



Odor-based real-time detection of maize plants under attack by pests: proof of concept

Marine Mamin^{a,1} , Carla C.M. Arce^{a,1} , Gregory Röder^a , Arooran Kanagendran^a, Thomas Degen^a , Emmanuel Defossez^b , Sergio Rasmann^b , Terunobu Akiyama^c, Kosuke Minami^d , Genki Yoshikawa^{d,e} , Felipe Lopez-Hilfiker^f , Priyanka Bansal^f , Luca Cappellin^{f,g} , Yunhe Li^h , Ted C.J. Turlings^{a,h,i,*}

^a Laboratory for Fundamental and Applied Research in Chemical Ecology (FARCE), Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel, 2000, Switzerland

^b Functional Ecology Laboratory, Institute of Biology, University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel, 2000, Switzerland

^c NanoWorld AG, Rue des Saars 10, Neuchâtel, 2000, Switzerland

^d Research Center for Macromolecules and Biomaterials, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki, 305-0044, Japan

^e Materials Science and Engineering, Graduate School of Pure and Applied Science, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8571, Japan

^f Tofwerk AG, Thun, Switzerland

^g Department of Chemical Sciences, University of Padua, Padua, Italy

^h Laboratory of Plant-Insect Research for Sustainable Agriculture (PIRSA), State Key Laboratory of Cotton Bio-breeding and Integrated Utilization, School of Life Sciences, College of Agriculture, Henan University, Kaifeng, 475004, China

ⁱ Department of Entomology, The Pennsylvania State University, University Park, Pennsylvania, USA

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ABSTRACT

Rapid detection of crop pests and diseases can enable timely, targeted interventions, and help reduce pesticide use. Plants under biotic stress are known to rapidly emit characteristic blends of volatile compounds that could potentially serve as fast and attacker-specific cues for precise pest monitoring. Here, we evaluated the feasibility of this approach using two complementary, state-of-the-art sensing technologies: a handheld nanomechanical membrane-based sensor array and chemical ionization time-of-flight mass spectrometry. Under laboratory conditions, with enclosed headspace sampling, both technologies readily distinguished undamaged maize plants from plants infested by caterpillars or infected with a fungal pathogen. Under semi-controlled outdoor open-air conditions, where volatile concentrations were strongly diluted, the membrane-based sensor no longer retained discriminatory power, whereas mass spectrometry predicted herbivory status with high accuracy using one-second measurements. Finally, a preliminary one-day field trial indicated that a compact, field-deployable, real-time mass spectrometer may discriminate between undamaged maize plants and plants subjected to simulated herbivory using spectral information averaged over 60-s measurement periods. Together, these results provide initial evidence for the potential of real-time mass spectrometry as a promising tool for crop monitoring to detect plants under attack by pests. However, further field validations are needed and several critical challenges remain to be addressed before translation to practical field applications.

1. Introduction

Current agricultural practices depend heavily on synthetic agrochemicals, a reliance widely acknowledged as unsustainable and a major contributor to environmental pollution, habitat degradation, biodiversity loss, and climate change [1,2]. Reducing agrochemical use while

safeguarding yields is therefore an essential focus of current agricultural research [3]. Despite decades of innovative control efforts, pests and diseases still cause up to 40% of potential crop losses [4], further underscoring the need for more effective and sustainable approaches to plant protection [5–7].

Efficient detection of pests and diseases through real-time

* Corresponding author.

E-mail address: ted.turlings@unine.ch (T.C.J. Turlings).

¹ These authors contributed equally to this work.

monitoring can greatly facilitate timely and precise interventions, reducing pesticide use while maintaining crop protection [8–10]. Precision agriculture has advanced several non-destructive monitoring techniques, including methods that directly detect attackers (e.g. acoustic sensors for insect activity) and those that monitor changes in plant phenotypes through imaging technologies [11–13]. Despite significant progress, key challenges remain, particularly detecting attacks at early stages and discriminating between multiple stressors under field conditions [14].

Inducible plant volatiles represent promising warning signals of pest and disease presence [15–21]. In response to arthropod herbivory or infection by pathogenic agents, plants rapidly emit characteristic blends of volatiles, often just within hours of attack [22–25]. These emissions are shaped by plant recognition of elicitors produced by the attackers, such as pathogen-derived molecules or compounds present in oral and egg secretions of herbivores [26–29]. Because different elicitors activate distinct signaling cascades, the resulting odor blends can be highly specific [29,30]. This specificity is exemplified by the ability of natural enemies to locate plants carrying their preferred insect prey [31,32]. Thus, inducible plant volatiles could serve as attacker-specific “chemical fingerprints” that can potentially be exploited for both fast and precise crop surveillance [33].

Two main strategies have been proposed to exploit plant volatiles for monitoring crop health. Static systems, such as leaf-wearable sensors, could continuously track individual plants [34,35], while mobile systems could sample volatiles across fields, potentially through autonomous platforms [36]. A mobile solution, however, requires odor sensing technologies capable of fast, real-time measurements, robust to environmental fluctuations and with sufficient sensitivity to detect volatiles in open air, where they are diluted, variable, and intermingled with background odors.

Different portable electronic gas sensors have been developed for rapid onsite odor detection, using arrays of sensing elements whose chemical or physical properties change upon interaction with volatiles. These changes are transduced into electronic or optical signals and analyzed with pattern recognition algorithms to discriminate odor profiles [19,21,37–41]. While several of these small systems have demonstrated the ability to detect volatile signatures associated with pest or pathogen attack in agriculturally relevant plant species, most studies have been performed under laboratory or greenhouse conditions, and using enclosed sampling setups, minimizing dilution and background interference [16,17,20,41]. Although much larger, certain high-performance instruments can provide accurate capabilities for real-time odor detection. In particular, Chemical Ionization Time-Of-Flight Mass Spectrometry (CI-TOF-MS) is highly performant for online measurement of trace volatiles in open-air and complex environments, as showcased by its use in atmospheric field studies [42].

We investigated here the feasibility of real-time pest and disease detection based on volatiles emissions by maize (*Zea mays*), one of the most widely cultivated crops worldwide. Experiments progressed from laboratory assays using a dynamic headspace sampling system to open-air measurements in semi-controlled outdoor conditions and finally initial, preliminary, field measurements. We applied two complementary sensing technologies. The first was a handheld module incorporating Membrane-type Surface Stress Sensors (MSS – NIMS), which detect volatile-receptor interactions through membrane deformation that generates piezoresistive signals [43–45]. This compact electronic sensor has demonstrated potential for food, environmental, and medical diagnostics [46–49]. The second technology was CI-TOF-MS. Laboratory and open-air assays were conducted with an instrument based on proton transfer reaction (PTR-TOF; Vocus S – Tofwerk AG) [50], an ionization method previously used to study dynamic stress responses in plants [20, 51–53]. For field measurements we used a portable TOF instrument (Vocus C – Tofwerk AG) [54]), configured with benzene cation ionization [55–57]. Laboratory studies homed in on volatiles induced by two lepidopteran pests of maize, *Spodoptera exigua* and *S. frugiperda*, and the

fungal pathogen *Colletotrichum graminicola*, whereas subsequent experiments focused on herbivory by *Spodoptera* caterpillars, given their agricultural significance [58]. This study provides a preliminary assessment of the feasibility of odor-based pest and disease detection in maize using available technologies, offering early-stage evidence to guide future research on integrating this approach into agriculture, with the potential to optimize and reduce agrochemical use.

2. Methods

2.1. Plants and growth conditions for laboratory and open-air assays

Maize plants, *Zea mays* var. Delprim and var. Aventicum (seeds supplied by Delley Semences et Plantes SA, CH), were grown in plastic pots (4 cm diameter × 10 cm height for laboratory experiments; 10 cm diameter × 8.6 cm height for the outdoor experiment). Pots were filled with commercial potting soil (Ricoter, Erdaufbereitung AG, CH) and grown under greenhouse conditions (30 ± 5°C, 60–80% relative humidity). Natural sunlight was supplemented with LED lamps (luminous flux 8,800 lm), providing a 16 h photoperiod. Plants were watered every other day and fertilized once per week with an 8–8–6 NPK fertilizer (Maag Garden, Switzerland). Plants used for laboratory measurements were 10–12 days old and had 2–3 leaves. For the open-air measurements, plants were 20–25 days old and had 4–5 developed leaves. For all outdoor measurements, the maize variety Delprim was selected due to its well-documented physiological and volatile-emission responses to *Spodoptera* attack [59,60], providing a robust and well-characterized baseline. This choice minimized genotype-driven variability and uncertainty at this initial proof-of-concept stage.

2.2. Pathogen, insects and regurgitant collection

Fungus: The fungal pathogen *Colletotrichum graminicola* (Ces.) G.W., which causes maize anthracnose leaf blight disease, was used to inoculate maize plants for laboratory measurements. The inoculation was done following Balmer et al. [61]. Fungal colony (M1.001, obtained from Lisa Vaillancourt, University of Kentucky, Department of Plant Pathology, Lexington, KY) was maintained at 25°C on potato dextrose agar (Difco PDA, Becton Dickinson) under continuous light (70 μmol m⁻² s⁻¹). Three-week-old plates were used to harvest spores for infection. Twenty μL of a *C. graminicola* conidia suspension (6 × 10⁵ spores mL⁻¹ sterile water with 0.01% Silwet L-77, Lehle Seeds) were applied and spread over the leaves using a syringe. After inoculation, infected plants were kept under room conditions for 16h, without direct sunlight and in an open plastic box (50 × 30 × 35 cm) containing vaporized water used as a humid chamber (min 75% relative humidity, 23 ± 2°C), before transferring them back to an isolated place in the greenhouse. Fungus-inoculated plants were further monitored after odor measurements, as signs of a successful anthracnose leaf blight disease were hardly detectable at this stage. Therefore, plants that had not developed any visible disease symptoms by day 15 (i.e., one week after inoculation) were excluded from further analyses, ensuring that the volatile profiles analyzed reflected successful infection. This procedure was adopted because successful infection by *C. graminicola* leads to visible anthracnose leaf blight symptoms on maize leaves, which typically appear 4–7 days after inoculation and are well developed by 7–10 days [62–64]. Characteristic compounds of the fungal treatment were not found in the chromatograms of the mock-inoculated control plants (sterile water and sterile water with 0.01% Silwet L-77) indicating that their induction was caused by the pathogen and not by the inoculation procedure or the surfactant (Fig. S1).

Caterpillars: In-house colonies of the beet armyworm *Spodoptera exigua* and the fall armyworm *Spodoptera frugiperda* (FOEN permits A192632-07 and A192558-02) caterpillars were reared on a wheat germ-based artificial diet (Frontier Scientific Services, Newark, USA) in transparent plastic boxes under laboratory conditions (26 ± 2°C, 60 ±

5% relative humidity and 16 h photoperiod) [65]. For laboratory measurements, second instar to early third instar, depending on availability, *S. exigua* and *S. frugiperda* larvae were used to damage the plants. To achieve consistent levels of feeding damage, larval density was adjusted according to larval size and species-specific differences in feeding rate [59]. Fewer *S. frugiperda* larvae were used because it feeds more intensively on Poaceae [59,66], resulting in comparable feeding damage between species (Fig. S2). Because moderate differences in feeding intensity above the induction threshold do not drastically affect emissions of typical caterpillar-induced volatiles [67–69], we did not quantify leaf area loss but used adjusted larval densities to standardize damage. For open-air measurement, late third instar *S. exigua* were used. In all cases, volatile sampling was performed 24 h after the onset of infestation.

Regurgitant collection: Caterpillar regurgitant, used for simulating herbivory, was collected as previously described [65]. Briefly, third- to fourth-instar *S. frugiperda* larvae were fed on maize leaves (Delprim) for 24 h. Larvae were gently squeezed in the head region using feather-weight entomological forceps to induce regurgitant release, which was collected into Eppendorf tubes and stored at -80°C until use.

2.3. Laboratory assays using GC-MS, MSS and PTR-TOF

Volatile emissions from maize plants (Delprim and Aventicum) attacked by herbivores or pathogens were measured using dynamic headspace sampling, where each plant is enclosed in a one-port glass bottle as described by [70]. Charcoal-filtered, humidified air entered the bottle at 0.7 L min^{-1} . This was combined with three analytical techniques: gas chromatography–mass spectrometry (GC-MS), membrane-type surface stress sensors (MSS), and proton-transfer-reaction time-of-flight (PTR-TOF) mass spectrometry (Figs. S3A and S4). Plants were kept under artificial light (SLT-EDK 6400K, Edkfarm, China, $200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, 16 h photoperiod).

