Plant Biomarker Pattern

Phytochemical pattern in *Apera spica-venti* after exposure to six different herbicides using High Performance Thin-Layer Chromatography (HPTLC)

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Introduction

Biomarker patterns were detected as patterns of phytochemical changes in *Apera spica-venti* (Poaceae) after exposure to six herbicides with different modes of action.

The phytochemical effects were detected as a biomarker pattern using High Performance Thin-Layer Chromatography (HPTLC).

The phytochemical compounds in the group of phenolic- and other natural fluorescence compounds, amino acids and carbohydrates were detected. The composition and concentration of the phytochemical compounds in exposed and unexposed plants were compared.

In spite of the fact that the modes of action of the herbicides was different, a similar biomarker pattern was detected.

The biomarker pattern is correlated to the effects i.e. reduction in biomass and can therefore be used to forecast herbicide efficacy in the field shortly after spraying.

Plant Biomarker Pattern

A biomarker pattern is defined as the changes in the composition and the content of phytochemical compounds detected in plants after exposure to herbicides (Ravn *et al*., 2005).

Plant species/Herbicide

Apera spica-venti (ASV) which belongs to the Poaceae familie was used as test plant.

The herbicides used for the study were:

- Boxer EC(Syngenta Crop Protection A/S) (**prosulfocarb**)= PRO
- Stomp (BASF) (pendimethalin) = PEN
- Primera Super (Bayer CropScience) (**fenoxaprop-p-ethyl**) = FEN
- Hussar OD (Bayer CropScience) (**iodosulfuron**) = IOD
- Monitor (Monsanto Crop Science Denmark A/S) (**sulfosulfuron**) = SUL
- Roundup Bio (Monsanto Crop Science Denmark A/S) (**glyphosate**) = GLY

Sample preparation

Freeze-dried plant material 100 mg was extracted with 2.00 ml 75% ethanol in ultrasonic bath with ice for two hours. The extracts were centrifuged before application on stationary phase (TLC-plate).

Cultivation & Exposure

The plants were cultivated in the greenhouse. At a growth stage of 4-5 leaves and 1-2 tillers, the plants were exposed with 50% of recommended field dose of the herbicide. The plants were harvested 14 days after exposure. Immediately after harvest the plants were frozen and freeze-dried

HPTLC

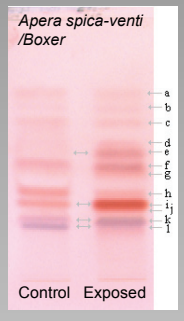
Stationary phase/Solvent/Derivatisation reagent

System 1: 47/49% 1-propanol+ 0.024M Na_2CO_3/**A**
System 2: 86/69% 1-propanol+ 1-propanol+0.024M Na_2CO_3/**B**
System 3: 47/ 40% 1-butanol+ 10% acetic acid (upper phase)/**C**
System 4: 52/49% 1-propanol+ 5.6% NH_3/**n.d.**
System 5: 92/69%1-propanol+0.024M Na_2CO_3 /**D**
System 6: 52/67% 1-butanol + 17% formic acid/**D**

Stationary phase:
52: Merck 1.05552, Cellulose (TLC)
92: Merck 1.6092, Cellulose (HPTLC)
47: Merck 1.05547, Silica Gel (HPTLC)
86: Merck 1.05586, LiCrospher Si 60 F_{254s}(HPTLC)

Derivatisation reagents:
A: 2-aminoethyl diphenylborinat + polyethylenglycole 4000 (phenolic compounds)
B: Vanillin and sulfuric acid (carbohydrates & related compounds)
C: Anisaldehyde + sulfuric acid (carbohydrates & related compounds)
D: Ninhydrine + copper sulphate (amino acids)
n.d.: no derivatisation (UV-254 & 366 nm) (natural fluorescence compounds)

Advanced HPTLC CAMAG equipment for quantification



HPTLC plate System 5: Amino acids
a: Leu, b: Val, c: Phe, d: Tyr, e: Pro, f: Ala, g: Thr, h: Gly, i: Gln, j: His (grey), k: Lys, l: unidentifed
Grey double arrows indicate biomarkers.

