149

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OptiScreen - Basic investigations for a mycotoxin screening sensor system usable in industrial grain sorting

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INTRODUCTION

Mycotoxins are toxic and under suspicion to be carcinogenic and mutagen, to influence the endocrine system negatively, to promote bleedings, to weaken the immune system, to damage skin and kidneys and they can attack the nervous system. In worst cases mycotoxins can pass undetected through the whole production chain of grain and cereal products and damage the health of humans and animals even in low concentrations.

The aim of the project "OptiScreen" is the development of a screening concept to detect mycotoxins in storage side and to improve the elimination of contaminated grain during industrial grain sorting.

The optical sensor for real time detection above a grain stream will enable to sort-out contaminated grains and will be supplemented with an ion mobility spectrometry (IMS) sensor for the analysis of the gas phase in grain silos for early and quantitative detection of fungal contamination.

BASIC ANALYTICS AND RESULTS

For the development of the described screening sensor system several fundamental investigations are necessary.

The microbiological analysis gives information about the microbial load of grains including the specific mold contamination and identification in correlation to the quantitative mycotoxin determination via ELISA and HPLC. The generated basic information is in general used for the calibration of the sensor system.

Figures 1 - 8 demonstrate typical results of applied analytical methods for mold contamination and identification as well as the total viable count.

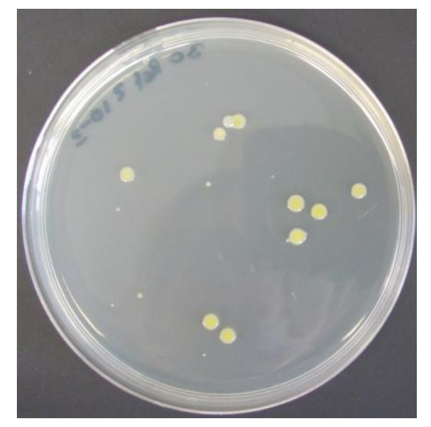
Fig. 1: Total viable count (barley) Standard-I-Agar

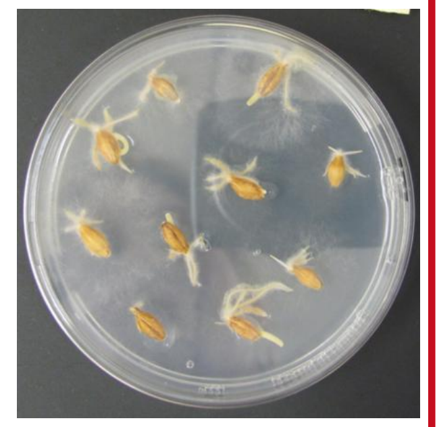
Fig. 2: Mold analytic of barley on Czapeck-Dox-Agar

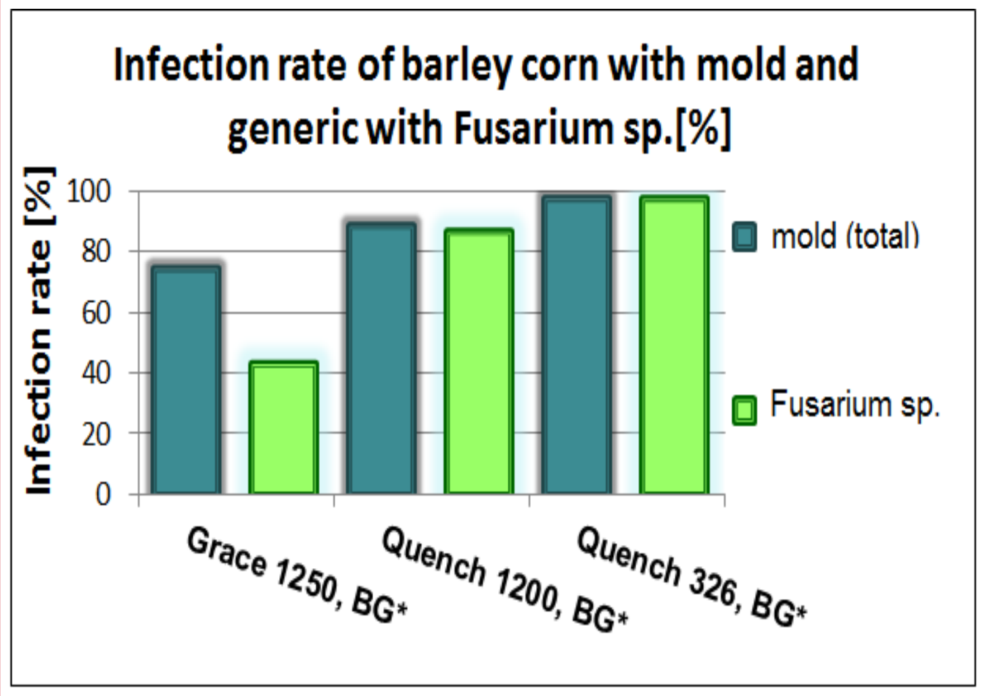

Fig. 3: Mold analytic of barley on Czapeck-Dox-Agar and on Malachite green agar (Fusarium sp.)

