

# Plant Biomarker Pattern

## Herbicide exposure in buffer zones

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### Background

In March 2000 the Danish Government issued the Pesticide Action Programme II. With respect to buffer zones, the goal before the end of 2002 is to lay out 20.000 hectare as buffer zones along brooks, water streams and lakes over 100 square meters.

### Concept

Buffer zones are investigated using a new biomarker method to show pesticide effects on plants.

### Method for plant study

The studies were performed in autumn 2000 and spring 2001. At six different buffer zones, three transects with three to eleven test spots in each transect were investigated. Plants were sampled one time before and three times after the field treatment.

The plant material was frozen and pressed before screening for a biomarker pattern using Thin Layer Chromatography (TLC).

A Roundup 2000+Teamup exposed field in Tudbæk.



### Plants

A herbicide sensitive plant, scarlet pimpernel *Anagallis arvensis* (Primulaceae) was used as model plant. Plants were:

- Cultivated in green-house for two months.
- Transplanted into the buffer zones in each test spot a month before exposure.
- Control plants were planted in a pesticide free area.
- Natural growing plants present in the buffer zone were sampled at the same time as the model plants.
- Control plants were from a pesticide free area.

### Biomarker method

Five different TLC-systems were used for each sample to ensure and confirm the presence of a biomarker pattern, elucidating the following groups of phytochemical compounds:

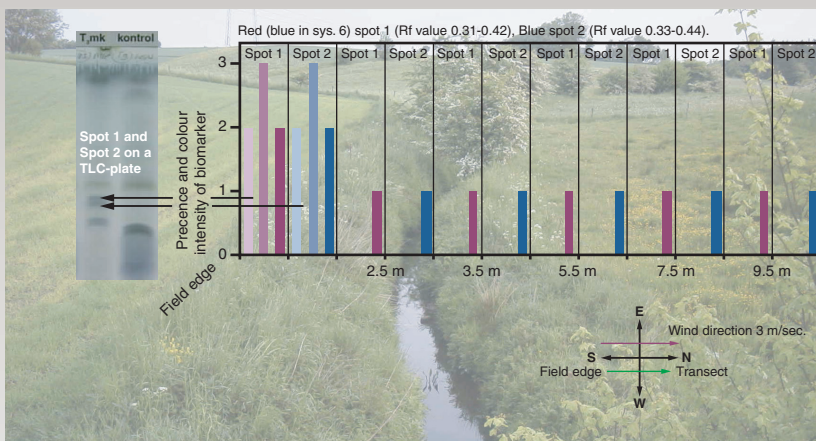
- Unspecific compounds (two TLC-systems)
- N-containing compounds
- Lipids and terpenoids
- Phenolic compounds

### Vegetation analysis

The ten most frequent plant species in the buffer zone:

- *Urtica dioica*
- *Elytrigia repens*
- *Poa pratensis*
- *Epilobium hirsutum*
- *Galium aparine*
- *Cirsium sp.*
- *Filipendula ulmaria*
- *Alopecurus pratensis*
- *Arrhenatherum elatius*
- *Ranunculus acris*

### Brook of Hesselbæk – Biomarkers in *Anagallis arvensis*



The position and the intensity of the biomarkers.

### Regulation

A two meter buffer zone shall be established for certain water streams and lakes in Denmark.

### Distance from field to field across the brook.

- Sampling 1 spot 1. (7 days after pesticide exposure)
- Sampling 1 spot 2. (7 days after pesticide exposure)
- Sampling 2 spot 1. (15 days after pesticide exposure)
- Sampling 2 spot 2. (15 days after pesticide exposure)
- Sampling 3 spot 1. (21 days after pesticide exposure)
- Sampling 3 spot 2. (21 days after pesticide exposure)

Buffer zones, location, wind direction, pesticides used and results.

Buffer zone	Max. m per transect	Location of buffer zone	Wind direction at the time of exposure	Pesticides	Results
Tudbæk (2000)	16 m	S	S/SW, 7 m/sec.	Roundup 2000, Teamup	No effect
Tudbæk (2001)	29 m	S	S/SW, 3-4 m/sec.	Stomp, Basagran MP	No effect
Smedholdt (2000)	12 m	N	SE, 3 m/sec.	Oxtril, Stomp, Boxer	≥12 m
Smedholdt (2001)	11.9 m	ENE	NW, 2 m/sec.	Rival	No effect
Ellerup (2001)	4.3 m	NNE	S/SW, 2 m/sec.	Express, SP 50	≥4.3 m
Hesselbæk (2001)	10.2 m	N	S, 3m/sec.	Basagran 480, Stomp	≥10.2 m
Danstrup (2001)	7.4 m	NE	NW, 2m/sec.	Corbel, CCC	≥7.4 m
Skovgård (2001)	29 m	N	S/SW, 3m/sec.	Laddok TE (Renol)	≤14 m

### Conclusion

Five out of six buffer zones close to and across brooks showed a biomarker pattern in plants up to 14 m.

This indicates a herbicide effect on the buffer zones from the treated area, although no visible injuries were observed.

The new biomarker method is sensible.

The diversity of the vegetation is dependent on the size of the buffer zone. Few plant species are present in narrow buffer zones (2 m) compared with broad zones, where the botanic diversity is larger.



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In 2000 an 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) was filed at the European Patent Office with the purpose of international patent protection.