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Automated dynamic sampling system for the on-line monitoring of biogenic emissions from living organisms

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Abstract

An automated system for continuous on-line monitoring of biogenic emissions is presented. The system is designed in such a way that volatiles, emitted as reaction to biotic or abiotic stress, can be unequivocally elucidated. Two identical sampling units, named target and reference bulb, are therefore incorporated into the system and consecutively analyzed in monitoring experiments. A number of precautions were considered during these experiments to avoid the application of unwanted stress onto both organisms. Firstly, the system is constructed in such a way that both bulbs are continuously flushed, i.e. before, during and after analysis, with high purity air to avoid any accumulation of emitted volatiles. Moreover, the air is pre-humidified by bubbling it through water to sustain the biological samples for longer periods in the in vitro environment. Sorptive enrichment on polydimethylsiloxane (PDMS) was used to trap the headspace volatiles. The hydrophobic nature of this material permitted easy removal of trapped moisture by direct flushing of the sampling cartridge with dry air before desorption. The system was used to monitor the emissions from in vitro mechanically wounded ivy (*Hedera helix*) and of in vitro grown tomato plants (*Lycopersicon esculentum* Mill.) upon cotton leafworm (*Spodoptera littoralis*) feeding. Differences in light and dark floral emissions of jasmine (*Jasminum polyanthum*) were also studied.

Keywords: Biogenic emissions; Headspace analysis; Sorptive enrichment; Dynamic headspace analysis; Automation; Polydimethylsiloxane

1. Introduction

An extremely exciting field of research comprises the search for the overall biological significance of components released by biological materials, i.e. biogenic emissions. Owing to the large diversity in emission sources (animals, plants) as well as in compound types (permanent gases, volatile organics), the entire field of interest is very wide and covers many different scientific disciplines. Insect ecology, phytochemistry, plant physiology, entomology, food and perfume chemistry are some of the areas where investigation of volatile emissions is carried out regularly.

The potential impact generated results may exhibit is far-reaching and not restricted solely to these areas. It is quite well known, for instance, that the communication within troops of social insects (bees, ants, wasps) primarily passes via volatile secretions (pheromones) [1], an effect that has been employed

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already in specific insect lures [2]. Moreover, also the field of plant volatile research has experienced great progress. It has been demonstrated, for example, that certain plant volatiles inhibit fungal proliferation [3], perhaps indicating future directions for genetic improvement. Additionally, particular volatile organics serve as critical volatile markers in such insect behaviors as host finding, feeding and ovipositioning [4,5], and can be eventually exploited to craft effective methods for control of insect pests, as an alternative to the toxic agrochemicals applied today [6]. A comprehensive overview of the various classes of plant volatiles, their occurrence in crops and non-crops and their overall significance in environmental interactions was provided by Charron et al. [7].

As can be expected from the literature dealing with these topics, presented results primarily focus on the final outcome of the above mentioned effects and although the analytical part is really critical with respect to the reliability of the final results, it is often merely of secondary importance. The prime portion of biogenic emissions are analyzed using dynamic adsorptive enrichment, a technique which was developed nearly 40 years ago for the analysis of apolar to medium polar volatiles in air. The success of this approach is primarily due to the significantly higher sensitivity that can be attained compared to the alternative (static) methods such as solid-phase microextraction (SPME) [8] and headspace sorptive extraction (HSSE) [9]. Unfortunately, dynamic enrichment is not free of hazards, especially when applied without full knowledge of its potential drawbacks. However, all of these problems are a direct consequence of the use of adsorbents and originate from the strong interactions that occur between trapped analytes and the adsorbent surface. Therefore, sufficient retention and high enrichment factors hardly are an issue, though more harsh desorption conditions are required to remove the analytes.