GC-MS: Volatiles were collected from maize plants infested with either six *S. exigua* ($n = 7\text{--}8$) or four *S. frugiperda* ($n = 7$) larvae per plant. Sampling was conducted 24 h after infestation. For fungal treatments, plants inoculated with *C. graminicola* were sampled seven days post-inoculation ($n = 8\text{--}9$). Control plants received no treatment ($n = 8$). Air was pulled through a 25 mg of Porapak Q 80/100 mesh Haysep-Q adsorbent filter (Ohio Valley Specialist Company, arietta, USA) at 0.4 L min^{-1} . Volatiles were collected for 2 h. Filters were eluted with $150\text{ }\mu\text{L}$ dichloromethane (Honeywell, Riedel-de Haën, Germany), and $10\text{ }\mu\text{L}$ of internal standard (n-octane and n-nonyl acetate, $20\text{ ng }\mu\text{L}^{-1}$ each) were added. Samples were stored at -80°C until analysis. Analyses were performed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977B mass spectrometer operating in total ion current (TIC) mode. A $1.5\text{ }\mu\text{L}$ aliquot was injected in pulsed splitless mode onto an HP-5MS column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$). The oven temperature program was 40°C for 3 min, increased to 100°C at $8^{\circ}\text{C min}^{-1}$, then to 230°C at $5^{\circ}\text{C min}^{-1}$. Helium was used as carrier gas at 1.1 mL min^{-1} . Compounds were identified by comparison with commercial standards and NIST 17 library spectra and quantified by using relative response factors obtained from calibration curves of commercial standards in comparison to nonyl acetate.

MSS: A nanomechanical Membrane-type Surface stress Sensors standard measurement module ($7.5 \times 11.5 \times 3\text{ cm}$, developed by MSS Alliance/Forum)[43–45,71,72] provided by National Institute for Materials Science (NIMS; Tsukuba, Japan) was used to sample volatiles from maize plants infested with three *S. exigua* larvae ($n = 15\text{--}18$), two *S. frugiperda* larvae ($n = 18$), or inoculated with *C. graminicola* ($n = 18$). Control plants were untreated ($n = 12$). The outlet port of the glass bottles was directly connected to the MSS module via Teflon tubing (50 cm length, $1/16''$ OD, 1 mm ID), and an empty bottle was used as source of purging air (Fig. S4A). The module consists of a compact micro-fabricated array with twelve channels, each containing a $3\text{ }\mu\text{m}$ -thick silicon membrane with a diameter of $300\text{ }\mu\text{m}$ coated with different receptor materials, such as functional silica/titania hybrid

nanoparticles or polymers (Table S1), conferring selectivity for gas mixtures [43,73,74]. Molecular adsorption and desorption on the receptor layers deform the membranes, altering the resistance of piezoresistors embedded in four sensing beams. These resistance changes are measured (in mV) using a full Wheatstone bridge with a -1.0 V bridge voltage [44] and recorded at 100 Hz. Measurements are continuous and rely on the alternance of sampling (adsorption phase of the analytes) and purging (acceleration of desorption) phases. Flow rates of both sampling and purging phases were set at 0.030 L min^{-1} . Each laboratory measurement consisted of 10 cycles of 10-second sampling and 10-seconds purging. Of the 12 available channels in the module, two showed unstable responses throughout measurements (channels 2.2 and 3.4, Table S1) and were therefore excluded from the analyses. Data from the sampling phase of the 8th cycle were used, extracting one value per second over the final 9 s (141–150 s).

PTR-TOF: volatiles were analyzed using a PTR-TOF instrument (Vocus S, ToFwerk AG, Thun, Switzerland). Plants were infested with six *S. exigua* ($n = 8$), four *S. frugiperda* ($n = 8$), or inoculated with *C. graminicola* ($n = 5$). Control plants were untreated ($n = 12$). Air was sampled from the bottles port at 0.1 L min^{-1} through a 1 m inlet heated to 100°C to minimize losses (Fig. S4B). Volatile compounds were ionized by proton transfer from H_3O^+ reagent ions. Further details about the Vocus S technology can be found elsewhere [50,75]. The ion–molecule reactor was operated at 1.8 mbar and 100°C , with front and back voltages of 500 V and 38 V, respectively, and an RF field of 1.3 MHz at 450 V. Mass spectra were recorded at 1 Hz over an m/z range of 20–486.

Raw data were processed using Tofware 3.2.3 (ToFwerk AG, Thun, Switzerland) for mass calibration, peak fitting, and quantification. Targeted ions included parent and characteristic fragment ions of plant volatiles (Tables S2 and S3), selected based on GC-MS data, prior experience, and literature [76,77]. Signal intensities were converted to concentrations (ppb) using instrument responses derived from a standard mixture with known proton-transfer rate constants, as described in Lopez-Hilfiker et al. [78]. Calibration was performed prior to measurements using a 20-ppb gas mixture (Apel-Riemer Environmental, Miami, USA). Sensitivity calibration was based on acetone, xylene, toluene and methyl ethyl ketone, while mass-dependent transmission efficiency was determined using acetaldehyde, acetonitrile, acrylonitrile, acetone, benzene, 1,2,4-trimethylbenzene, toluene, xylene and methyl ethyl ketone. The estimated uncertainty of this quantification method is approximately 30% [78].

2.4. Open-air sampling using MSS and PTR-TOF in semi-controlled outdoor conditions

Open-air volatile sampling, without headspace enclosure, was conducted using the same PTR-TOF and MSS systems and under the same instrumental settings as described above over three days (1, 2, and 5 July 2024) at the Faculty of Science of the University of Neuchâtel, Switzerland ($47^{\circ}00'00.5''\text{ N}$, $6^{\circ}57'00.5''\text{ E}$) (Fig. S5). Measurements were performed between 09:00 and 14:00 under mostly sunny conditions, with occasional overcast periods. Ambient temperature ($19\text{--}26^{\circ}\text{C}$), relative humidity (42–60%), and wind speed ($1\text{--}10\text{ km h}^{-1}$) were recorded manually every 15–20 min using a portable weather station (TFA Spring Breeze, Dostmann GmbH & Co. KG, Germany). Sampling was carried out immediately outside a university building, where plants were exposed to natural airflow and ambient environmental conditions, while remaining in a semi-sheltered setting: the proximity of the building limited wind exposure to fewer directions. This facilitated positioning the MSS module and PTR-TOF sampling inlets downwind of the plant whenever possible.

Plants were grown in the greenhouse as described above and transferred to a laboratory room one day prior to measurements, where they were maintained under artificial light (SLT-EDK 6400K, Edkfarm, China, $200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$, 16 h photoperiod, $26 \pm 2^{\circ}\text{C}$). Half of the plants were infested with two to three late third-instar *S. exigua* larvae for 24 h, while

the remaining plants served as undamaged controls. To prevent larvae from falling off plants during infestation without interfering with feeding behavior, infested plants were partially enclosed in open-ended PET cooking bags secured around the stem. One quarter of control plants were also bagged; this treatment did not induce detectable volatile emissions (Table S4). Bags and larvae were removed 2–3 h before sampling. Feeding damage was assessed visually as the percentage of leaf area removed.

Volatiles were sampled from the space between leaves, 5–15 cm above the whorl, targeting the youngest fully expanded leaf, regardless of whether that leaf itself was damaged (Fig. S5). Measurements alternated between control and damaged plants. Each plant was first sampled using PTR-TOF, followed immediately by MSS measurements. PTR-TOF sampling was conducted for 60 s at a distance of 1–2 cm from the leaf and subsequently at 4–5 cm from the same leaf. MSS sampling was performed at 1–2 cm for seven cycles of 10 s sampling followed by 10 s purging. The MSS inlet was reduced to a 16 cm Teflon tube, and technical air was used for purging. The PTR-TOF inlet was fixed on a clamp stand and the maize leaves were delicately held with gloved-fingers to maintain the proper distance, despite the wind. The MSS inlet was similarly maintained at the right distance from leaves (Fig. S5).

On day 1, we measured a total of 52 plants (control $n = 25$, damaged $n = 27$), on day 2 a total of 50 plants (control $n = 22$, damaged $n = 28$) and on day 3, a total of 56 plants (control $n = 30$, damaged $n = 26$).

2.5. Field measurements with portable TOF

The field assay was conducted in an agricultural maize plot in Mathod, Switzerland (46.7493° N, 6.5545° E) (Fig. S6). The experiment took place on 2 May 2025 and was limited to a single day due to weather conditions and instrument availability. Maize plants (var. Delprim) grew under standard agronomic conditions. Seeds were sown at the end of March with 25 cm spacing within rows and 50 cm between rows. The field plot measured $\sim 60 \text{ m} \times 18 \text{ m}$. Two rows were used for the experiment, separated by one untreated row. The first experimental row was located 3 m from the plot edge, and only plants situated at least 3 m from the remaining plot borders were used. Plants were used at the three leaves stage, with the fourth leaf emerging.

Measurements were performed using a portable TOF instrument (Vocus C, ToFwerk AG, Thun, Switzerland) [54]. It weighs approximately 30 kg and has a low power consumption of $\sim 250 \text{ W}$. The instrument sensitivity and detection limits are comparable to the aforementioned Vocus S PTR-TOF instrument. For this experiment the instrument was powered using the battery of a hybrid car. A catalytic zero air generator (ToFwerk AG, Thun, Switzerland) was used to generate clean air, which was further purified with a VOC trap (VICI Metronics) allowing the instrument to be independent of any auxiliary gas supplies. While the compact design of the instrument balances sensitivity, weight and power consumption its mass resolving power is somewhat limited (1200 Th/Th) and is best coupled with a rather selective reagent ion to maintain relatively simple mass spectra. The instrument was operated with benzene cation ionization which is a more selective chemical ionization approach than PTR based ionization [55–57]. This ionization method enables detection predominantly through charge transfer processes or benzene adduct formation, depending on the compound properties.

For the simulated herbivory treatment, plants were treated in alternating treatment blocks across two rows, with blocks generally comprising five undamaged plants or three plants subjected to simulated herbivory, such that the two treatments were interspersed along the rows. Herbivory damage was simulated by combining mechanical wounding with the application of *S. frugiperda* regurgitant. On the day before measurements (1 May 2025), between 15:00 and 17:00, the first leaf was wounded on both sides (six scratches per half leaf using serrated forceps) and the entire wounded surface treated with 8 μL of regurgitant. The second leaf was wounded on one side and treated with 4 μL . On the

day of measurements (2 May 2025), induction of the first row was performed between 07:30 and 08:30 and induction of the second row between 09:30 and 10:00. On this second day, the remaining half of leaf 2 was wounded and treated with 4 μL , and leaf 3 was wounded on both sides and treated with 8 μL . Because sampling progressed along the rows in a similar order as treatment application, plants were sampled approximately 4–5 h after the most recent damage.

Volatile emissions were measured individually for each plant on 2 May 2025 between 11:15 and 14:45, including 65 damaged plants across 31 blocks and 64 undamaged plants across 32 blocks). Within each treatment block, typically two plants were sampled: one located near the center and one near the border of the block. Sampling progressed systematically along the rows, alternating between damaged and undamaged blocks, with the first row completed before moving to the second row. Treatment sampling was approximately balanced within each row (row 1: 42 undamaged and 41 damaged plants; row 2: 22 undamaged and 24 damaged plants). For sampling, air was drawn through 1 m of unheated PTFE tubing (1/4 in ID) at 10 L min⁻¹ corresponding to a residence time of $\sim 190 \text{ ms}$. We further placed a glass funnel (10 cm diameter) at the inlet to increase the sampling area and reduce sensitivity to exact inlet positioning.

Meteorological variables were automatically recorded every 2 min using a small weather station (DNT Pro, GmbH & Co. KG, Germany). During the sampling time, solar radiation ranged from 343.4 to 708.2 W m⁻² under clear, sunny conditions, air temperature ranged from 22.5 to 27.5°C, and relative humidity from 34 to 54%. Wind speeds were low, ranging from 0 to 2.6 m s⁻¹, corresponding to light wind conditions. However, wind direction was highly variable throughout the measurement period (2–338°, median 257°), preventing consistent downwind positioning as achieved in the simpler open-air experiment. Sampling was performed from one of the sides of the rows and kept constant for each row. Based on recorded wind direction and sampling side, the angular difference between approximate inlet orientation and airflow direction (0–180°, where 0° indicates downwind and 180° upwind sampling) had a median of 125° (range 30–180°), reflecting variable and often non-optimal sampling position relative to wind. Because inlet positioning was approximate and a 10 cm diameter funnel was used to broaden the sampling cone, inlet–wind alignment should be considered indicative rather than exact.

2.6. Data analysis

Statistical analyses and modeling were performed using a combination of R (version 4.3.2; [79]) and Python (version 3.10.12). Classical statistical analyses and Random Forest classification for laboratory assays were primarily conducted in R, whereas machine-learning analyses for open-air and field measurements were performed in Python using the scikit-learn library (version 1.5.0; [80]).

