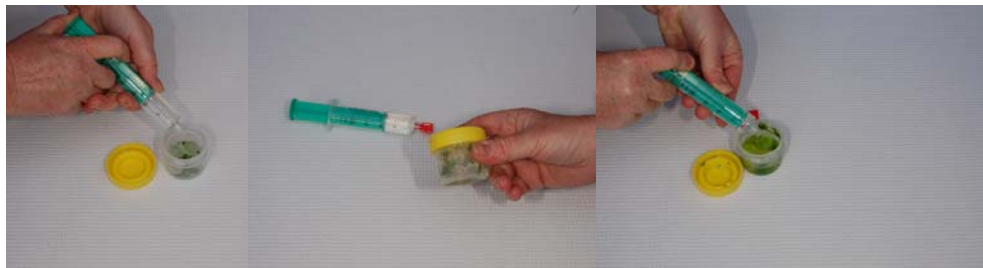


HWR-Test[®] S

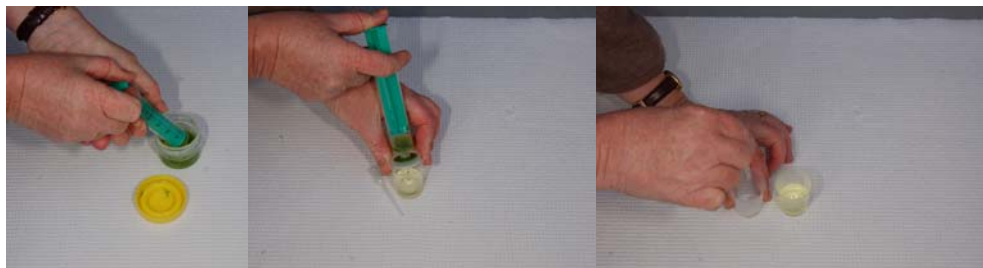
3 steps



1) Sample preparation



2) Extraction



4) Chemical reaction and detection



HWR-Test[®] Semi-field tests

Correspondence between plant biomass reduction and colour reaction of phytochemical biomarkers



Response of weed plants to herbicide treatment

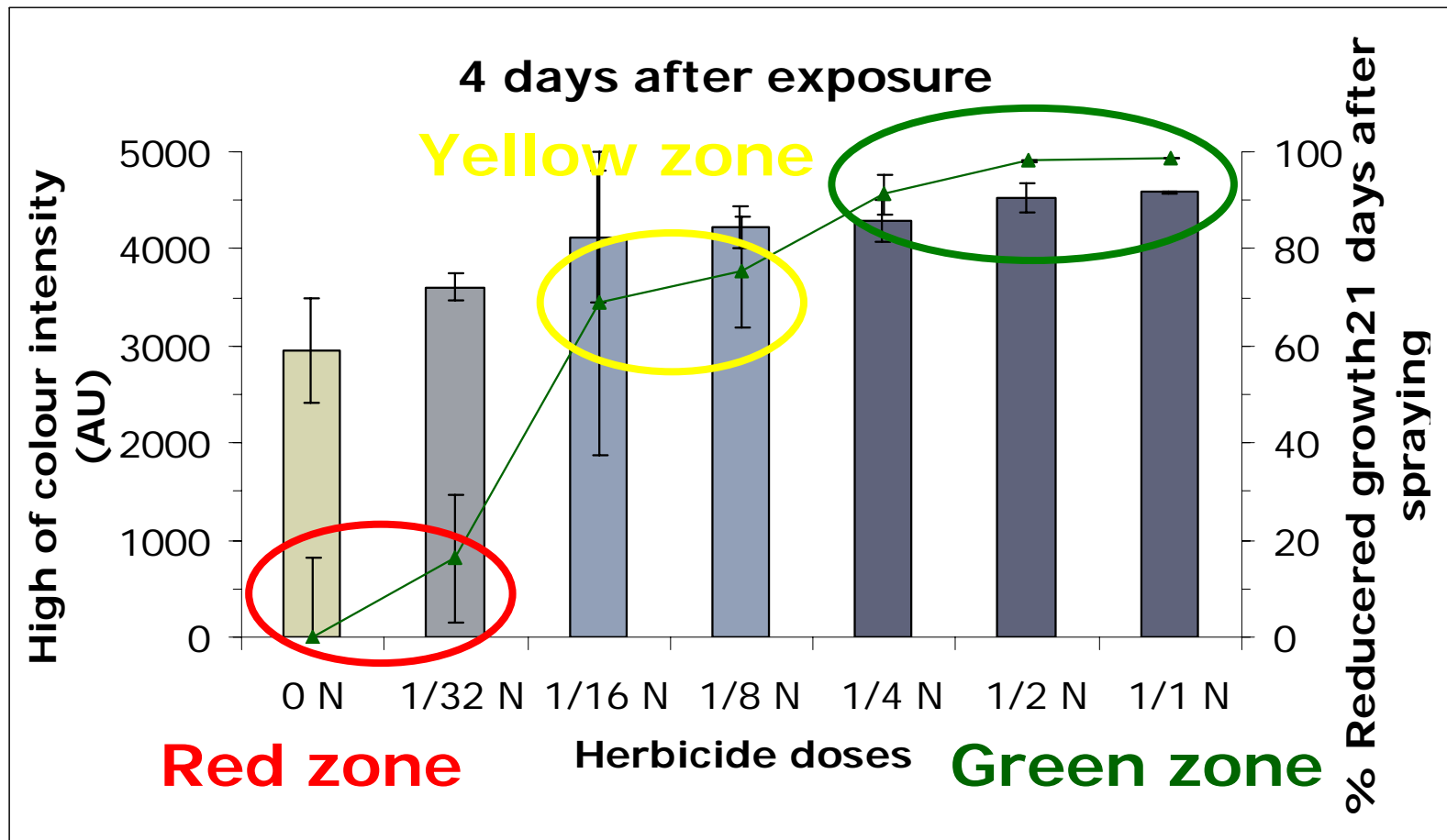


Control 1/32N 1/16N 1/8N 1/4N 1/2N 1N



HWR-Test®

Semi-field study on *Lolium perenne* exposed to Hussar (iodosulfuron) – Dose/Responses