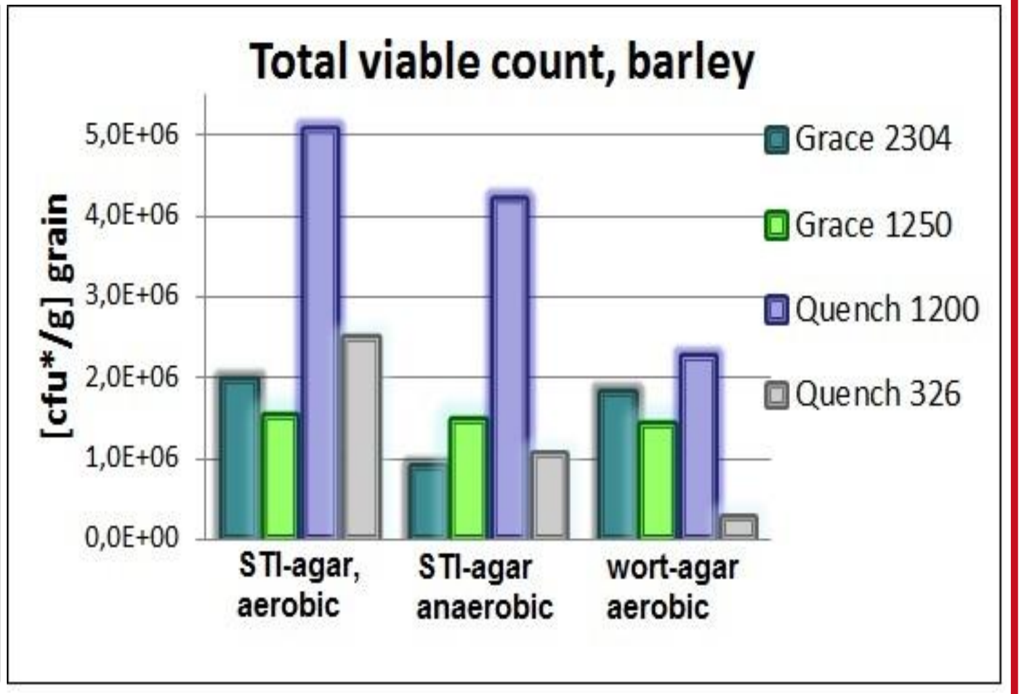

Fig. 4: Total viable count of barley on STI-agar under aerobic and anaerobic conditions and on wort-agar

Figure 5-8 illustrate typical strains of mold wich were isolated from barley.

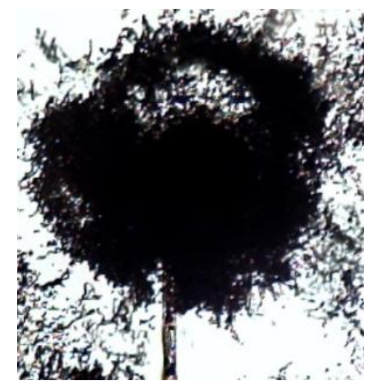
Fig. 5: Aspergillus sp.


Fig. 6: Penicillium sp.


Fig. 7: Fusarium sp.


Fig. 8: Alternaria sp.

BASIC ANALYTICS AND RESULTS

The amount of the mycotoxins DON, ZEA, and OTA of natural and specifically contaminated barley will be analyzed by HPLC analysis using immunoaffinity columns. Additionally, the amount of Aflatoxin B1 will be analyzed by ELISA. The analyses of spiked barley revealed a recovery rate of 84% for DON (Fig. 9) and of 80% for ZEA.

