



# Plant Biomarker Pattern

## High Performance Thin-Layer Chromatography (HPTLC) used to detect a phytochemical pattern in five different plant species after exposure to glyphosate

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### Introduction

High Performance Thin-Layer Chromatography (HPTLC) was used to detect phytochemical changes as a biomarker pattern in five different plant species after exposure to glyphosate.

Glyphosate is a systemic herbicide and the main mode of action is inhibiting the production of aromatic amino acids in plants (Ravn *et al.*, 2005).

The phytochemical effect was detected as a biomarker pattern. Phytochemical changes 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 phytochemical composition of the five plant species 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

The weed plant species tested were all from the plant familie (Poaceae):

- *Apera spica-venti* (ASV)
- *Lolium perenne* (LP)
- *Poa annua* (PA)
- *Bromus hordeaceus* (BH)
- *Alopecurus myosuroides* (AM)

The herbicide used for the study was: Roundup Bio (Monsanto Crop Science Denmark A/S)

### 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 the 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



Advanced HPTLC CAMAG equipment for quantification

### Results/Conclusion

A common biomarker pattern of six different compounds with a content higher/lower than 25% (content of biomarker in exposed minus content of biomarker in control) was detected in all the plant species exposed to glyphosate. Unexpected in relation to the mode of action of glyphosate, the content of phenylalanine was increased for two of the plant species.

### HPTLC

**Stationary phase/Solvent/Derivatisation reagent**

**System 1:** 47/49% 1-propanol+ 0.024M Na<sub>2</sub>CO<sub>3</sub>/A

**System 2:** 86/69% 1-propanol+ 1-propanol+0.024M Na<sub>2</sub>CO<sub>3</sub>/B

**System 3:** 47/ 40% 1-butanol+ 10% acetic acid (upper phase)/C

**System 4:** 52/49% 1-propanol+ 5.6% NH<sub>3</sub>/none

**System 5:** 92/69% 1-propanol+0.024M Na<sub>2</sub>CO<sub>3</sub> /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<sub>254s</sub> (HPTLC)

**Derivatisation reagents:**

**A:** 2-aminoethyl diphenylborinat + polyethyleneglycole 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)

### 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.

### Biomarker in per cent of the content

(exposed minus control)(positive values = blue;negative values = green)

Rf-value/colour of spot /HPTLC-system	Biomarker	AM	ASV	PA	BH	LP
0.78 +/- 0.04/orange/1	Unidentified		25%	-25%		
0.85 +/- 0.00/blue/1	Unidentified				50%	25%
0.05 +/- 0.02/black/2	Unidentified	50%				50%
0.09 +/- 0.09/black/2	Unidentified	50%	50%			
0.14 +/- 0.02/black/2	Unidentified					
0.21 +/- 0.01/black/2	Unidentified		50%	50%		50%
0.26 +/- 0.00/orange/2	Unidentified	-50%	-50%	-50%	-50%	-50%
0.32 +/- 0.02/black/2	Unidentified	50%	50%	50%	50%	50%
0.47 +/- 0.00/black/3	Unidentified	-25%	-25%	-50%	-25%	-25%
0.60 +/- 0.00/violet/3	Unidentified	25%		25%	25%	25%
0.79 +/- 0.00/black/3	Unidentified	-25%	-25%	-25%	-25%	-25%
0.86 +/- 0.00/black/3	Unidentified		-25%	-25%		
0.67 +/- 0.03/blue/4	Unidentified				50%	50%
0.15 +/- 0.00/violet/5	Unidentified		-50%	-25%		-50%
0.25 +/- 0.00/red/5	Glycine	50%	50%	50%	50%	50%
0.45 +/- 0.00/yellow/5	Proline	75%	75%	75%		50%
0.50 +/- 0.00/red/5	Methionine	25%	25%	25%	25%	
0.60 +/- 0.00/violet/5	Phenylalanine	25%		25%		
0.65 +/- 0.00/red/5	Isoleucine		25%		25%	
0.25 +/- 0.00/red/6	Glycine	50%	50%	50%	50%	25%
0.30 +/- 0.00/violet/6	Unidentified		-25%	-25%	-25%	
0.45 +/- 0.00/yellow/6	Proline	75%	75%	50%	50%	50%
0.50 +/- 0.00/violet/6	Phenylalanine	50%		25%	25%	
0.60 +/- 0.00/red/6	Methionine	25%	25%	25%	25%	
0.75 +/- 0.00/red/6	Isoleucine		25%		25%	

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