2.6.1. Laboratory measurements

To assess the discriminant capacity of the three odor-sensing methods under controlled conditions, we applied the same multivariate workflow to all laboratory datasets. We performed non-metric multidimensional scaling (NMDS) and a permutational multivariate analysis of variance (PERMANOVA) to assess differences in overall volatile profiles among treatments, using the vegan package [81]. Bray–Curtis dissimilarities were used for GC–MS and PTR–TOF data to assess overall differences in the volatile profiles between treatment, as commonly used for chemical datasets [82]. Gower dissimilarities were used for MSS data because sensor responses were continuous measurements in time with different shape that included both positive and negative values, allowing differences among sensor responses to be compared on a standardized scale. NMDS ordination was visualized constrained to two dimensions. Homogeneity of multivariate dispersion among treatments was assessed using the *betadisper* function, with significance evaluated by permutation tests (*permutest*) with 9,999

permutations, without additional restriction. PERMANOVA was performed using *adonis2* function, also with 9,999 permutations, without additional restriction. For reproducibility, a random seed of 6 was used for NMDS ordination and permutation-based tests of multivariate dispersion and PERMANOVA.

In parallel, univariate treatment effects were assessed for each quantified compound (GC-MS) or targeted ion (PTR-TOF) by fitting linear models with treatment as the explanatory variable. Overall treatment effects were evaluated using ANOVAs with heteroscedasticity-consistent covariance estimators, implemented with the *Anova* function (*car*; [83]) in combination with heteroscedasticity-consistent covariance estimators (*sandwich*; [84]). For compounds/ions for which a treatment group contained only zero values, half of the zero observations were randomly replaced by one-half of the smallest positive value detected for that compound/ion to avoid zero within-group variance in the robust model estimation. ANOVA p-values were adjusted across compounds/ions using the Benjamini–Hochberg false discovery rate (BH-FDR) procedure. For compounds/ions showing a significant overall treatment effect after this correction, post-hoc pairwise comparisons were performed using *glht* (*multcomp*; [85]), with p-values from the pairwise comparisons additionally adjusted for multiple testing using the BH-FDR procedure. To explore whether volatile profiles contained information that could be used to discriminate among treatments, we used Random Forest (RF) classification implemented in the *randomForest* package [86]. The RF model combines multiple decision trees, each trained using a bootstrap sample of the observations (i.e. samples drawn with replacement from the original dataset). For each tree, observations not included in its bootstrap sample (out-of-bag, OOB observations) were used to evaluate classification performance. Models consisted of 500 trees and used the default number of predictor variables considered at each split ($mtry = \sqrt{p}$, where p is the number of predictor variables). The random seed was set to 6 before RF classification. Model performance was summarized using confusion matrices, showing the numbers of correctly and incorrectly classified samples for each treatment. Because the number of samples was small, the RF analysis was considered exploratory, and the OOB classifications were used to assess classification patterns within the present dataset rather than to evaluate the model's predictive performance on new samples.

2.6.2. Open-air measurements with MSS

A PCA was performed on sensor responses extracted from the 6th sampling phase to visualize variation among treatments, sampling days, and gradients of temperature and humidity. For these analyses, temperature and humidity were obtained from the internal sensor of the MSS module. Multiple feature extraction strategies from MSS response curves and alternative preprocessing approaches were evaluated, including the laboratory strategy (features extracted from one sampling phase) and previously described MSS workflows [47,49]. For each processed dataset, classification performance was evaluated using a set of standard machine-learning classifiers in five-fold cross-validation and compared with dummy-classifier baselines representing different random and class-based prediction strategies.

2.6.3. Open-air measurements with PTR-TOF

In total, 158 plants were measured over three measurement days: Day 1, $n = 52$ (25 control, 27 damaged); Day 2, $n = 50$ (22 control, 28 damaged); and Day 3 $n = 56$ (30 control, 26 damaged). PTR-TOF measurements were acquired over 60 s, with the resulting data obtained at 1-s resolution. Analyses targeted the same ions as the laboratory measurements, restricted to caterpillar-induced compounds (Tables S7), representing a total of 31 features. Ion concentrations were quantified using a calibration run performed daily before measurements, as described above.

Exploratory analyses: Data structure was explored using principal component analysis (PCA; *scikit-learn*) applied to ion concentrations averaged over the 60-s sampling period and standardized to zero mean

and unit variance. PCA was used to visualize variation among sampling days, across temperature and humidity gradients, and between treatment groups. Temperature and humidity measurements were obtained using the small weather station described above. To identify ions showing differences in abundance between damaged and control plants, Wilcoxon rank-sum tests were performed on the 60-s averaged ion concentrations. P-values were adjusted for multiple testing across targeted ions using the BH-FDR procedure.

Predictive analyses: Analyses were primarily focused on the greater sampling distance (4–5 cm), as this distance yielded smaller differences in volatile emissions between control and damaged plants and therefore represented the more challenging classification setting. It was also considered more representative of potential field applications, where measurements may be less likely to be conducted at very short distances from the plant. First, to assess how integration duration affected classification performance, we used the first 1, 5, 15, 30, or 60 s of each plant's 60-s recording, averaging targeted ion concentrations within that duration to yield exactly one observation per plant per duration. In a second analysis, we assessed the robustness of the first-second result to the specific second chosen, by randomly selecting one second from each plant and repeating this procedure 20 times. Finally, we performed a Leave-One-Day-Out cross-validation to evaluate the robustness of classification to acquisition-day variability.

Classification and preprocessing: Classification was performed using L1-regularized logistic regression (Lasso) implemented in *scikit-learn* (*LogisticRegression*). L1 logistic regression limits model complexity and performs embedded feature selection by shrinking weakly informative coefficients toward zero, facilitating model interpretation [87,88]. Models were fitted using the *liblinear* solver [89]. The regularization parameter C was selected by inner cross-validation from a predefined grid (0.01, 0.1, 1, and 10), within a nested cross-validation framework [90], as described below. Model fitting was allowed a maximum of 1000 iterations, with convergence determined using the default *scikit-learn* convergence tolerance. Other model hyperparameters were kept at their *scikit-learn* defaults. For reproducibility, a base random seed was fixed at 10 for all stochastic procedures. Ion features were standardized to zero mean and unit variance (*StandardScaler*). Standardization was implemented within the model pipeline (*Pipeline*) so that scaling parameters were estimated independently within each training partition and subsequently applied to the corresponding validation or test partition.

Repeated nested cross-validation and bootstrap uncertainty estimation: Model performance was assessed using repeated nested 5-fold stratified cross-validation (*StratifiedKFold*), with five repeats, resulting in 25 outer train/test splits. The PTR-TOF dataset consisted of 158 unique plants, including 77 undamaged and 81 damaged plants. Five independent repetitions of 5-fold outer cross-validation were performed. Because the number of plants was not exactly divisible by five, the number of plants assigned to each partition differed slightly between folds. Number of plants in each partition is summarized in Table 1. For each outer split, the regularization parameter C was selected by five-fold stratified cross-validation within the corresponding training set from four candidate values (0.01, 0.1, 1, and 10) using mean inner-CV balanced accuracy as the selection criterion. The selected C was then used to refit the model

Table 1

Number of plants in the nested cross-validation partitions for the open-air dataset. Values represent the minimum–maximum number of plants observed across the 25 outer train/test splits and the corresponding inner cross-validation folds.

Cross-validation level	Partition	Experimental units (plants)
Outer CV	Training	126–127
	Test	31–32
Inner CV	Training	100–102
	Validation	25–26

using the complete outer training set, after which predictions were generated for the held-out outer test set. Thus, information from the outer test set was not used during preprocessing, feature filtering or hyperparameter selection. The same outer cross-validation partitions were used across all five datasets (durations of measurement integration). Because the cross-validation was repeated five times, each plant received five predictions from held-out test sets, one from each repeat. These predictions were averaged to obtain a final predicted probability for each plant, and the corresponding final plant-level classification was obtained using a probability threshold of 0.5. Classification performance was then calculated at the plant level. Balanced accuracy was used as the primary performance metric, while accuracy, precision, recall (sensitivity), F1-score, specificity and ROC-AUC were calculated as secondary performance metrics.

Sampling uncertainty in plant-level classification performance was estimated from the final set of cross-validated predictions using non-parametric bootstrap resampling of plants [91]. Following repeated cross-validation and aggregation of predicted probabilities, plants were resampled with replacement 5000 times, and the performance metrics were recalculated for each bootstrap sample. The 2.5th and 97.5th percentiles of the resulting bootstrap distributions were used to define the 95% percentile bootstrap confidence intervals. Thus, the resulting confidence intervals reflect uncertainty in the estimated performance associated with variation among the sampled plants.

Feature stability and importance: Feature selection stability and contribution to models were assessed across the 25 outer-CV models generated by the repeated nested cross-validation. A feature was considered selected when its L1 coefficient was non-zero. Selection frequency was calculated as the percentage of outer-CV models in which each feature was selected. Mean standardized coefficients were calculated across the models in which each feature was retained.

Permutation test: For computational efficiency, a permutation test was performed only for the first-second analysis. The test assessed whether classification performance exceeded that expected by chance under the null hypothesis of no association between the PTR-TOF targeted features and treatment [92]. Because measurements were taken over three different days, treatment labels were randomly reassigned within measurement day, thus preserving the original treatment composition of each day while breaking the association between data and treatment. For each permutation, the complete repeated nested cross-validation was rerun, with parameter C reselected within the inner cross-validation for each outer split. A total of 250 permutations was performed, and statistical significance was assessed by calculating the permutation p-value as the proportion of permutation-based balanced-accuracy values equal to or greater than the observed plant-level balanced accuracy, using a +1 correction for the observed statistic and number of permutations.

Leave-One-Day-Out cross-validation: To evaluate the robustness of classification to acquisition-day variability, leave-one-day-out validation was performed using the first measured second per plant. In each of three iterations, all plants from one acquisition day were held out as a test set, while the remaining two days were used for model training. Within the training data, the regularization parameter C was selected from 0.01, 0.1, 1, and 10 using five-fold stratified cross-validation. Feature standardization was performed using parameters estimated from the training data only. The final L1-regularized logistic regression model was refitted using all plants from the two training days with the selected C and evaluated on the completely unseen acquisition day.

2.6.4. Field measurements with portable TOF

In contrast to previously analyzed TOF datasets, for which fitted peak areas were quantified in absolute concentration units, the field analysis used the raw mass-spectral intensities provided by the portable instrument for nominal m/z features, spanning from 7 to 556 Da. The data consisted of one intensity value per nominal m/z feature for each one-second spectrum; no targeted peak fitting was applied. For each 60-

second measurement, the intensity at each nominal m/z feature was averaged across the one-second spectra, resulting in one value per feature. This approach allowed an exploratory assessment of the spectral information recorded by the portable instrument and the identification of mass-to-charge features associated with plant damage under field conditions. This resulted in 550 features for a dataset of 129 plants, including 64 undamaged plants and 65 damaged plants, distributed across 63 independent experimental blocks (32 with undamaged plants and 31 with damaged plants). Damaged and undamaged blocks were interspersed along the field rows, with measurements typically obtained from two plants per block. The two treatment groups were approximately balanced within each row (row 1: 42 undamaged and 41 damaged plants; row 2: 22 undamaged and 24 damaged plants), minimizing the potential for treatment effects to be confounded with row-specific spatial effects.

Classification and preprocessing: Classification was performed similarly to the PTR-TOF open-air data set, using logistic regression with an L1 (Lasso) penalty. Here, five preprocessing strategies were evaluated: no normalization, total ion current (TIC), probabilistic quotient normalization (PQN) and TIC or PQN followed by a base-10 logarithmic transformation (TIC-log10, PQN-log10). TIC and PQN were used to account for differences in overall signal intensity, while the logarithmic transformation was used to reduce skewness and differences in feature magnitude. The preprocessing approaches were motivated by practices used in untargeted metabolomics [93]. Preprocessing was performed independently within each training/test split to avoid information leakage. Within each training split, features with zero variance were first removed based on the training data only (one feature). The selected normalization and, where applicable, logarithmic transformation were then applied to the retained features. For PQN, a reference spectrum was calculated as the median intensity of each m/z feature across the training samples and then applied unchanged to the corresponding test samples. For each sample, a scaling factor was calculated as the median ratio of its intensities to those of the reference spectrum. Finally, the features were standardized to zero mean and unit variance using parameters estimated from the training data and applied unchanged to the corresponding test data. For reproducibility, a base random seed of 10 was used for stochastic procedures.

Repeated nested blocked cross-validation and bootstrap uncertainty estimation: To account for the experimental structure of the field dataset, with plants grouped within experimental blocks, repeated nested cross-validation was performed using experimental block as the grouping variable to assess model performance (*StratifiedGroupKFold*). The dataset included 129 plants distributed across 63 experimental blocks; 32 blocks with undamaged plants and 31 blocks with damaged plants. Five repeats of five-fold stratified grouped cross-validation were used, resulting in 25 outer train/test splits. Experimental blocks were kept intact within each partition, such that no block contributed plants to both the training and test sets. The numbers of experimental blocks and plants in each partition are summarized in Table 2. Within each outer training set, the regularization parameter C was selected using the same nested cross-validation procedure described for the PTR-TOF dataset, using five-fold grouped stratified cross validation, with experimental block kept as grouping variable, and was then used to refit the model on

Table 2

Number of plants and experimental blocks in the nested grouped cross-validation partitions for the field dataset. Values represent the minimum–maximum number observed across the 25 outer splits and corresponding inner folds. Experimental blocks were kept intact within each partition.