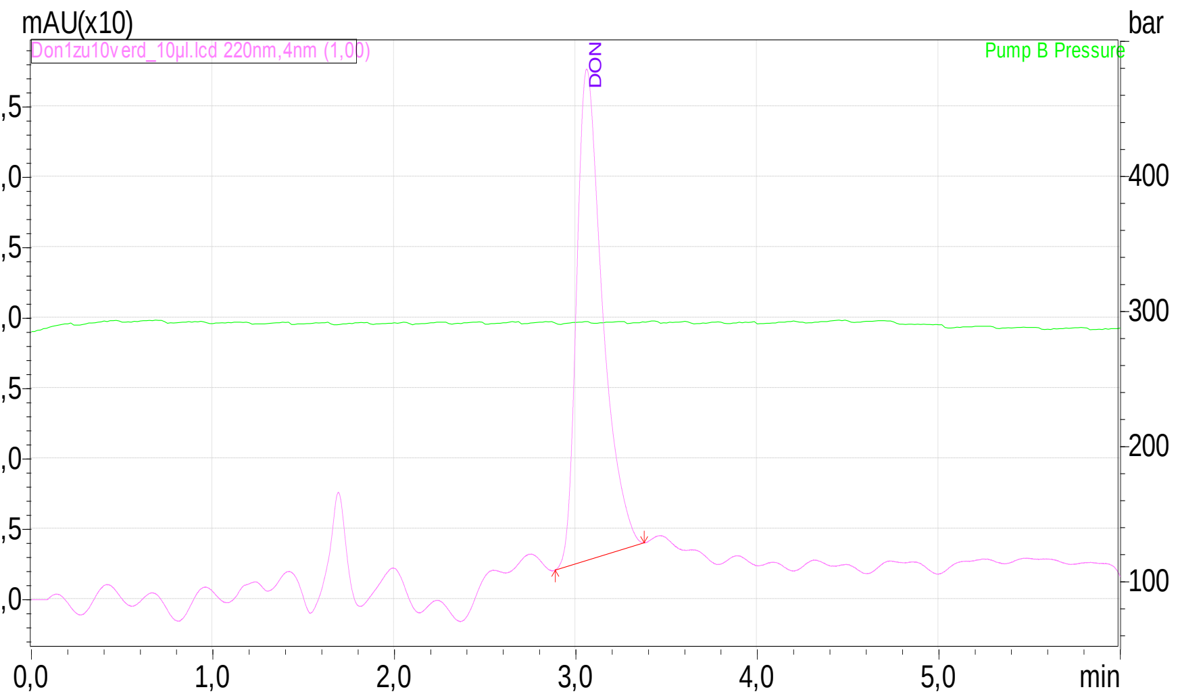

Fig. 9: HPLC chromatogram of spiked barley with DON

EXPERIMENTAL PART 1 : OPTICAL SPECTROSCOPY

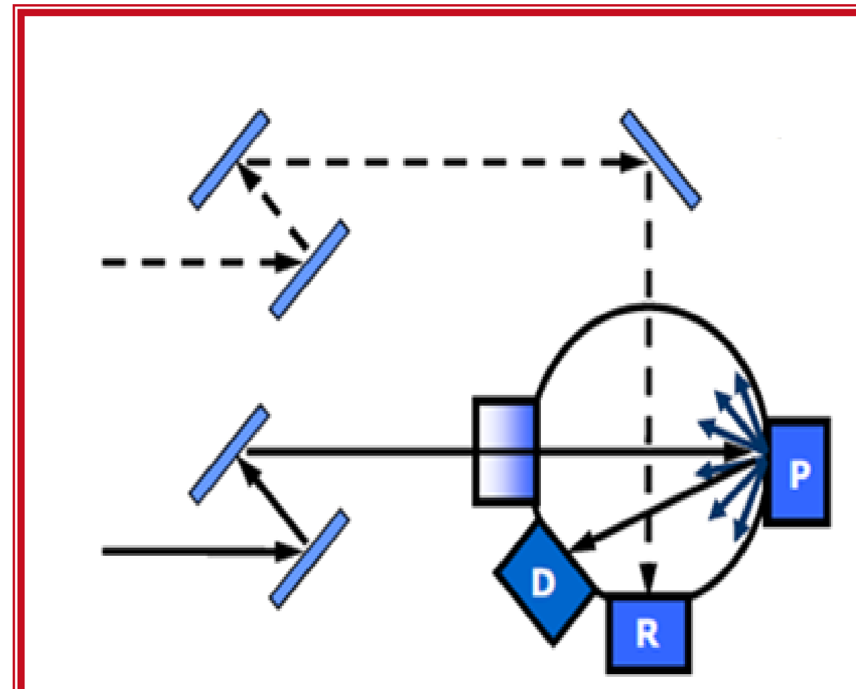

Fig 10: NIR-reflection

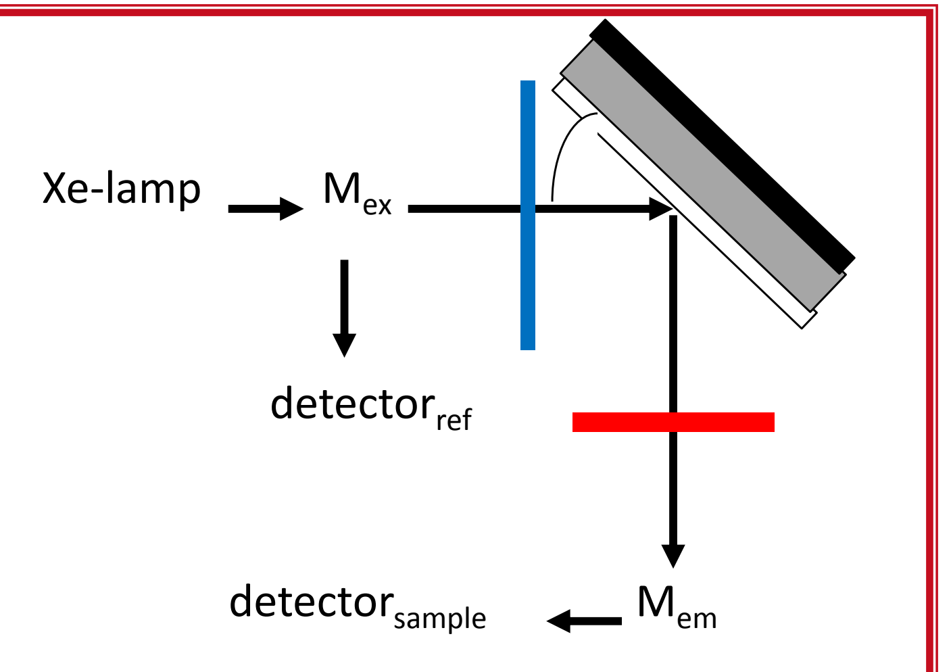

Fig. 11: Front-face detection

Due to the highly complex matrix of grain, the sensitive analysis of mycotoxins is based on a combination of high-resolution UV/VIS fluorescence spectroscopy and reflection spectroscopy in the NIR range. Therefore, two detection systems are used: a