In general, liquid extraction of the adsorbent bed with a suitable organic solvent is sufficiently invasive to allow complete desorption. Unfortunately, a great part of the enrichment effect acquired through sampling has to be sacrificed since only a small portion of the final liquid extract is available for subsequent analysis. Consequently, relatively long sampling times and/or high sampling flow-rates are necessary to deliver sufficient sensitivity in a reasonable amount of time.

From a sensitivity standpoint, it is much more attractive to employ the alternative method, i.e. thermal desorption. Here, the adsorbent is inserted in a small oven placed on top of the gas chromatograph (GC), which is subsequently heated to elevated temperatures. When properly designed, this set-up allows complete transfer of the sample from trap to analytical column. However, the strength of the analyte–surface interactions calls for high desorption temperatures, which can lead to the formation of artifacts and incomplete desorption, especially when polar and/or higher molecular mass compounds (MW) are involved [10–12].

Recently, sampling tubes packed with 100% polydimethylsiloxane (PDMS) particles were introduced for the enrichment of volatile emissions [13,14]. With this material, preconcentration occurs by sorption or dissolving of the target analytes in the bulk of the liquid phase instead of adsorption onto a porous surface. Since this process is less energetic compared to adsorption, trapped volatiles are easily desorbed from the trap using thermal desorption, even at relatively mild temperatures. In addition, the absence of active sites avoids displacement effects and catalytic modifications of trapped components from occurring [12]. The performance of the dynamic configuration with PDMS has been evaluated for the analysis of plant volatiles, where it performed superior to Tenax, which was characterized by a significant interference of degradation products arising from decomposition of the polymeric skeleton and incomplete removal of higher MW analytes [15].

Just recently, we described a completely automated and stand-alone system for the analysis of gaseous emissions, e.g., ethylene, released by plants [16]. However, in order to be able to analyze regular volatile emissions, an adapted version of this system had to be developed. These adaptations were carried out successfully and the system was immediately used for determining the response of *Arabidopsis thaliana* to oxidative damage [17]. The results presented in that particular paper mainly dealt with the various problems and strategies encountered when identification of unknown volatiles has to be performed, without paying too much attention to the

practical operation and additional possibilities of the device. These particular matters will be discussed in the present paper.

2. Experimental

2.1. Biological materials

Ivy (*Hedera helix*) and jasmine plants (*Jasminum polyanthum*) were purchased from a local florist and placed in an isolated room under four TL-lamps (Osram, 32 W/lamp) equipped with a time controller to obtain a 16 h photoperiod (day, 4.00 a.m. to 8.00 p.m.). The temperature inside the room fluctuated around 25°C during the light period and 18°C during the dark period. Plants were kept in this chamber until analyzed.

Three-week-old tomato plants (*Lycopersicon esculentum* Mill.) of the cultivar Moneymaker were obtained from the Department of Plant Production — Horticulture of Ghent University, Belgium where special facilities are present to achieve aseptic plant growth. Plants were directly grown in especially designed sampling units, which were placed inside a growth chamber with full control of temperature (25±1°C), photoperiod (16 h), light strength (60 $\mu mol/m^2/s$) and relative humidity (85%). Plants were brought to the laboratory 1 day before experiments were started. During the total duration of these experiments four TL lamps (16 h photoperiod) were placed in the vicinity of both units, in order to preserve the day–night cycle.

Cotton leafworm caterpillars (*Spodoptera littoralis*) were obtained from a colony, which was maintained at the Department of Crop Protection of Ghent University, Belgium. The colony was exclusively fed with Moneymaker leafs. Animals were delivered together with the tomato plants and placed in-between two petri dishes. In order to initiate immediate feeding after introducing the animals into the plant containers, they were famished during the overnight period the plant was left to stabilize.