Cross-validation level	Partition	Experimental units (blocks)	Plants
Outer CV	Training	50-51	101-106
	Test	12-13	23-28
Inner CV	Training	39-41	79-86
	Validation	9-11	18-23

the complete outer training set. The same outer cross-validation partitions were used across all five preprocessing strategies. For each plant, the five predicted probabilities from the repetitions were averaged to obtain a single plant-level predicted probability, and plant-level classifications were assigned using a probability threshold of 0.5. All final performance metrics were then calculated at the plant level, with balanced accuracy used as the primary performance measure. Accuracy, precision, recall (sensitivity), F1-score, specificity and ROC-AUC were also calculated.

Sampling uncertainty in plant-level performance was estimated from the aggregated predictions for the held-out outer test sets as described for the PTR-TOF -dataset, using non-parametric bootstrap resampling with 5000 replicates. Because plants within the same experimental block were not considered independent, experimental blocks were used as the bootstrap sampling unit. Blocks were resampled with replacement, with all plants belonging to a sampled block retained together. Thus, the resulting confidence intervals reflect uncertainty associated with variation among experimental blocks while preserving the grouping of

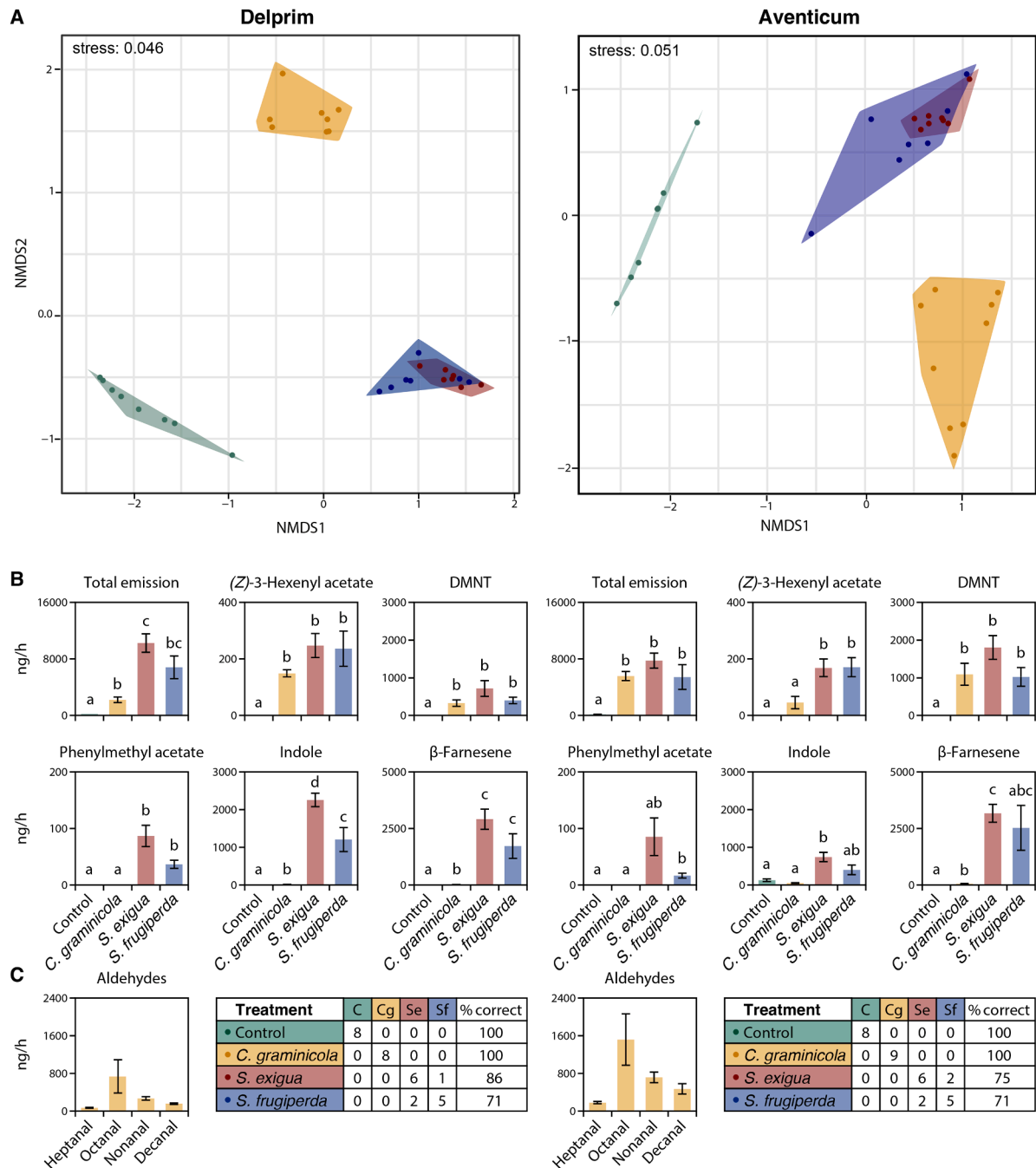


Fig. 1. Characterization of the volatile profiles of maize plants infested with different pests. Volatile emissions were collected under laboratory conditions using classical push-pull headspace sampling and analyzed by GC-MS. Undamaged maize plants (green) of the varieties Delprim and Aventicum were compared with plants infested by a fungal pathogen (*Colletotrichum graminicola*; yellow) or by caterpillars (*Spodoptera exigua*, red; *Spodoptera frugiperda*, blue). (A) NMDS ordination illustrating differences in overall volatile profiles among treatments. Ellipses are shown for visualization only and do not represent statistical confidence regions. (B) Emission rates (ng h⁻¹; mean $\pm$ s.e.) of selected major volatile compounds. Different letters indicate significant pairwise differences after FDR correction; full results in Supplementary Information. (C) Confusion matrix showing the results of the exploratory Random Forest classification according to treatment. Classification was based on out-of-bag (OOB) predictions, using observations not included in the bootstrap sample used to build each tree.

plants within blocks.

Feature stability and importance: Feature selection stability and contribution to models were assessed as described for the PTR-TOF dataset above.

Permutation test: A block-level permutation test was performed to assess whether classification performance was greater than expected by chance under the null hypothesis of no association between the measured m/z features and plant damage. Because plants within an experimental block were not independent, treatment labels were randomly permuted among experimental blocks rather than individual plants, while preserving the original number of blocks assigned to each class. For each permutation, the complete analysis pipeline was repeated, including the five preprocessing strategies, repeated nested blocked cross-validation, selection of parameter C within the inner cross-validation, generation of predictions for the held-out outer test sets, and aggregation of predicted probabilities to the plant level. Because the preprocessing strategy yielding the highest balanced accuracy was selected for the observed performance, the same selection step was incorporated into each permutation. Specifically, plant-level balanced accuracy was calculated separately for the five preprocessing strategies, and the maximum balanced accuracy across these was retained in each permutation to generate the null distribution, which was then compared with the observed maximum balanced accuracy. This procedure accounts for the selection among the five preprocessing strategies when assessing whether the observed classification performance could have arisen by chance. A total of 250 permutations was performed. The permutation p -value was calculated as described above for the PTR-TOF dataset.

3. Results

3.1. Laboratory measurements

3.1.1. Volatile profiling of maize plants using GC-MS

Volatile emissions from undamaged maize plants and plants infected with *C. graminicola* or infested with *S. frugiperda* or *S. exigua* were first characterized under laboratory conditions using dynamic headspace sampling and GC-MS analysis (Fig. S3). Two commercial hybrids were examined (var. Delprim and Aventicum). Nonmetric multidimensional scaling (NMDS) showed clear separation among volatile profiles of undamaged, fungus-infected, and caterpillar-infested plants for both varieties (Fig. 1A). PERMANOVA detected significant effects of treatment on volatile profiles (Delprim: $F_{3,26} = 27.047$, $R^2 = 0.76$, $p < 0.001$; Aventicum: $F_{3,26} = 21.729$, $R^2 = 0.70$, $p < 0.001$). Tests of multivariate dispersion showed no significant differences among treatments in Delprim ($F_{3,26} = 1.471$, $p = 0.247$). In Aventicum, multivariate dispersion differed significantly among treatments ($F_{3,26} = 3.655$, $p = 0.025$), indicating that the observed treatment effect may reflect differences in both profile location and within-treatment variability.

Undamaged plants constitutively released only small amounts of linalool in Delprim or indole in Aventicum, whereas fungal infection and herbivory strongly increase emissions rates and blend complexity. Caterpillar infestation induced characteristic herbivore-associated blends dominated by green leaf volatiles (GLVs; e.g., (*E*)-2-hexenal), monoterpenes (e.g., β -myrcene), sesquiterpenes (e.g., (*E*)- β -farnesene), homoterpenes (e.g., (*E*)-4,8-dimethyl-nonatriene; DMNT) and aromatic compounds (e.g., indole) (Fig. 1B; Fig. S3; Tables S5 and S6). Induced blends differed between maize varieties; for example, caterpillar infestation elicited the detectable emission of (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) in Delprim but not in Aventicum (Fig. 1B; Tables S5 and S6). Volatile profiles induced by *S. frugiperda* and *S. exigua* showed partial separation in the NMDS ordination. Although the induced blends were qualitatively similar, *S. frugiperda* generally elicited lower emission rates of several compounds, including indole, with these differences being more pronounced in Delprim than in Aventicum (Fig. 1B; Tables S5 and S6). In contrast, *C. graminicola*

infection induced a subset of compounds also detected in caterpillar-infested plants, but typically at lower emission rates. Fungal infection was further distinguished by the release of fatty aldehydes (e.g., octanal), which were not detected in caterpillar-induced profiles, as well as a small number of infection-associated terpenes such as D-limonene (Fig. 1B; Fig. S1 and S3; Tables S5 and S6). Consistent with these patterns, exploratory Random Forest (RF) classification based on induced maize volatile profiles indicated discriminations among undamaged, fungus-infected, and caterpillar-infested plants, with partial separation of the two caterpillar species (Fig. 1C).

3.1.2. MSS module

An MSS module comprising twelve channels coated with different adsorbent materials (Table S1) was used to measure maize volatile emissions under laboratory conditions, using the same dynamic headspace sampling setup as for GC-MS (Fig. S4A). MSS measurements generated distinct electrical response patterns across channels (Fig. 2A). Using MSS responses extracted from a 10-s sampling period, NMDS revealed clear separation among undamaged, fungus-infected, and caterpillar-damaged plants for both maize varieties (Fig. 2B; PERMANOVA: Delprim, $F_{3,59} = 90.086$, $R^2 = 0.82$, $p < 0.001$; Aventicum, $F_{3,61} = 66.422$, $R^2 = 0.77$, $p < 0.001$). Dispersion also differed significantly among treatments in both Delprim ($F_{3,59} = 9.169$, $p < 0.001$) and Aventicum ($F_{3,61} = 7.560$, $p < 0.001$), indicating that the observed multivariate treatment effects could reflect differences in both response profiles and within-treatment variability. Only limited overlap was observed between control and fungus-infected plants in Delprim and between *S. exigua*- and *S. frugiperda*-infested plants in Aventicum. Similarly, exploratory RF classification indicated that samples could be distinguished according to treatment, including between plants attacked by the two closely related caterpillar species.

3.1.3. PTR-TOF (Vocus S)

Laboratory measurements of maize volatile emissions were performed with PTR-TOF under laboratory conditions, using the same dynamic headspace sampling setup as for GC-MS (Fig. S4B). Volatile compounds previously identified by GC-MS, along with additional ions, were quantified from spectra acquired during 60-s measurements and analyzed as time-averaged concentrations (Fig. 3A; Tables S2 and S3).

Several targeted ions showed significant increases in caterpillar-infested plants and corresponded to GC-MS-identified marker compounds, including indole ($C_8H_8N^+$) and sesquiterpenes ($C_{15}H_{25}^+$), which together represented the most abundant signals, as well as other herbivory-associated compounds such as DMNT ($C_{11}H_{19}^+$) and monoterpenes ($C_{10}H_{17}^+$) (Fig. 3A; Tables S2 and S3). In addition, PTR-TOF detected several ions associated with caterpillar herbivory that were not observed in the GC-MS analyses, including ions consistent with protonated aliphatic aldoximes (e.g., isobutyraldoxime, $C_4H_{10}NO^+$; methylbutanal oxime, $C_5H_{12}NO^+$) and other nitrogen-containing compounds such as isobutyronitrile ($C_4H_8N^+$). These signals were particularly associated with *S. exigua* damage in both maize varieties, although responses were more variable in Aventicum (Fig. 3A; Tables S2 and S3). In contrast, ions corresponding to fatty aldehydes characteristic of *C. graminicola* infection in the GC-MS analyses showed little differentiation among treatments in the PTR-TOF data, with the exception of decanal ($C_{10}H_{21}O^+$), which was more elevated in infected Delprim plants (Fig. 3A; Tables S2 and S3).