classical UV-VIS-NIR spectrometer (Lambda 750, Perkin Elmer) to determine water content and specific metabolites and a fluorescence spectrometer for *front-face detection* (Fluoromax 3, Horiba) to monitor the emission signature of the superimposed spectra of grain and mycotoxins.

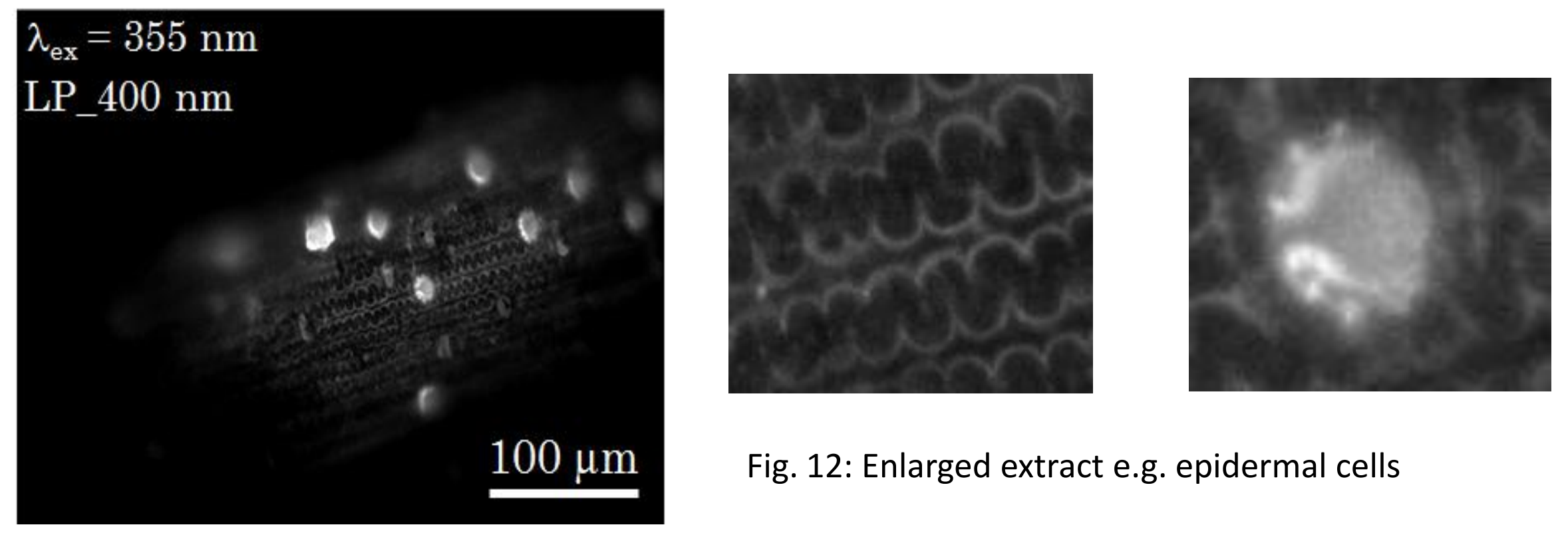

Fig. 12: Enlarged extract e.g. epidermal cells

A detailed knowledge of sample autofluorescence (*e.g. hotspots or heterogeneity)* is of great importance as correction parameters with regard to the PCA.