2.2. Sampling units

A schematic drawing of the sampling units is part of Fig. 1. Plant materials were placed underneath two identical pyrex glass bulbs (1 and 2), both tightly pressed with clamps (3) onto separate glass plates (25×25 cm, with 5 cm high glass side plates) (4). After pressing the bulb onto the plate, the latter was filled with a small amount of purified water (Milli Q, Millipore, Bedford, MA, USA), which was not able to enter the interior, for perfect sealing. Larger plants were placed inside a 9.4 L bulb (30 cm height×20 cm I.D.), while smaller ones were placed inside a 3.5 L one (20 cm height×15 cm I.D.). Each bulb had a gas inlet (5) and outlet (6) made from Duran GL 14 screw-thread tubes (Schott Glas, Mainz, Germany), equipped with GL 14 plastic nuts and Teflon-lined septa (Supelco, Bellefonte, PA, USA). High purity air (N50 grade) obtained from Air Liquide (Schelle, Belgium) was fed to the bulbs (7). Flow-rates were chosen as function of the volume of the sampling units and approximated a refresh rate of 1 bulb volume per hour to avoid excessive flow stress. Before entering the system, air was humidified by bubbling through Milli Q water (8).

Analyses of tomato plants were performed directly on dedicated sampling units. Plants were grown in vitro, i.e. under aseptic conditions, in order to eliminate the influence of microbiological contaminations onto plant volatile emissions. Details concerning these sampling units are presented elsewhere [18].

2.3. Experimental set-up

A schematic representation of the set-up for automated analysis of biogenic emissions is given in Fig. 1. The heart of the system is a TDS-G device (Gerstel, Mülheim a/d Ruhr, Germany) for on-line sampling and thermodesorption. Basically, this device consists of two programmable temperature vaporization (PTV) injectors placed in series. The first injector (9) is the actual thermodesorption system (TDS) in which the sampling tube (10) is contained. A glass tube packed with 100% PDMS particles (ca. 300 mg, Gerstel) was used as enrichment cartridge. The second PTV (11) is a CIS-4 injector (Gerstel), which is used as a cryotrap to refocus the analytes during desorption. After desorption, the cryotrap is flash heated to inject the sample as a small plug onto the analytical column. Automatic switching between sampling (—) and thermo-