For the Delprim variety, NMDS revealed clear separation among volatile profiles of all treatments (Fig. 3B; PERMANOVA, $F_{3,29} = 17.869$, $R^2 = 0.65$, $p < 0.001$), with limited overlap observed between control plants and *S. frugiperda*-infested plants. Multivariate dispersion did not differ significantly among treatments ($F_{3,29} = 1.356$, $p = 0.275$), indicating that the observed treatment effect was not associated with differences in within-treatment dispersion. Accordingly, exploratory RF classification distinguished treatments reasonably well, with misclassifications occurring primarily between these two groups (Fig. 3C).

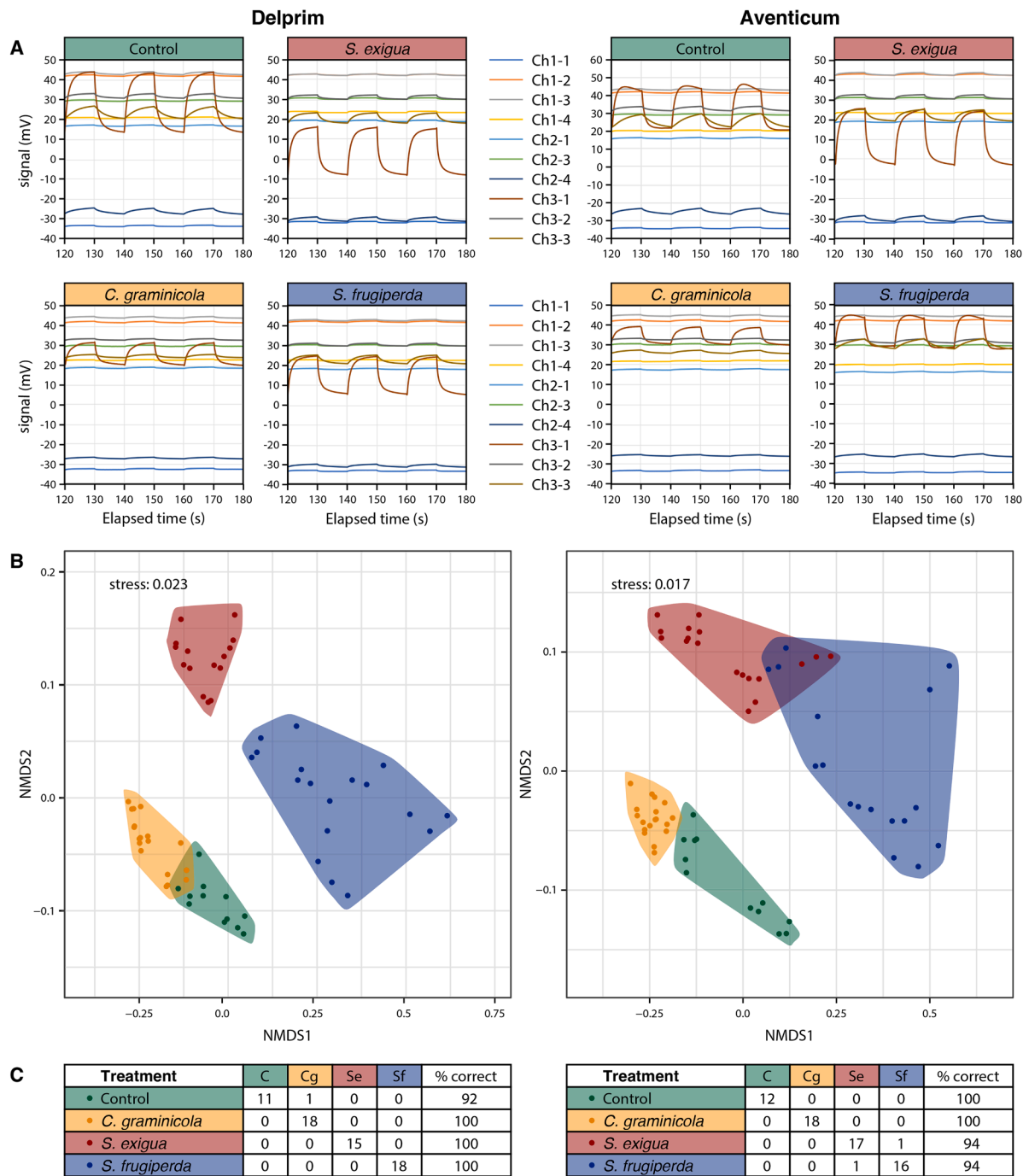


Fig. 2. Distinction among maize volatile profiles using the MSS module under laboratory conditions. Volatile emissions were collected under laboratory conditions using the same headspace sampling system as for GC–MS analyses. Undamaged maize plants (green) of the varieties Delprim and Aventicum were compared with plants infested by a fungal pathogen (*C. graminicola*; yellow) or by caterpillars (*S. exigua*, red; *S. frugiperda*, blue). (A) Sensor signals recorded over three consecutive sampling–purging cycles from ten independent MSS channels with membranes coated with different sensing materials; data from a single 10-s sampling cycle were used for analysis. (B) NMDS ordination illustrating differences in sensor response patterns among treatments. Ellipses are shown for visualization only and do not represent statistical confidence regions. (C) Confusion matrix showing the results of the exploratory Random Forest classification according to treatment. Classification was based on out-of-bag (OOB) predictions, using observations not included in the bootstrap sample used to build each tree.

For Aventicum, NMDS showed more extensive overlap among treatments, although differences in volatile profiles remained detectable ($F_{3,29} = 9.229$, $R^2 = 0.49$, $p < 0.001$; Fig. 3B), notably between *S. exigua*-infested plants and undamaged or *C. graminicola*-infested plants. Multivariate dispersion did not differ significantly among treatments ($F_{3,29} = 0.776$, $p = 0.529$), indicating no evidence that differences in within-treatment dispersion contributed to the observed treatment effect. Consistent with this pattern, RF classification indicated

discrimination of *S. exigua*-infested plants from the other treatments and more generally distinguished caterpillar-infested plants from other treatments but did not discriminate *C. graminicola*-infested plants from undamaged controls (Fig. 3C).

3.2. Open-air measurements

Following the encouraging performance of the MSS module and PTR-

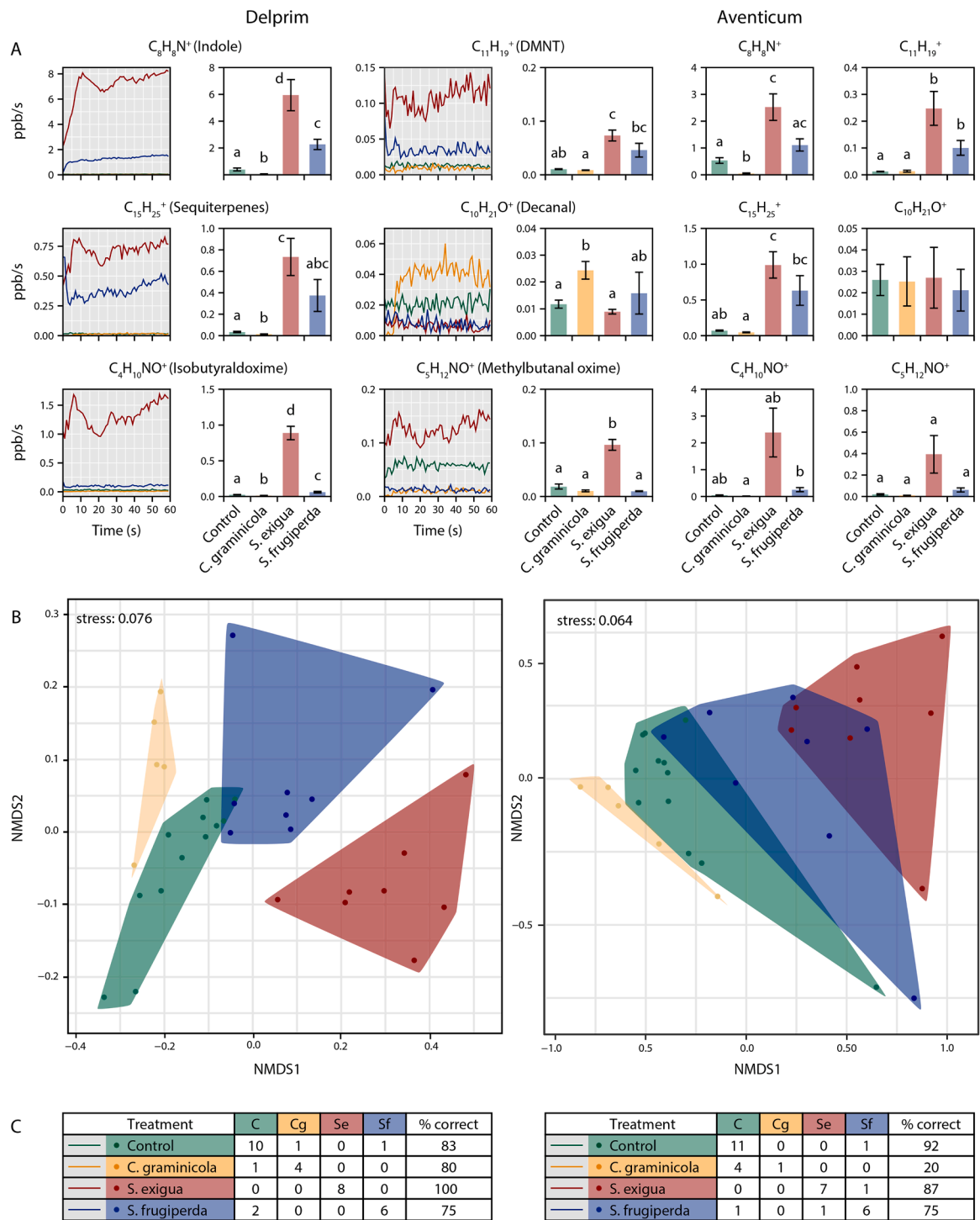


Fig. 3. Distinction among maize volatile profiles using PTR-TOF under laboratory conditions. Volatile emissions were measured under laboratory conditions using the same headspace sampling system as for GC-MS analyses. Undamaged maize plants (green) of the varieties Delprim and Aventicum were compared with plants infested by a fungal pathogen (*C. graminicola*; yellow) or by caterpillars (*S. exigua*, red; *S. frugiperda*, blue). (A) Time series of concentrations (ppb) of selected volatile compounds measured by PTR-TOF over 60-s measurements (1 spectrum s⁻¹), together with bar plots showing mean ± s.e. of concentrations averaged over the 60-s measurement. Different letters indicate significant pairwise differences after FDR correction; full results in Supplementary Information. (B) NMDS ordination illustrating differences in volatile profiles among treatments. Ellipses are shown for visualization only and do not represent statistical confidence regions. (C) Confusion matrix showing the results of the exploratory Random Forest classification according to treatment. Classification was based on out-of-bag (OOB) predictions, using observations not included in the bootstrap sample used to build each tree.

TOF in laboratory assays, volatile emissions from maize plants (var. Delprim) were measured outdoors under open-air conditions without headspace enclosure. Greenhouse-grown plants previously exposed to 24 h of *S. exigua* feeding in the laboratory and subsequently cleared of insects, as well as undamaged controls, were sampled individually immediately outside a campus building. Plants were thus exposed to ambient airflow and environmental conditions while sampling, while the surroundings remained relatively simple and non-natural (Fig. S5).

Measurements were conducted over three days.

3.2.1. MSS module

MSS measurements were performed at a distance of 1–2 cm from the leaves (Fig. S5). Principal component analysis (PCA) of MSS responses extracted from a 10-s sampling period showed that the dominant sources of variation were associated with sampling day and environmental factors such as temperature and humidity, with no clear separation between damaged and undamaged plants (Fig. S7). Consistent with this result, machine learning models trained on the MSS data did not perform better than random classification. These findings indicate that, under outdoor open-air conditions, MSS signals were strongly influenced by environmental variability and did not contain sufficient treatment-specific information to reliably discriminate plant damage status.

3.2.2. PTR-TOF (Vocus S)

PTR-TOF measurements were conducted for 60 s at distances of 1–2 cm and 4–5 cm from the leaves under open-air conditions (Fig. S5), focusing on ions previously identified as herbivore-induced in laboratory assays. PCA of concentrations averaged over the 60-s measurement period indicated that overall variability was primarily associated with sampling day, while plant treatment represented a clear source of variation. Temperature and humidity contributed comparatively little (Fig. S8). Visually assessed leaf damage varied substantially across sampling days (median damaged surface: 5%, 15%, and 30%) and ranged thus from minor to relatively severe [94–96]. However, concentrations of key herbivore-induced volatiles did not significantly increase with more extensive damage (Fig. S9), consistent with previous findings that even limited herbivory can already elicit strong volatile responses in maize [97].