DETECTION OF OCHRATOXIN AND AFLATOXIN B1 BY FLUORESCENCE SPECTROSCOPY

Grains with different moisture contents, mycotoxins on $MgSO_4$ as well as artificial mycotoxin contaminations on grains were measured to evaluate possible disturbances due to the matrix. A set of wavelength pairs (λ_{ex}= 330 nm/ λ_{em}= 460 nm und λ_{ex}= 380/ λ_{em}= 438 nm) for reference and sample measurements were determined by reduced excitation-emission spectra.

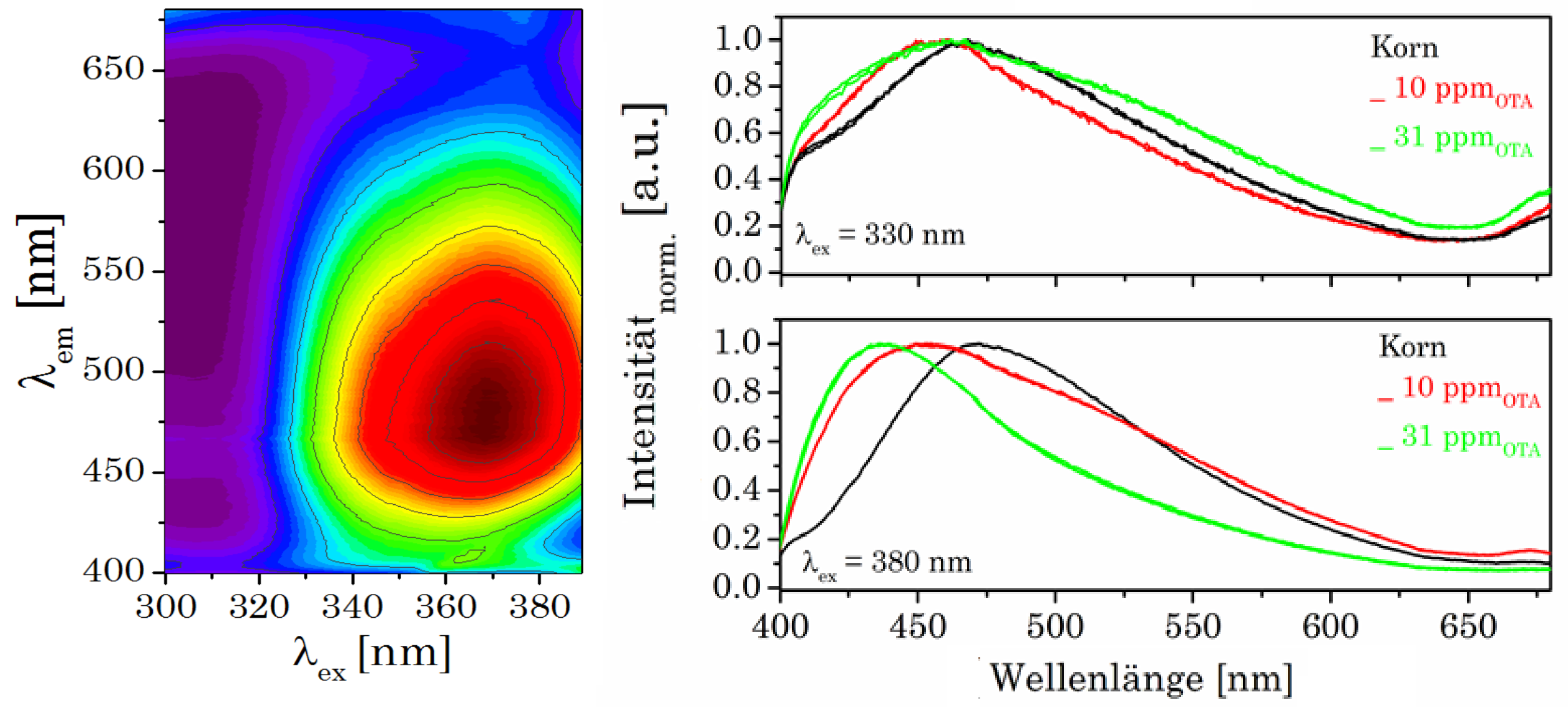

Fig. 13: Front-Face Fluorescence Spectroscopy

Distinct allocation of NIR bands to water, starch, lipids or carbohydrates, whereby parameters like moisture and clues of mycotoxins (e.g. lipid degradation) are accessible.

