BOOK OF ABSTRACTS

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PP 32: Volatile organic compounds as diagnostic biomarkers for grey mould on lettuce

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Abstract: *Grey mould is one of the most destructive diseases of lettuce. Volatiles of healthy and infected plants were analysed to find differences between them. Healthy and infected plants can be distinguished by the sesquiterpene compounds found in the volatilome of lettuce infected with grey mould; in the future, these compounds could be used as diagnostic biomarkers.*

Keywords: volatile organic compounds, grey mould, lettuce

Introduction: Lettuce (*Lactuca sativa*) is among the most vulnerable vegetables. Damages caused by phytopathogenic organisms can easily ruin its commercial value. Grey mould (*Bortytis cinerea*) is a major fungal disease of vegetable crops grown in greenhouses and it is one of the most destructive and economically important fungi affecting greenhouse lettuce. Infection usually begins at the seedling or transplanting stage and eventually leads to plant death [1].
Fungal pathogens can produce mycotoxins that are dangerous to humans in addition to economic damage. Volatile organic compounds as diagnostic biomarkers have been used to detect infected plants [2], but the volatiles released by lettuce infected with grey mould have not yet been studied.

Experimental methods: In the inoculum, conidia concentration was adjusted to 1×10^6 spores mL^{-1}. Leaves of 3-week old plants were inoculated with a microsprayer.
Volatiles were collected from 4 infected and 4 control plants in two stages: at the first symptoms (*e.g.* fading leaves) and at a heavily damaged stage (greyish and yellow spots appeared on the leaves, and oldest leaves began to wilt). Volatile collection traps filled with 50 mg Porapak Q (80-100 mesh) were used to collect volatiles in the headspace for 6 hours. The collected volatiles were immediately extracted with 300 µL of *n*-hexane.
An Agilent 6890 gas chromatograph (GC) coupled with a 5975 C MSD mass spectrometer (MS) was used with a HP-5 UI capillary column to analyze the collected volatiles. 1 µL sample was injected in splitless mode for 30 seconds with the injector temperature set to 250°C. Helium was used as carrier gas. Positive electron ionization (EI+) was used for detection. The detector was used in scan mode between 35-500 m/z values. Agilent MassHunter Workstation Qualitative Analysis B.08.00 software was used to analyse and compare the chromatograms. Kováts indices (KI) were calculated using the C8-C20 alkane calibration standard and the identification was also verified by comparison with Kováts indices (KI) values obtained from the Agilent NIST 2017 Mass Spectral Library. Statistical analysis was performed using Canoco 5.

Results and discussions: Already at the appearance of the first symptoms (FS), we could distinguish between the infected and healthy plants. In the volatilome of the infected plants, 56 compounds were detected, while in the headspace of the controls only 26 compounds appeared. Most of the compounds visible in the headspace of the infected plants were sesquiterpenes.
This large difference in the number of compounds detected did not change when the plants became heavily damaged (HD). In this condition, 57 compounds were detected in the volatilome of the infected plants and 27 compounds in the headspace of the control plants. The difference was also mainly due to sesquiterpenes, but the intensity of the detected sesquiterpene compounds decreased remarkably. PCA analysis was used to separate the control and infected groups. When all examples were examined together FS, HD and the control plants were clearly separated into 3 groups.
Sesquiterpene compounds have already been considered as pathogen induced volatiles [3].

Conclusions: The detection of volatiles identified at an early stage of infection could help to stop the spread of disease in greenhouses. Until now, compounds released from lettuce due to grey mould infection were not known. Based on our study, sesquiterpenes could be used as diagnostic biomarkers. In the long term, the use of these compounds for early detection of infections would increase the safety of fresh vegetables.

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