Several ions were significantly increased in damaged plants at both sampling distances, including indole ($C_8H_8N^+$), sesquiterpenes ($C_{15}H_{25}^+$ and fragment ions), isobutyraldoxime ($C_4H_9NO^+$), DMNT ($C_{11}H_{19}^+$), and jasmane ($C_{11}H_{17}O^+$) (Fig. 4A; Table S7). Others, such as monoterpenes ($C_{10}H_{17}^+$), showed significant differences only at the shorter sampling distance (Table S7). Unsurprisingly, concentrations detected during open-air measurements were markedly lower than those measured under laboratory enclosed headspace conditions, typically reaching low ppt levels (Table S7). Median indole ($C_8H_8N^+$) concentrations decreased by more than 200-fold from laboratory measurements to open-air sampling at 1–2 cm and by nearly 300-fold at 4–5 cm, while median sesquiterpene ($C_{15}H_{25}^+$) concentrations declined by 26-fold at 1–2 cm and 50-fold at 4–5 cm. Increasing the sampling distance from 1–2 cm to 4–5 cm reduced median concentrations by 1.3-fold for indole and 1.9-fold for sesquiterpenes (Table S8).

Given the smaller treatment-related differences in volatile concentration observed at greater sampling distance, subsequent prediction analyses focused on 4–5 cm as the more conservative scenario. We first examined whether classification performance was affected by the duration over which volatile signals were averaged, using the first 1, 5, 15, 30, or 60 s of each plant recording as one observation per plant. Plant-level balanced accuracy (aggregated predictions across repeated cross-validation runs) gradually improved with increasing integration duration, from 0.869 (95% CI, 0.816–0.917) at 1 s to 0.963 (95% CI: 0.933–0.988) at 60 s, (Fig. 4B). These confidence intervals reflect sampling uncertainty associated with variation among plants. The proportion of damaged plants correctly detected (recall/sensitivity) increased from 0.802 (0.714–0.884) at 1 s to 0.926 (0.866–0.976) at 60 s. The proportion of undamaged plants correctly classified as such (specificity) increased from 0.935 (0.875–0.986) to 1.000 (1.000–1.000). Consistent with these results, the ROC-AUC, which summarizes the model's ability to distinguish damaged from healthy plants across different classification thresholds, increased from 0.925 (0.877–0.965) to 0.991 (0.978–0.998) (Table S9).

Alongside the improvement in classification performance, we examined how many features were required to achieve it. The mean

number of features selected per outer-CV model was highest at 5 s (20.3, SD = 4.5) and declined gradually with longer integration duration thereafter, reaching 12.3 (SD = 3.9) at 60 s, roughly 40% fewer features than at the 5-s peak (Fig. 4C). Examining which specific ions contributed to classification across durations (Fig. S11) showed that indole ($C_8H_8N^+$) and sesquiterpenes ($C_{15}H_{25}^+$) were not only selected in nearly every outer-CV model at every duration (100% and 84–100%, respectively), but also consistently ranked among the two most influential predictors by mean absolute coefficient, regardless of integration duration. A third ion, likely corresponding to a sesquiterpene fragment ($C_{11}H_{17}^+$), also ranked consistently high and was selected in 80–100% of models at 5, 15, 30, and 60 s, though it did not emerge as a stable predictor at 1 s. Because subsequent analyses (statistical significance via permutation testing, assessing robustness to the specific second measured, and generalization across sampling days) focused specifically on the 1-s duration, we examined feature importance at this duration in more detail (Fig. 4D). As mentioned before, indole and sesquiterpenes ranked as the two strongest and most consistently selected predictors, with indole showing by far the largest coefficient (7.8), nearly 1.7-fold larger than sesquiterpenes (4.6), the next strongest predictor. The next most stable predictor at this duration was $C_{10}H_{15}^+$ (coefficient = 1.4, selected in 96% of models), corresponding to a sesquiterpene fragment.

To check that the classification performance reflected genuine treatment-related information rather than chance, we performed a permutation test on the first-second model, randomly reassigning class labels within measurement day across 250 permutations. The observed plant-level balanced accuracy (0.869) exceeded the null distribution obtained under label permutation (permutation $p = 0.004$; 250 permutations; Fig. 4E), indicating that classification performance was driven by treatment-associated signal rather than incidental structure in the data.

In addition, to assess whether the 1-s result depended on the second chosen, we repeated the analysis using 20 independently drawn random seconds (one per plant, per draw). Balanced accuracy across these draws averaged 0.900 (SD = 0.018, range: 0.868–0.938), consistent with the prespecified first-second result and confirming that a single second of measurement, regardless of which second, already carries sufficient information to distinguish damaged from undamaged plants in this simple open-air setting (Fig. 4F).

Finally, to evaluate how well classification generalized across sampling days, we performed leave-one-day-out validation using the first second, holding out each day in turn as a test set while training on the remaining two days. Plant-level balanced accuracy varied by held-out day, ranging from 0.749 (Day 1, $n = 52$ test plants) to 0.888 (Day 2, $n = 50$) and 0.829 (Day 3, $n = 56$) (Fig. 4G). Together, these results indicate that the classification model was able to discriminate damaged from undamaged plants when evaluated on entirely unseen sampling days, although performance varied among days and was lower when Day 1 was held out.

3.3. Preliminary field measurements with portable TOF (Vocus C)

The outdoor experiment showed that real-time mass spectrometry can detect volatile markers associated with herbivory damage when plants are sampled under open-air conditions. To test this approach under more realistic conditions we next performed measurements on field-grown plants (var. Delprim). As the logistical and infrastructure requirements of deploying a standard laboratory instrument are unrealistic for real-world field measurements in an off-grid scenario, we used a recently developed portable TOF (Vocus C) [54]. It weighs around 30 kg and consumes only 250 W of power, overcoming some of the practical constraints associated with field measurements. The instrument was operated with benzene cation ionization which is a more selective chemical ionization approach than PTR based ionization. Because the use of live caterpillars was not possible in the field, herbivory was simulated by repeatedly mechanically damaging the leaves

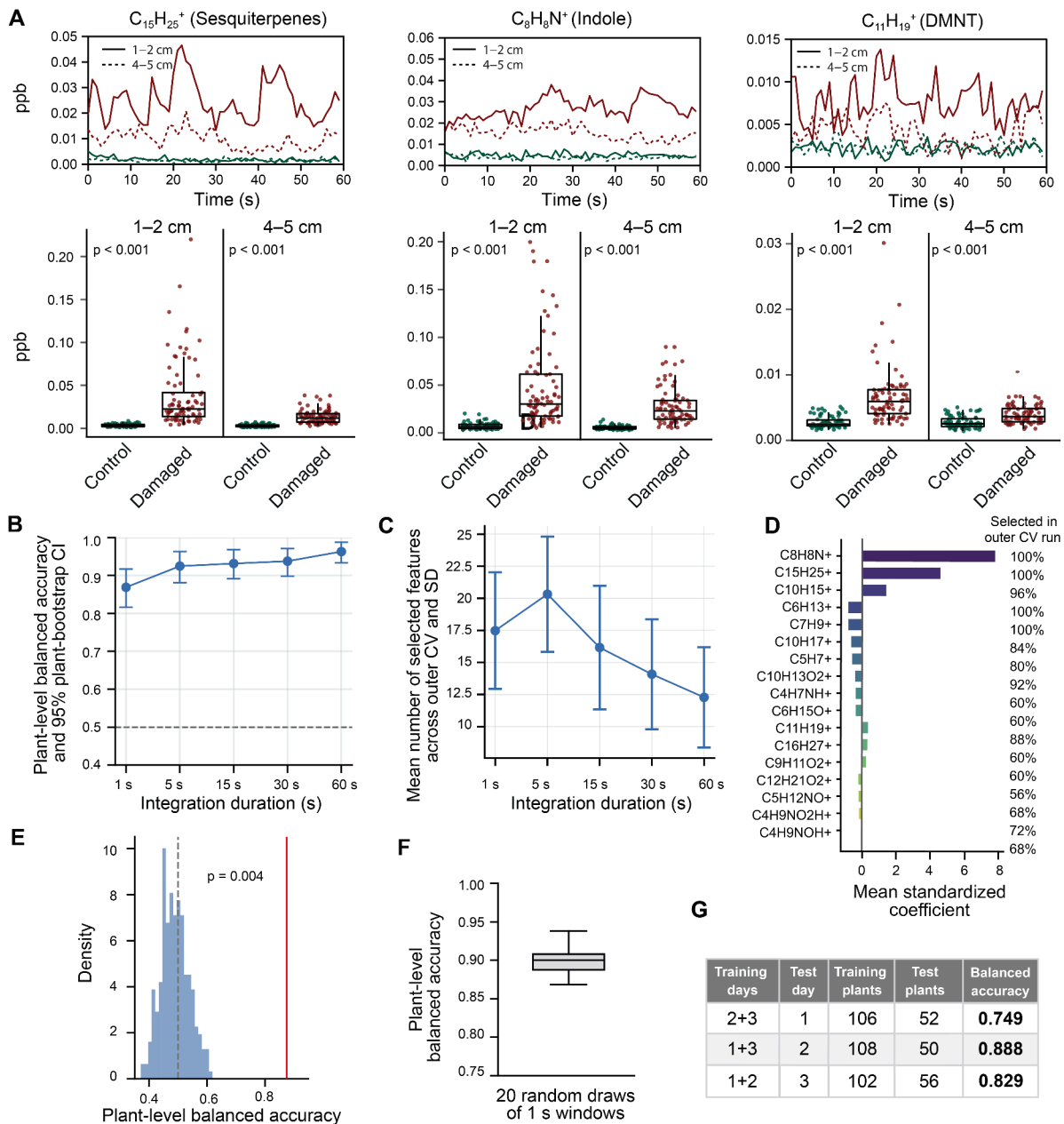
and applying caterpillar regurgitant, a treatment that is known to elicit similar volatile emissions to those induced by caterpillar feeding [98, 99].

We first evaluated the portable instrument in direct comparison with the PTR-TOF using greenhouse-grown maize plants subjected to simulated herbivory under the same open-air set-up as described above (Fig. S10). Under these conditions, the smaller TOF could detect signals consistent with herbivory-associated volatile markers detected by PTR-TOF, including indole (m/z 117; corresponding to m/z 118 for PTR-TOF) and sesquiterpenes (m/z 204; corresponding to m/z 205 for PTR-TOF) (Fig. S10).

We then explored whether the portable TOF could discriminate between the volatile emissions of damaged and undamaged plants under field conditions, in Mathod, Switzerland (Fig. S6). Over a single day, 129 plants were measured for 60 s each, including 64 undamaged plants and 65 plants subjected to simulated herbivory. The two treatment classes

were interspaced in 63 experimental blocks along the field rows; blocks contained plants from a single treatment and measurements were typically obtained from two plants per block. The average spectral intensities across each 60-s measurement were used as predictors (nominal m/z features from 7 to 556 DA; 550 features) in an L1-regularized logistic regression classifier, with five alternative pre-processing (normalization) strategies evaluated.

Plant-level balanced accuracy, was highest with TIC normalization at 0.667 (95% block-bootstrap CI: 0.578–0.751), compared with 0.581 (0.486–0.675) without normalization, 0.620 (0.523–0.711) with TIC-log10, 0.644 (0.547–0.735) with PQN, and 0.605 (0.502–0.704) with PQN-log10 (Fig. 5B; Table S10). Both TIC and PQN therefore improved performance relative to the not normalized data, with broadly similar overall discrimination. This suggests that variation in overall signal intensity, potentially reflecting differences in sample concentration or dilution, contributed to variability in the field measurements and that



(caption on next page)

Fig. 4. Distinction between volatile emissions from undamaged and damaged maize plants under outdoor, open-air sampling, conditions using PTR-TOF. Volatile emissions were measured from maize plants (var. Delprim). Control (undamaged) plants were compared with plants previously damaged in the laboratory for 24 h by *S. exigua* caterpillars, which were removed before measurements. In total, 77 control and 81 damaged plants were measured over three days. Each plant was measured for 60 s at 1-s resolution, close to the leaves (1–2 cm) or at a greater distance (4–5 cm). A total of 31 targeted volatile features were quantified as candidate predictors for classification. Classification used L1-regularized logistic regression with repeated nested cross-validation (5 repeats $\times$ 5-fold stratified cross-validation) and regularization strength C selected by inner cross-validation within each training set; predicted probabilities were averaged across the 5 repeats to obtain one final prediction per plant. Panel G used leave-one-day-out cross-validation. Feature standardization and selection of the regularization parameter C were performed within the training data only. Balanced accuracy was used as the primary performance metric throughout. Panel B–G use measurements collected at 4–5 cm from the leaves. Panels D, E, and G are based on the first 1 s of each plant's 60-s measurement; panel F additionally compares this against 20 random single-second draws. (A) Volatile emission dynamics and treatment comparison. Time series of concentrations (ppb) of selected volatile compounds recorded at 1-s resolution over 60 s for both measurement distances, together, with boxplots showing concentrations averaged over the full 60-s measurement (individual plants are shown as points). P-values indicate treatment differences (Wilcoxon rank-sum tests; full results in Supplementary Information). (B) Classification performance across integration periods. Plant-level balanced accuracy is shown as of measurement integration duration (1, 5, 15, 30 or 60 s). Points show plant-level performance with 95% percentile bootstrap confidence intervals from resampling plants with replacement, reflecting sampling uncertainty among plants. The dashed line indicates chance-level balanced accuracy (0.5). (C) Number of selected features across time integration periods. Mean number of features selected by L1-regularized logistic regression outer cross-validation models, shown as a function of integration period. Error bars indicate the standard deviation across outer cross-validation models. (D) Feature importance and selection stability. Volatile features are ranked by the absolute mean standardized model coefficient across outer cross-validation models, using the first 1 s of each measurement. Bars show mean coefficients, with positive and negative values indicating the direction of the model coefficient. Percentages indicate the proportion of outer cross-validation models in which each feature was selected. Only features selected in at least 50% of outer cross-validation models are shown. (E) Permutation test. Null distribution of plant-level balanced accuracy (using the first 1 s of each measurement) obtained from 250 permutations, randomly reassigning class labels within measurement day to preserve day structure. The complete procedure was repeated for each permutation. The solid red line indicates the observed plant-level balanced accuracy and the dashed black line indicates chance-level balanced accuracy (0.5). (F) Single-second robustness. Classification performance using 20 random draws, each using one randomly selected 1-s interval per plant. The boxplot shows the distribution of plant-level balanced accuracy across the 20 random draws. (G) Generalization across sampling days. Leave-one-day-out validation was performed using the first 1 s of each plant's 60-s measurement, with a single model fit per held-out day. Each sampling day was held out in turn for testing. Balanced accuracy is reported for the held-out day, together with the numbers of training and test plants.

correcting for this variation was important for robust classification.