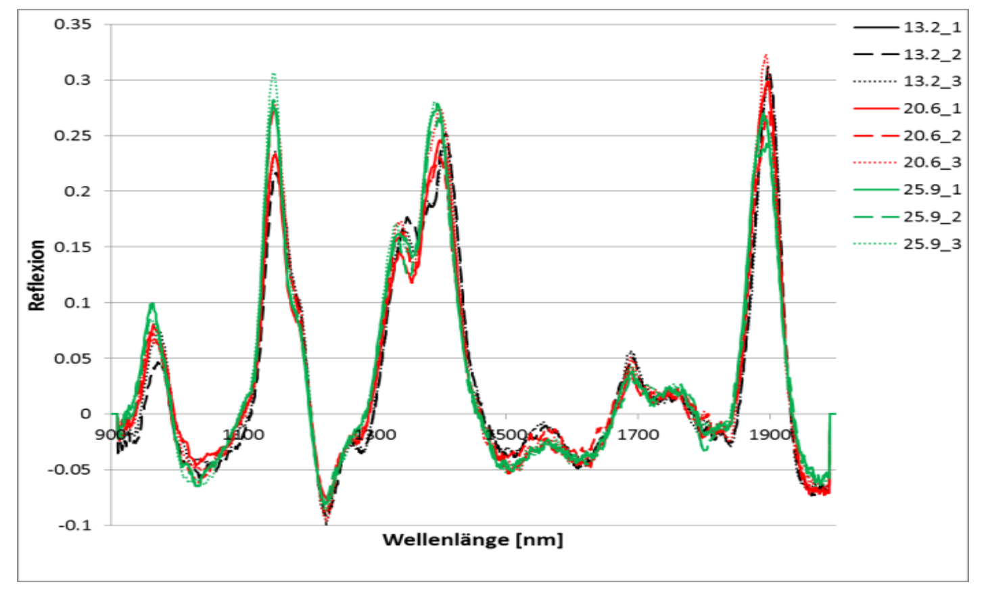

Fig. 14: NIR-Reflection Spectroscopy

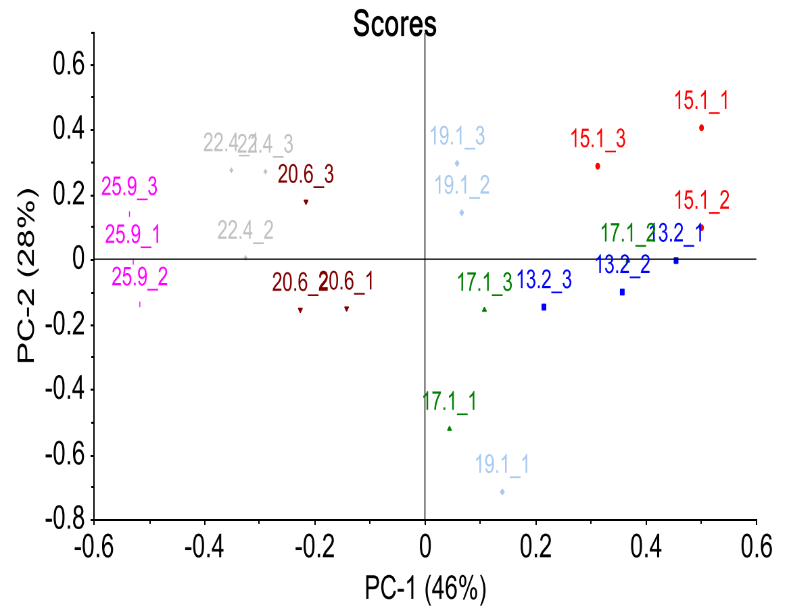

Fig. 15

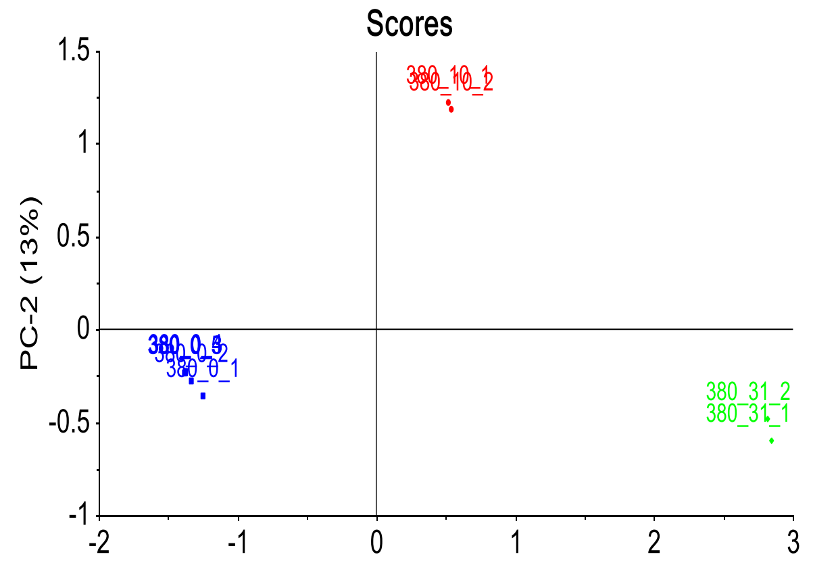

Fig. 16

Chemometric data analysis (PCA): A discrimination of grains with different moisture contents as well as mycotoxin contaminations was possible using two principal components and first/second derivative as preprocessing method (Fig. 15-16). In the subproject an ion mobility spectrometry (IMS) sensor is developed for the analysis of the gas phase in grain silos for early and quantitative detection of fungal contamination. Therefore different mold strains are bred on agar and brewing barley (Fig. 17) as soon as investigated with regard to characteristic volatile metabolites headspace.

DETECTION OF VOLATILE METABOLITES OF *FUSARIUM CULMORUM* ON AGAR AND GRAIN BY GC-APCI/EI-MS


Fig. 17: GC-Vials - barley with Fusarium culmorum /Aspergillus niger (r.)

The applied GC-APCI-MS uses a miniaturized x-ray lamp emitting soft x-radiation (2.7 keV) as an ion source which produces protonated molecular ions. The detection of these molecular ions in combination with the characteristic fragment spectra obtained by GC-EI-MS allows a reliable identification of volatile metabolites. Several characteristic volatile metabolites of fusarium culmorum are found in the headspaces of agar and grain as shown in figure 18-19 .This APCI source is also used in the RoadRunner IMS. Therefore the results can be transferred.

A second aim is the direct detection of mycotoxins on grain by a fast, mobile analytical technique. The RoadRunner IMS is a hand-held spectrometer which is able to carry out one measurement in 10 s. Aflatoxin B1 is detected as protonated monomer and dimer ions. The corresponding peaks are found at 15 and 22 ms. The identity of these ions could be confirmed in the MS spectrum.