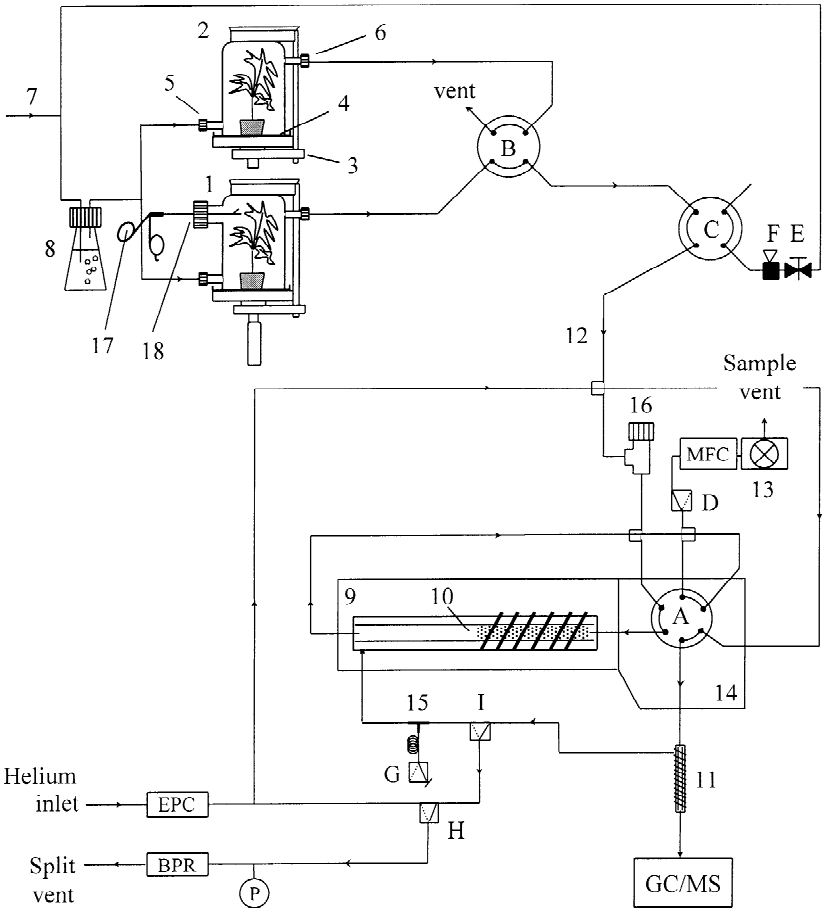

Fig. 1. Schematic representation of the sampling and thermodesorption system: (1) target bulb, (2) reference bulb, (3) clamp, (4) glass plate, (5) gas inlet, (6) gas outlet, (7) air inlet, (8) air humidifier, (9) thermodesorption unit, (10) PDMS tube, (11) PTV cryofocusing device, (12) sampling line, (13) vacuum pump, (14) heated compartment, (15) TDS backflush, (16) heated T-piece, (17) endoscopic pliers for in vitro wounding and (18) pliers inlet. The following valves control the different processes: (A) 6-port rotary valve for sorption/desorption, (B) 4-port rotary valve for bulb selection, (C) 4-port rotary valve for purging, (D) solenoid valve for purge vent, (E) open/close valve, (F) needle valve, (G) solenoid valve for TDS backflush on/off, (H) GC split/splitless valve and (I) TDS split/splitless valve.

desorption (---) is achieved by means of a 6-port rotary valve (valve A), which is located in-between both injection devices. Sampling line (12) and vacuum pump (13) complete the set-up. The valve block was maintained at 300°C, the complete compartment (14) was kept at 270°C. Connections towards valve A were made of 530 μm I.D. silicon steel tubing (Gerstel). The complete unit is controlled and programmed with Gerstel Master Software.

This TDS-G system is connected to two sample containers of which the headspace is analyzed consecutively. Run-to-run switching between both bulbs was achieved by means of a first 4-port rotary valve (valve B). This valve is controlled by a GC external event and is rotated at the start of each chromatographic run. During the sampling period the PDMS tube was held at 20°C. Sample volumes and sampling flow-rates will be provided in the respective parts of the Results and discussion section.

Water vapors, which accumulate during sampling, were removed automatically by purging the system with dry N50 air. The purge air enters the system at a second 4-port rotary valve (valve C), travels

through the system and is vented at solenoid valve D which serves as temporary split point. As soon as the sampling period has completed, valve C is activated and rotated into the purge position (---). The electrical signal required to achieve switching is delivered by the TDS-G electronics controller and is the same as the vacuum pump on/off signal. Simultaneously, the TDS-G controller also transmitted a specific purge output signal to valves D and E. Valve E is a regular open/close valve which is used to prevent the purge air from escaping the system during sampling and analysis. The flow-rate of the purge air is regulated with a fine metering valve (valve F). The split point at valve D was used as measuring point for the flow meter. When the purge period is completed, the same purge signal is retransmitted so that valves D and E are returned to their original position. The total duration of the purge period is controlled with the Master software. A 5 min purge at 400 ml/min was used to dry the PDMS tube.

After purging, valve A rotates into the desorption position directing the carrier gas in the opposite direction over the PDMS bed. A small portion of the carrier gas (3 ml/min) is vented from the TDS to backflush the sampling tube exterior (15). During sampling this outlet is closed with valve G. Compounds released during thermodesorption, were swept towards the inlet (empty baffled liner) at 100 ml/min, with the GC split valve (valve H) open (solvent vent mode). After complete thermodesorption (ca. 10 min) the CIS-4 was ramped to 350°C with valve H closed (splitless mode). *Hedera helix* samples were desorbed with the TDS split valve (valve I) open, followed by regular splitless injection. Together with the start of the GC run, valve A is repositioned, ready for a new sampling period.