The proportion of damaged plants correctly detected (recall/sensitivity) increased from 0.585 (0.453–0.714) without normalization to 0.662 (0.526–0.787) with TIC normalization. PQN normalization, however, gave the highest specificity, with 0.734 (0.594–0.866) of undamaged plants correctly classified, compared with 0.672 (0.557–0.786) for TIC. Similarly, ROC-AUC increased from 0.613 (0.495–0.727) without normalization to 0.698 (0.584–0.800) with TIC, indicating improved discrimination between damaged and undamaged plants. However, the relatively wide block-bootstrap confidence intervals reflected substantial sampling uncertainty associated with variation among the experimental blocks, and their overlap provided no clear evidence that one normalization strategy consistently outperformed the others. Nevertheless, given its highest observed balanced accuracy and ROC-AUC (Table S10), TIC normalization was selected for further evaluation.

We next asked whether the observed classification performance could plausibly have arisen by chance. In a block-level permutation test, damage labels were randomly reassigned between experimental blocks and the complete classification procedure was repeated for each permutation across all five preprocessing strategies. For each permutation, the maximum plant-level balanced accuracy across the five strategies was retained to generate the null distribution. The observed maximum balanced accuracy of 0.667 was greater than that obtained under this null distribution in 246 of the 250 permutations (permutation $p = 0.020$; Fig. 5C), indicating that the observed discrimination was unlikely to result from a random association between spectral profiles and damage labels.

We examined which spectral features contributed most consistently to the classification. Although the number of features retained by the L1 models varied substantially among cross-validation splits and preprocessing strategies (Fig. S12), a limited set of features showed high selection stability. Across preprocessing strategies, m/z 204 and m/z 117 showed the highest selection stability (Fig. S13). Under TIC normalization, both features were selected in all outer cross-validation models and were the two features with the largest mean absolute coefficients (Fig. 5D). These stable m/z features were consistent with the nominal masses of indole and sesquiterpenes, which were detected as markers of herbivory in the preceding open-air and laboratory experiments, although compound identities could not be confirmed from the field

measurements alone. The distributions of their normalized intensities showed some separation between damaged and undamaged plants, although they overlapped substantially between the two groups (Fig. 5E). Thus, these features contributed reproducibly to classification while not providing complete separation between damaged and undamaged plants at the individual-plant level.

4. Discussion

Plants rapidly emit specific volatile compounds in response to biotic threats, a well-documented process central to plant defense and ecological signaling [22,33,100,101]. This has motivated the idea of harnessing plant odors as reliable indicators of biotic stress in crops [102–104]. In this study, we evaluated the feasibility of such an approach in maize using currently available sensing technologies. We adopted a stepwise experimental strategy, progressing from laboratory assays with enclosed plants to open-air measurements in a simple outdoor setting and, finally, to a proof-of-concept preliminary field trial. This allowed us to assess whether induced plant volatile signatures remain detectable and informative as conditions shift from controlled to progressively more realistic settings.

4.1. Volatile signatures of biotic attacks in maize

Laboratory GC-MS analyses confirmed that maize plants emit distinct volatile signatures in response to herbivory and fungal infection. Caterpillar feeding induced the release of multiple terpenes, GLVs, and aromatic compounds, whereas undamaged plants emitted few volatiles at low abundance, consistent with prior studies [25,59,70,105–111]. The two *Spodoptera* species elicited quantitatively distinct responses, as previously described [59], illustrating that volatile signatures can be sufficiently nuanced to potentially distinguish between closely related pests. While such discrimination may not be essential for practical pest detection if management strategies are similar, it highlights the rich information content of herbivore-induced volatile blends. Fungal infection elicited a subset of compounds also observed during herbivory, but additionally induced characteristic fatty aldehydes and monoterpenes, consistent with previous reports for *Colletotrichum* sp. in beans [112], although volatile emission responses of maize to infection by *Colletotrichum* pathogens remain poorly characterized and largely

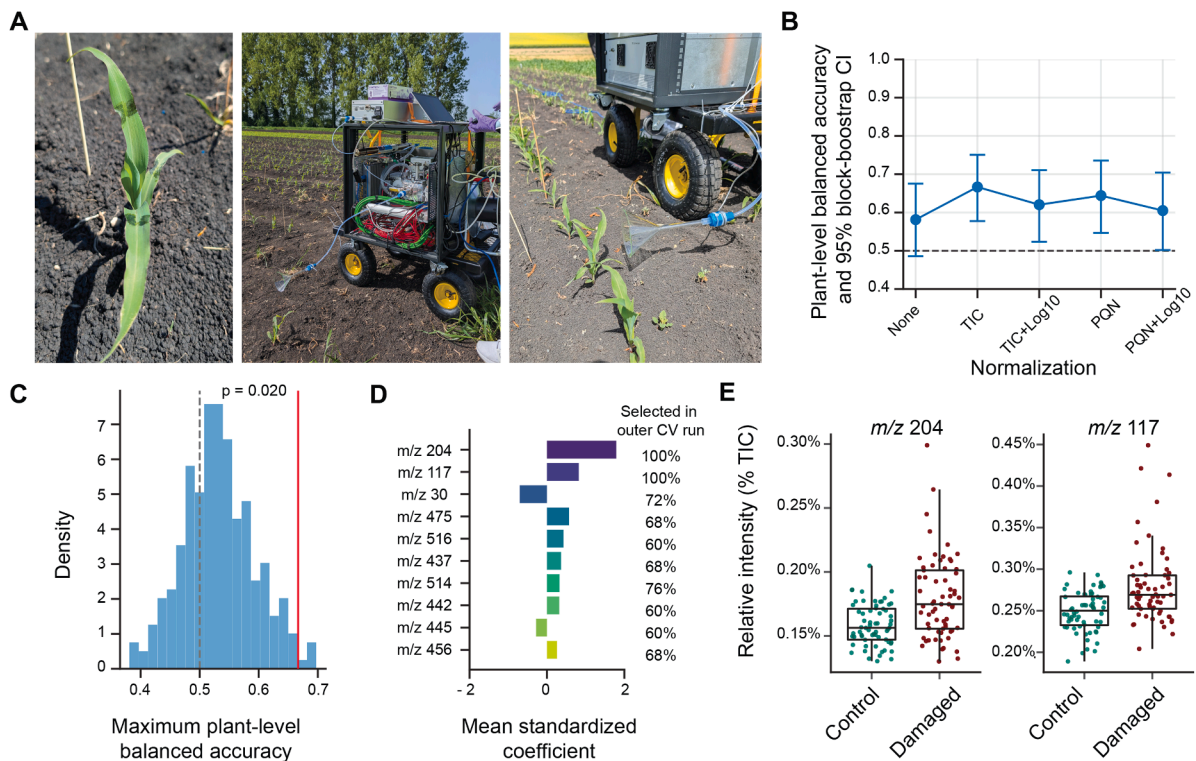


Fig. 5. Preliminary one-day field evaluation of maize simulated herbivory damage classification using portable TOF mass spectrometry. Measurements were performed on maize plants under field conditions using a portable TOF instrument, comparing undamaged with plants subjected to prior simulated herbivory. Simulated herbivory consisted of mechanical leaf damage combined with the application of *S. frugiperda* regurgitant. Each plant was measured for 60 s. For each nominal m/z value from 7 to 556 Da, the mean spectral intensity across the 60-s measurement was used as a predictor. In total, 129 plants were analyzed, including 64 undamaged and 65 damaged plants distributed across 63 alternating experimental blocks. Classification used L1-regularized logistic regression with nested five-fold cross-validation, with experimental block used as a grouping variable at both the outer and inner levels. Preprocessing, feature filtering, and selection of the regularization parameter C were performed within the training data only. Balanced accuracy was used as the primary performance metric. (A) Field sampling setup. Photographic overview of the experimental setup, showing (i) a maize plant subjected to simulated herbivory in the field, (ii) the portable instrument mounted on a mobile cart, and (iii) the sampling configuration. (B) Classification performance. Plant-level balanced accuracy of L1-regularized logistic regression models evaluated using five preprocessing strategies: no normalization, total ion current (TIC) normalization, TIC normalization followed by log10 transformation, probabilistic quotient normalization (PQN), and PQN followed by log10 transformation. Predictions from held-out outer cross-validation folds were aggregated to obtain one prediction per plant. Error bars indicate 95% percentile bootstrap confidence intervals from resampling experimental blocks with replacement, reflecting sampling uncertainty among blocks. The dashed line indicates chance-level balanced accuracy (0.5). (C) Permutation test. Null distribution of the maximum plant-level balanced accuracy obtained across the five preprocessing strategies from 250 permutations, after randomly reassigning treatment labels among experimental blocks. For each permutation, the complete analysis procedure was repeated. Plant-level balanced accuracy was calculated separately for each preprocessing strategy, and the maximum balanced accuracy across the five strategies was retained to generate the null distribution. The solid red line indicates the observed plant-level balanced accuracy obtained from the TIC model and the dashed black line indicates chance-level balanced accuracy (0.5). (D) Feature importance and selection stability. Features are ranked by the absolute mean standardized model coefficient across outer cross-validation models after TIC normalization and standardization. Bars show mean coefficients, with positive and negative values indicating the direction of the model coefficient. Percentages indicate the proportion of outer-CV models in which each feature was selected. Only features selected in at least 50% of outer-CV models are shown. (E) Distribution of informative spectral features. Distribution of TIC-normalized signal intensities for the two most stable and informative m/z features identified in (D), shown separately for undamaged and damaged plants. Boxes indicate the interquartile range (25th–75th percentiles), with the horizontal line indicating the median; individual points represent individual plants.

undocumented. In this study, only plants that subsequently developed visible symptoms were included as evidence of successful inoculation. Future work should assess volatile profiles across different stages or severities of infection, including less symptomatic plants, to evaluate their usefulness for detection of diseases, with infection status confirmed using molecular diagnostics.

Importantly, volatile responses differed between maize varieties, with one variety showing clearer separation among volatile profiles than the other. Such intraspecific variation is well documented in maize and other crops [67,113–115], and has been linked to genetic variation in terpene synthase gene expression and copy number among maize genotypes [116–118], as well as differences in the strength of herbivore-induced defense signaling pathways, with some maize genotypes exhibiting primed or constitutively elevated jasmonate signaling that results in faster and stronger induced responses upon herbivory compared to others [119,120]. Our results further suggest that the

performance of odor-based monitoring tools may depend on plant genotype, implying that detection approaches should be evaluated and calibrated across multiple varieties to ensure robustness and general applicability under field conditions. From a practical standpoint, this genotype-dependence poses both a challenge and an opportunity for deployable sensing systems. On one hand, monitoring tools calibrated on a single genotype may require re-calibration or genotype-specific reference libraries when applied to other cultivars, adding complexity to large-scale deployment. On the other hand, this variability creates an opportunity to prioritize or select cultivars with stronger or more distinct stress-induced volatile signatures, either for targeted deployment of existing sensing technology or, longer term, as a trait of interest in breeding programs aimed at integrating precision pest-monitoring capacity into commercial maize lines. Future work, combining volatile phenotyping across diverse germplasm with sensor validation will be needed to determine how broadly detection algorithms generalize

among odor phenotypes, and whether machine-learning approaches trained across multiple genotypes can offset genotype-specific signal variability. This approach could complement ongoing efforts to identify or breed genotypes with reliably strong indirect defense signaling for pest management purposes [121,122].