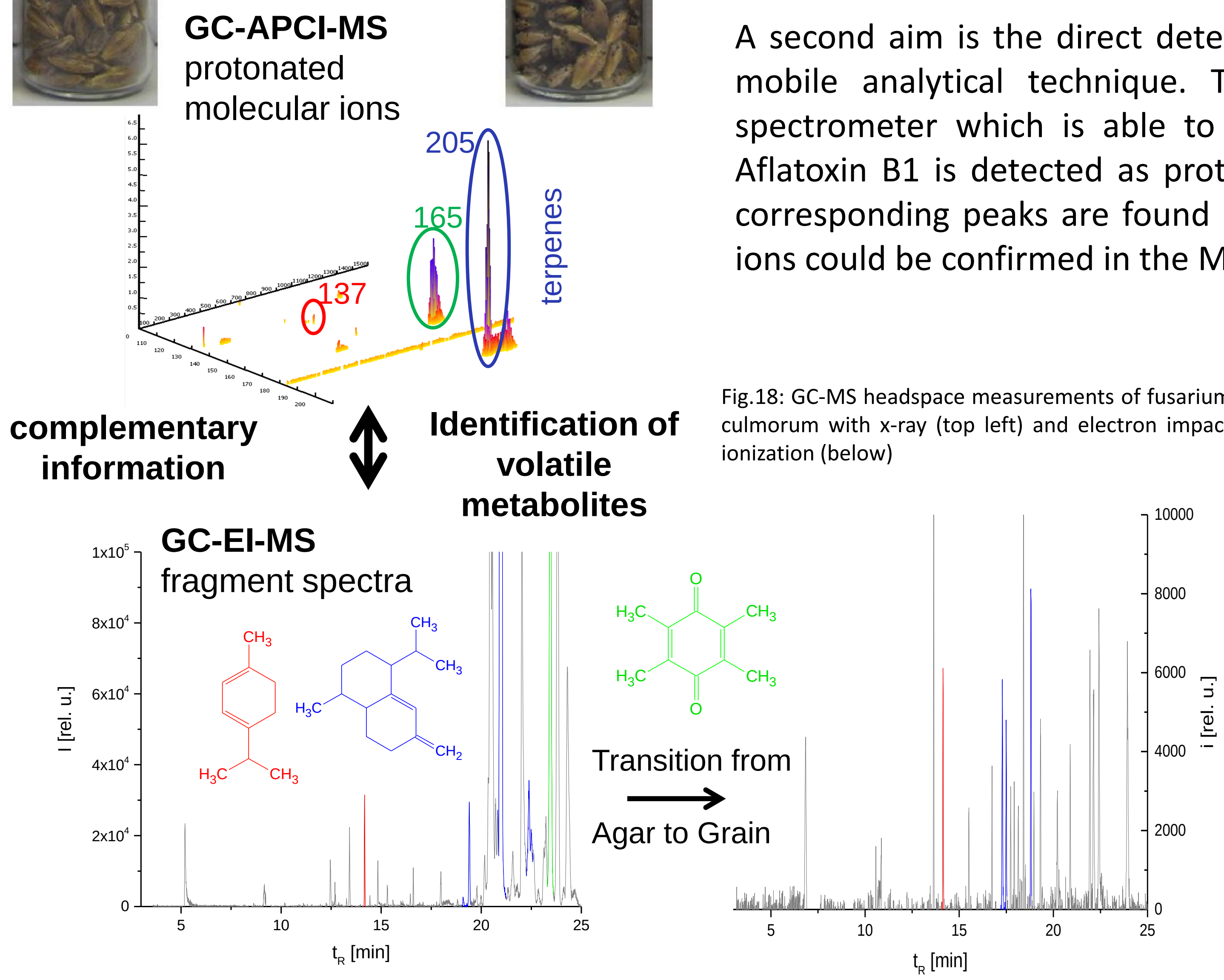

Fig.18: GC-MS headspace measurements of fusarium culmorum with x-ray (top left) and electron impact ionization (below)