Results/Conclusion

A common biomarker pattern of 10 different compounds with a content higher/lower than 25% was detected in *Apera spica-venti* exposed to the 6 different herbicides.

Biomarker in per cent of the content (exposed minus control)(positive values = blue;negative values = green)

Rf-value/colour/system	Biomarker	FEN	GLY	IOD	SUL	PRO	PEN
0.75 +/- 0.00/orange/1	Unidentified	75%	25%		75%		
0.07 +/- 0.01/black/2	Unidentified	50%	50%	50%	50%		50%
0.13 +/- 0.01/black/2	Unidentified	50%	50%	50%	50%	50%	50%
0.17 +/- 0.01/black/2	Unidentified				50%	50%	
0.22 +/- 0.01/black/2	Unidentified	50%	50%	50%	50%	50%	50%
0.26 +/- 0.00/orange/2	Unidentified	-50%	-50%	-50%	-50%	-50%	-50%
0.28 +/- 0.01/black/2	Unidentified	50%	50%	50%			
0.32 +/- 0.01/black/2	Unidentified	50%	50%	50%	50%	50%	50%
0.36 +/- 0.01/black/2	Unidentified				50%	50%	50%
0.37 +/- 0.00/violet/3	Unidentified	-25%	-25%	-25%	-25%	-25%	
0.47 +/- 0.00/black/3	Unidentified	-25%	-25%	-25%	-25%		
0.60 +/- 0.00/violet/3	Unidentified	-25%				-25%	
0.79 +/- 0.00/black/3	Unidentified	-25%	-25%	-25%	-25%	-25%	
0.86 +/- 0.00/black/3	Unidentified	-25%	-25%			-25%	
0.20 +/- 0.00/turquoise/4	Unidentified	-75%	-75%	-75%	-75%	-75%	-75%
0.30 +/- 0.00/yellow-blue/4	Unidentified	-50%	-50%	-50%	-50%	-50%	-50%
0.35 +/- 0.00/turquoise/4	Unidentified	-50%	-50%	-50%	-50%	-50%	-50%
0.15 +/- 0.00/violet/5	Unidentified	-25%	-50%	-25%	-25%	-50%	-25%
0.25 +/- 0.00/red/5	Glycine	50%	50%	50%	50%	50%	50%
0.40 +/- 0.00/violet/5*	Unidentified	-50%	-50%	-50%	-50%	-50%	-50%
0.45 +/- 0.00/yellow/5	Prolin	50%	75%	50%	50%	50%	
0.50 +/- 0.00/red/5	Methionine		25%		-25%	25%	
0.65 +/- 0.00/red/5	Isoleucine		25%		-25%	25%	
0.25 +/- 0.00/red/6	Glycine	50%	50%	50%	50%	50%	
0.30 +/- 0.00/violet/6	Unidentified	-25%	-25%			-25%	-25%
0.45 +/- 0.00/yellow/6	Prolin	50%	75%	75%	50%	50%	
0.60 +/- 0.00/red/6	Methionine		25%			25%	
0.65 +/- 0.00/violet/6	Tyrosine		25%			25%	
0.75 +/- 0.00/red/6	Isoleucine		25%			25%	

*no derivatisation detection in UV-light 366 nm

International patent application/Reference

Ravn, 2000: International PCT patent application:"An assay method and kit for testing biological material for exposure to stress using biomarkers" (WO 01/92879 A1, PCT/DK01/00377)

Ravn, H.W.; Hjorth, M., Lauridsen, L.,Kudsk, P., Mathiassen, S.K., Mondolot, L (2005). New Phytochemical Screening Method for Biomarkers in Plants Exposed to Herbicides. *Bull. Environ. Contam. Toxicol.*, 75, 236-245.

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