Field-tests (DJF/NERI)

Biomarker/Effect studies af BioTest-Kits

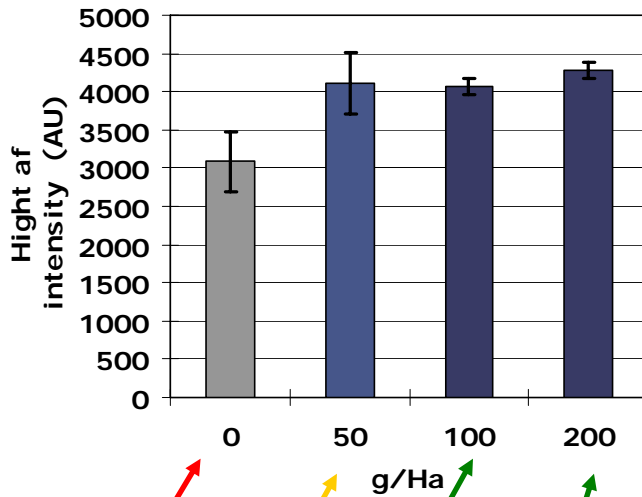


Plant Biomarkers - Dr. Helle Weber Ravn



Results of field validation

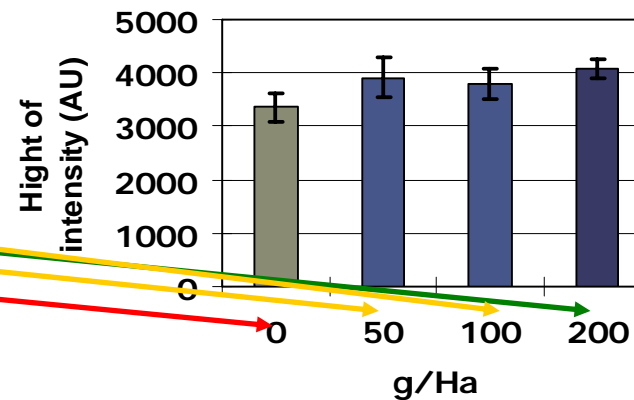
Poa annua - Atlantis - 7 days after exposure



Red Yellow green
zones

Differences in
sensitivities

Lolium perenne - Atlantis - 7 days after exposure



ON $\frac{1}{4}$ N $\frac{1}{2}$ N 1N



Horizontal development of BioTest-kit 1 (effect): Weed plants & herbicides

Species

- Common chickweed (*Stellaria media*)
- Wall speedwell (*Veronica arvensis*)
- Common poppy (*Papaver rhoeas*)
- Field pansy (*Viola tricolor*)
- Loose silky bent (*Ápera spica-vénti*)
- Black-grass (*Alopecurus myosuroides*)

Herbicides

- Express ST (tribenuron-methyl)
- Monitor (sulfosulfuron)
- Safari (triflusulfuron-methyl)
- Ally ST (metsulfuron-methyl)
- Harmony Plus (thifensulfuron-methyl + tribenuron-methyl)
- MaisTer (foramsulfuron + iodosulfuron)
- Titus WSB (rimsulfuron)



Vertical development

1. Step

Development of simple field technological equipment – collaboration with HH-AU, Herning 3th semester 6 engineer students in autumn 2008 at NERI (financed by Frisenette ApS, Knebel

2. Step

A business Ph.D. (if financial support can be identified) at PhytoChemLab



Herbicide Resistance Test – HR-Test

Coordinator: Dr. Helle Weber Ravn, NERI

Goal

A new simple and fast tool to
detect herbicide resistance in
weed plants after exposure

Collaboration project between Aarhus University
and a private company: Frisenette ApS



Coordinators and Collaborators

National Environmental Research Institute, University of Aarhus, Silkeborg

Dr. Helle Weber Ravn (project leader), Lise Lauridsen & Anni Christiansen

Danish Faculty of Agricultural Science, University of Aarhus Flakkebjerg

Mr. Per Kudsk & Dr. Solvejg K. Mathiasen

Hobro - Aalborg Union of Farmers, Hobro

Mrs. Hanne K. Kristensen

Frisenette ApS, Knebel

Ervind Müller & Søren H. Christensen (product development & forhandler)

Faculty of Pharmacy, University of Montpellier I, France

Dr. Laurence Mondolot (Histochemical investigations)



Summary:

- A cheap, fast and easy tool to control the effect of a given herbicide dose only a week after spraying and before visual signs (**HWR-Test[®]**) is developed
- Cheap, fast and easy tools to detect herbicide resistance in weed plants short time after spraying (**HR-Test**) is in development
- Other simple test-kits can be developed