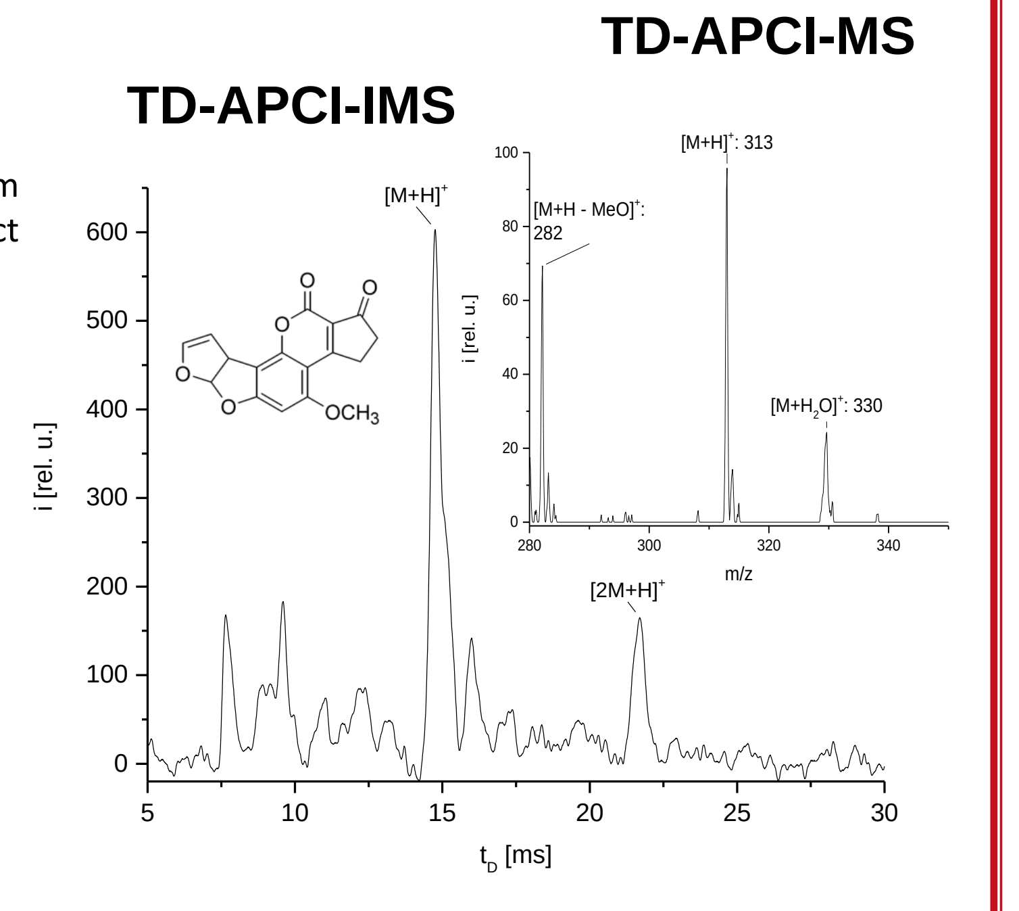

Fig.19: Thermodesorption spectra of Aflatoxin B1 with IMS and MS (inlet) using x-ray ionization

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