

# Monitoring the Effects of GMO - Standardized Technical and Biological Pollen Sampling

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## I. Introduction

Pollen dispersal plays a significant role in the spread of genetically modified organisms (GMO). Tasks like GMO monitoring according to the directive 18/2001 EC or the surveillance of pollen transfer for coexistence both require procedures that enable quantification and documentation of GMO input and spread through pollen in a comparable way.

For this, methods for biological and technical pollen sampling as well as PCR-screening procedures were developed in a research project [5] to prove GMO input. The methods were finally standardized in VDI guidelines on pollen monitoring that consist of two parts:

- Part 3 describes the technical pollen sampling with the pollen mass filter PMF and the Sigma-2 [6].
- Part 4 presents the biological pollen sampling with colonies of honey bees [7].

The guidelines are intended to serve as technical aids to standardize the methods. Primary aim is to guarantee comparable results between different researchers, laboratories and sites of investigation.

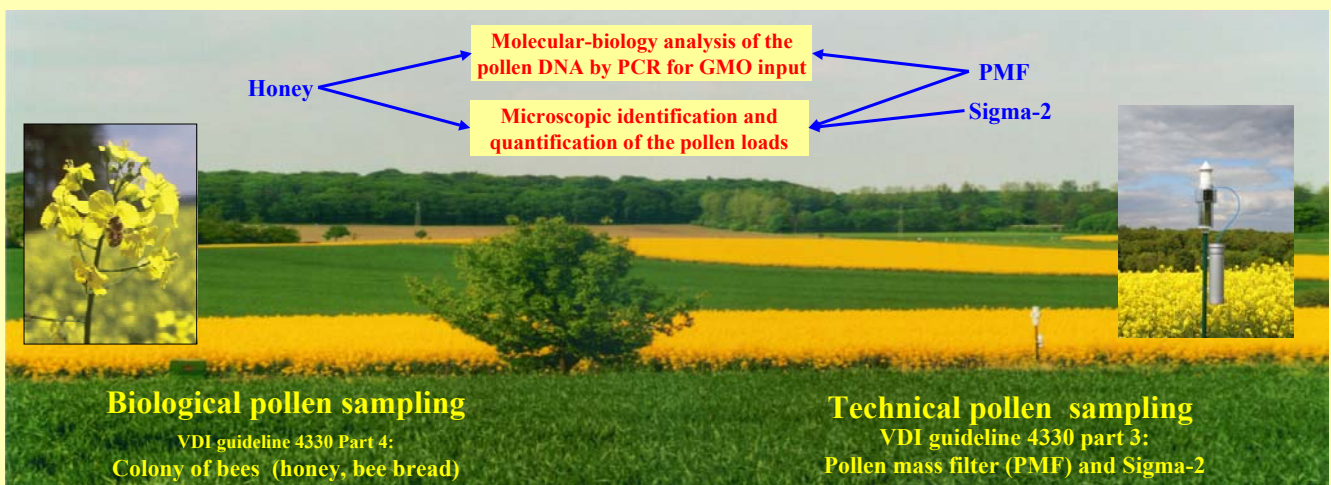
## III. Validation in field tests

Validation was performed using defined pollen gradients in the areas around sites where transgenic HR rape, Bt corn and VR sugar beet were grown. In 2001, physical-mechanical traps and beehives were installed at a total of 81 sites in the vicinity of GMO test fields, in cells of up to 8 x 8 km<sup>2</sup> in area as well as in corresponding reference areas, on the basis of findings from pollen distribution models, at various distances and wind directions, in such a way that gradient tests could be performed over several different orders of magnitude. From 2002 to 2004, further tests were conducted, including comparative measurements with the standard volumetric pollen trap (Burkard type).

The results in bee's honey as biological pollen sampler showed typical collection areas dependent on distance. This reflected the active collection behaviour of the honey bees. Pollen data from bee's honey were thus an indication of the blooming pollen-producing plants established within a certain radius around the sampling point.

GMOs were detected by PCR in the sample matrices of both the technical and biological pollen collectors (PMF, honey). Sensitivity, reproducibility and detection limits were statistically evaluated. Based on these results, sample sizes were derived that are required for a reliable monitoring.

The results showed that both methods, technical and biological pollen sampling, complemented each other in manifold ways.



## II. Description of the project

The PMF pollen mass filter was developed for the project and deployed in combination with the Sigma-2 passive sampler as the technical pollen trap. The biological pollen sampler used was the honey bee. The pollen samples obtained with the Sigma-2 collector were analysed visually with the help of image analysis methods to determine the pollen deposition. The PMF samples were used for molecular biological DNA analysis of GMO loads using PCR screening. The pollen in the honey and bee bread samples were counted under a microscope, and the pollen DNA analysed with PCR.

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- 5 Hofmann, Schlechtriemen, Wosniok, Foth (2005): GVO-Pollenmonitoring – Technische und biologische Pollensammler und PCR-Screening für ein Monitoring von gentechnisch veränderten Organismen. BfN Scripten 139, 275p. URL: [www.bfn.de](http://www.bfn.de)
- 6 VDI guideline 4330 part 3 (2005-07): Monitoring the effects of GMO – Pollen monitoring – Technical pollen sampling using pollen mass filter (PMF) and Sigma-2 samplers. VDI, Beuth Verlag, Berlin. URL: [www.beuth.de](http://www.beuth.de)
- 7 VDI guideline 4330 part 4 (2005-07): Monitoring the effects of GMO – Pollen monitoring – Biological pollen sampling using colonies of honey bees. VDI, Beuth Verlag, Berlin. URL: [www.beuth.de](http://www.beuth.de)
- 8 VDI guideline 4330 part 1 (2005-04): Monitoring the effects of GMO – Basic principles and monitoring strategy. VDI, Beuth Verlag, Berlin. URL: [www.beuth.de](http://www.beuth.de)

## IV. Results

Pollen from the core species of rape, corn and sugar beet, as well as numerous other cultivated and wild plant species were identified in the samples obtained from the technical traps as well as by biological sampling in the honey and bee bread. In total, more than 150 pollen species were collected. The pollen input could be quantified by amount and species.

In the technical pollen traps, the gradients of the rape pollen loads from GMO fields were mirrored, depending on wind direction, wind speed and distance, down to a clearly identifiable background regional load corresponding to the intensity of crop cultivation in the area.

## V. Conclusions

For environmental monitoring of GMOs, the technical and biological methods are an ideal combination. In total, using the technical and biological collectors enables pollen from approximately 150 plant species or genera to be identified and differentiated.

The pollen monitoring by the technical and biological sampling methods can be applied almost anywhere in the open field. No expensive installations are needed. Due to the comparably low costs a high number of samples sites can be realised, sufficient for an effective monitoring of GMO transfer.

The methods were successfully validated in field experiments and finally standardised in VDI guidelines on monitoring the effects of GMO. The series started to be published in April 2005 [7, 8, 9].

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