4.2. Odor sensing under laboratory conditions with enclosed headspace sampling

Under enclosed headspace sampling, the MSS module performed very well in laboratory assays, reliably distinguishing healthy maize plants from infested and infected plants and discriminating among different attackers. This finding adds to a few studies demonstrating successful use of portable odor sensors for discriminating different types of biotic stress through plant induced volatiles, which have likewise relied on enclosed sampling conditions, either static or dynamic [123–127].

PTR-TOF also distinguished among treatment-specific volatile signatures in laboratory assays, with particularly strong performance for herbivory. In addition to detecting the major compounds identified by GC-MS, it provided complementary information on the herbivore-induced volatile blend by capturing likely aldoximes and other nitrogen-containing compounds that were not detected by GC-MS. Such compounds have been reported as components of herbivore-induced volatile emissions in several plant species [128] and, occasionally, in maize [111,129]. These results underscore that the full volatile response of plants can be richer than is typically captured by GC-MS alone, and that minor compounds may contribute to valuable chemical information that can improve the accuracy and specificity of odor-based plant stress detection tools. This has direct implications for the real-time mass spectrometry approach evaluated here. Techniques such as PTR-TOF-MS offer high sensitivity and sub-second temporal resolution that, in principle, make them well suited to capturing low-abundance, transient volatile signals that conventional GC-MS sampling may under-represent [20,51–53]. Yet, PTR-TOF-MS also has inherent limitations. We found that its performance in identifying fungal infection was less consistent and varied between maize varieties. Fatty aldehydes, which were characteristic markers of fungal infection in GC-MS analyses, were poorly detected, likely because their parent ions undergo extensive fragmentation during PTR ionization, generating overlapping masses that reduce chemical specificity [130–132]. Newer real-time mass spectrometry techniques such as those with an adduct ionization mechanism have been shown to cover complementary chemical space (e.g., lipid- and amino-acid-derived signals) that PTR-based ionisation underrepresents [57,133]. Maximizing the diagnostic value of odor-based monitoring may ultimately depend not on a single analytical platform, but on strategically combining or selecting sensing techniques with complementary coverage of the volatile profiles, a direction that should be prioritized for future work building on the present findings.

4.3. Odor sensing in open-air conditions

Under open-air conditions, volatile concentrations were strongly diluted relative to laboratory assays, as expected. PTR-TOF measurements indicated that herbivore-induced compounds occurred at low ppt levels, more than two orders of magnitude lower than in enclosed headspace experiments. Under these conditions, the MSS module did not capture treatment-specific volatile signatures of herbivory damage. This lack of discrimination likely reflects a combination of strong dilution and the influence of fluctuating environmental factors, such as humidity and temperature, on sensor performance. These observations are consistent with previous reports identifying sensitivity constraints and environmental dependence as major challenges for deploying portable gas sensors for plant odor detection under field conditions [16,19,37,38,41]. In addition, as an equilibrium-based sensor, MSS may be particularly susceptible to rapid concentration fluctuations because the

receptor layer requires time to equilibrate with the surrounding air, reducing signal strength under dynamic open-air conditions. Although the MSS module can reach ppt-level detection limits for some compounds [134], the time required to accumulate sufficient signal at very low concentrations currently exceeds one minute [48,135], limiting its suitability for mobile, continuous monitoring in crop environments. Nonetheless, the compact size, ease of use, and strong performance under more controlled sampling conditions at detecting induced plant volatile signatures suggest potential for other on-site applications where volatile concentrations are higher or sampling can be stabilized, such as inspections in containers of imported plant material [136–138].

PTR-TOF detected several herbivore-induced volatiles under open-air conditions, even at low ppt concentrations. In this simple experimental setup, data collected at one-second intervals already contained sufficient information to distinguish herbivory-damaged from undamaged plants with high balanced accuracy of 87%, rising further to 96% with longer integration. This suggests that, at least under these semi-controlled open-air conditions, even brief measurements already capture much of the relevant signal, though longer sampling further improves the reliability of detection. Ions corresponding to indole and sesquiterpenes were by far the most influential and consistently selected markers. This strong predictive performance reflects the experimental and biological simplicity of the system. Here, plants were grown under optimal and constant greenhouse conditions and assessed in a simple outdoor setting that does not capture the complexity of agricultural fields. Under these simplified conditions, undamaged plants emitted little to no detectable volatiles, whereas damaged plants produced clear signals, effectively reducing discrimination to the presence or absence of a small number of dominant characteristic compounds. Moreover, plants were measured individually, without interference from neighboring plants, making odor attribution straightforward. In field settings, by contrast, plants grow while being exposed to multiple and variable biotic and abiotic factors, introducing numerous sources of variation that can affect their volatile emission [33,101,139,140]. Moreover, emissions could overlap with odor plumes from other plants, as well as other sources, such as non-crop vegetation, within or surrounding the field. Thus, these results are best interpreted as indicating that real-time mass spectrometry can resolve low-abundance plant odor signals emitted by greenhouse-grown plants when sampled without a containment system, under very moderate environmental variability (e.g., changes in humidity, temperature, and wind) during sampling.

4.4. Odor sensing under field conditions

To begin evaluating odor-based detection under more realistic agricultural conditions, we conducted an initial exploratory field trial. Although the experiment was limited to a single day and one field plot and therefore cannot support general conclusions about performance under field conditions, the results provide an encouraging first indication that herbivory-related spectral information can be detected under field conditions.

Using a portable TOF, plants with simulated herbivory could be partially distinguished from undamaged plants, with balanced accuracy reaching 67%, using the averaged information of 60-s measurements. Permutation analysis indicated that the observed classification performance was unlikely to arise by chance. Moreover, spectral features consistent with the nominal masses of sesquiterpenes and indole were repeatedly selected across models. This is in line with *Spodoptera*-associated volatile compounds detected in our laboratory and semi-controlled open-air experiments, suggesting that components of the herbivory-associated odor signature remained detectable under true field conditions.

Nevertheless, the ability to distinguish damaged from undamaged plants was limited and subject to substantial sampling uncertainty. In addition, it remains to be determined how short measurements can be while still capturing sufficient information, as the average spectral

information over 60 s was used here. The PTR-TOF open-air experiment showed that discrimination decreased with shorter integration durations, suggesting a likely trade-off between measurement speed and predictive performance.

However, these results were obtained without optimization of either instrumentation or sampling strategy specifically for field-based measurements. Thus, they represent an encouraging initial proof-of-concept rather than evidence of field-level diagnostic performance and motivates more extensive field validation. Overall, this single-day preliminary field trial based on simulated herbivory suggests that a compact, potentially field-deployable, real-time mass spectrometer can capture odor-related spectral information that partially discriminates damaged from undamaged maize plants under field condition.

5. Limitations

The main limitation of this study is the exploratory nature of the field evaluation. Restricted to a single day and plot, its scope is too limited to draw firm conclusions about the feasibility of plant volatile-based pest detection using real-time mass spectrometry for practical field application. Substantial further work and broader field evaluation are needed to assess generalizability, robustness, and specificity across the conditions encountered in agricultural environments that can influence volatile emissions. The approach therefore needs to be tested across a broad range of variability sources, including abiotic environmental factors, plant developmental stages, and dynamic, co-occurring biotic stressors, requiring notably sampling across different plots and time points. Moreover, different agricultural settings and practices introduce further variation through mixed cropping, variable plant density, and diverse background vegetation. As multiple stresses can co-occur in agricultural systems, further testing will also be needed to evaluate the capacity of the approach to distinguish among different stress-induced volatile profiles. Detecting and distinguishing among multiple antagonists may benefit from AI-based approaches to handle complex odor patterns. Unlike medical or explosive-detection applications, agricultural use may tolerate some error, particularly false positives, and fine-scale distinctions between similar pests (e.g., different lepidopteran species) may not always be necessary when the same control measure applies to all.

The field evaluation was also conducted under a simplified experimental setup that does not fully reflect natural herbivory conditions. Although mechanical wounding with regurgitant application is a well-established proxy for caterpillar herbivory, and previous studies have shown that regurgitant elicits volatile responses that are not fully induced by mechanical wounding alone [98,99,105], the absence of a wound-only treatment in our experiment implies that a contribution of a general wound response to the discrimination between treated and undamaged plants cannot be excluded. Validation using real herbivory, ideally under naturally occurring infestations, and comparison with related stresses including physical damage will therefore be important to determine whether the results translate to agricultural conditions with sufficient specificity and reliability. On the other hand, the simplified simulated herbivory treatment may also have been conservative with respect to signal strength. Our experimental layout, consisting of small sets of damaged and undamaged plants positioned adjacent to each other along a row, represents a particularly challenging configuration requiring fine-scale detection, whereas natural infestations typically occur in spatial clusters rather than as isolated plants [141–143], potentially creating larger, aggregated volatile sources that may generate stronger signals and be less sensitive to small variations in wind direction. Continuous herbivory is also expected to produce stronger, more sustained volatile emissions than the temporally limited artificial damage applied here [111].

In addition, two main challenges were encountered during this preliminary field trial that should be considered in future experiments. First, the absence of independent reference volatile measurements, such as GC-MS analyses of collections from individual plant headspace

samples, limited our ability to investigate the origin of misclassified plants. We could not determine whether misclassification reflected limitations in the portable instrument's detection of relevant volatile signals, or biological variation in plant responses, such as undamaged plants producing induced volatiles or damaged plants showing weak or no response. Although highly labor-intensive, integrating complementary GC-MS analyses in future experiments would help distinguish between these possibilities.

Second, practical sampling was constrained by airflow conditions, which were more variable in the field than in the simplified outdoor experiments. During the latter, wind direction was relatively stable, likely because it was partly constrained by surrounding buildings, allowing the sampling inlet to be positioned consistently downwind of individual plants. Under field conditions, wind speeds were relatively low that day ($< 3 \text{ m s}^{-1}$), but wind direction was variable. This variability could have diluted or redirected emissions and increased contributions from neighboring plants, complicating odor attribution. These effects may become more pronounced under stronger winds, though refined sampling strategies, such as partial airflow shielding, adaptive inlet positioning, or incorporating wind direction data into odor source-mapping, could help mitigate wind-related limitations [144–146].

6. Outlook

This study represents a preliminary proof-of-concept and an encouraging early step towards odor-based agricultural pest detection using induced plant volatile emissions, highlighting the potential of real-time mass spectrometry for this application. It enabled sensitive and fast detection of pest-induced plant volatiles under semi-controlled open-air conditions, while preliminary field data support the potential feasibility of in situ detection of plant herbivory damage, although further field validation is needed.

Still, CI-TOF instruments remain costly, bulky, and currently require expert knowledge for their operation, likely limiting their direct near-term use in agricultural settings. Nevertheless, their ability to identify and quantify informative marker volatiles under realistic conditions could provide valuable guidance for developing next-generation portable sensors. Because many crop species emit overlapping herbivory-induced volatile profiles [70], these insights may be transferable beyond maize.

More broadly, odor-based monitoring could complement existing precision-agriculture tools aimed at reducing agrochemical inputs, such as robotic weeders and selective sprayers [10,147,148], and support more targeted, cost-effective deployment of biological control agents, including microbial insecticides and entomopathogenic nematodes [103,149,150]. These applications will require further interdisciplinary research to improve the robustness, applicability, and scalability of odor-based detection under agricultural conditions, together with assessment of operational, logistical, and economic feasibility. Broader field validation will ultimately be needed to determine whether real-time chemical-ionization mass spectrometry could contribute to practical, early, and non-destructive pest monitoring as a component of sustainable crop protection.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Statement for studies with humans / animals

Our study did not involve any human or animal use. We only used insects, under quarantine laboratory conditions which Prof. Ted

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CRediT authorship contribution statement

Marine Mamin: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Carla C.M. Arce:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Gregory Röder:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Arooran Kanagendran:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **Thomas Degen:** Visualization, Writing – review & editing. **Emmanuel Defosse:** Formal analysis, Visualization, Writing – review & editing. **Sergio Rasmann:** Formal analysis, Writing – review & editing. **Terunobu Akiyama:** Resources, Writing – review & editing. **Kosuke Minami:** Resources, Writing – review & editing. **Genki Yoshikawa:** Resources, Writing – review & editing. **Felipe Lopez-Hilfiker:** Resources, Methodology, Investigation, Data curation, Writing – review & editing. **Priyanka Bansal:** Resources, Methodology, Investigation, Data curation, Writing – review & editing. **Luca Cappellin:** Resources, Writing – review & editing. **Yunhe Li:** Formal analysis, Writing – review & editing. **Ted C.J. Turlings:** Funding acquisition, Project administration, Resources, Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: at the time of the study, Felipe Lopez-Hilfiker and Priyanka Bansal were employees of Tofwerk AG, and Terunobu Akiyama was affiliated with NanoWorld AG. The MSS technology used in this study was developed through collaborations involving NIMS and NanoWorld AG. Authors involved in the development of the sensing instruments contributed to instrument development, technical advice, and experimental measurements and data extraction for the portable TOF, but did not contribute to the downstream statistical analyses, machine-learning modeling, or interpretation of the results. The authors declare no other competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.atech.2026.102604.

Data availability

<https://github.com/MaminMar/OdorBasedDetection> ().

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