The lines connecting the different valves to the TDS-G system were made from 1/8″ PTFE tubing. The tube connecting valves A and F had a 1/16″ I.D. to minimize carry-over between subsequent analyses (internal volume <1 ml). A schematic overview of the different valve positions during a sampling/desorption sequence is summarized in Table 1.

In all experiments the TDS was heated from 20°C (1 min) to 225°C (5 min) at 60°C/min. The CIS-4 was ramped from −150°C (0.5 min) at 12°C/s to 350°C (5 min). The splitless time was 2.5 min.

2.4. Capillary GC–MS

The TDS-G unit was mounted on top of an Agilent 6890 GC/5973 MSD (Agilent Technologies, Little Falls, DE, USA). The MSD was operated in the electron impact mode (70 eV), generating full scan spectra between 50 and 250 amu at 7 scans/s. The system was also equipped with a Wiley database for spectral identification. A HP-5MS capillary column (30 m L×250 μm I.D.×0.25 μm d_f) was used during the experiments. Helium was the carrier gas at 35 cm/s. The GC oven was programmed from 40°C (2 min) to 280°C (5 min) at 15°C/min for all experiments.

3. Results and discussion

The relative complexity of the final set-up (see Fig. 1) is a direct consequence of the necessity for appropriate accommodation of the organisms during analysis. When dealing with plant material, this primarily implied a regular supply of water and nutrients, control of temperature, relative humidity

Table 1
Schematic representation of the valve positions during a complete sampling/desorption cycle

Event	A	B	C	D	E	G	H	I
Sampling bulb 1	———	———	———	———	C[a]	———	———	———
Purge signal	———	———	·····	·····	O[a]	———	———	———
Thermodesorption	- - -- - -	———	·····	———	C	- - -- - -	———	———
Injection (=GC start)	———	- - -- - -	- - -- - -	———	C	- - -- - -	- - -- - -	———
Sampling bulb 2	———	·····	———	———	C	———	———	———

Characters refer to the valves in Fig. 1. Valves H and I are used in the splitless injection and thermodesorption mode. Valve C is controlled by the vacuum pump on/off signal.

[a] C=closed; O=open.

(RH), light strength and photoperiod. The two latter factors were easily implemented by means of a set of lamps, which were equipped with a time-controlled power switch. Practical implementation of the other factors was less straightforward to realize.

The main feature of the set-up is the use of two identical sample containers, namely target and reference unit. The target unit contains the plant that has been subjected to the specific stress situation under investigation (wounding, insect feeding, herbicide spraying, etc.). The use of two sample containers was preferred over a singular set-up for two reasons. Firstly, accurate control of temperature and RH, which requires dedicated facilities, is less crucial, since both plants constantly experience identical environmental fluctuations. In addition, specific stress-related volatile emissions are easily recognized by continuously comparing the emissions emanating from the target plant with these of the reference plant.

Water supply was taken care of by feeding both containers with humidified air. Air is delivered continuously to the systems, i.e. before, during and after analysis a constant supply is guaranteed. Continuous air supply was also necessary to preclude accumulation of volatile emissions inside one of the containers. Although frequently applied to increase the amount of analytes in the headspace (and mask the insensitivity of the sampling technique), accumulation has a profound effect on plant behavior which should not be underestimated. It has been demonstrated, for example, that ethylene is able to influence its own emission via a positive feedback loop [19]. Since the sensitivity of other compounds towards this effect is hard to predict in advance, the incorporation of continuous headspace refreshments was necessary to provide a sure safety margin.

Subsequent water removal was achieved by purging the sampling tube with dry air after the sampling period. In the initial stage of instrumental development two alternative approaches, i.e. Nafion membrane permeation and condensation in a cryostat, were also briefly considered. The key advantage of both approaches is the limited load on the instrumental side of development. However, their operation principle, which is based on in-line moisture removal, is particularly dangerous when polar compounds are present in the sample. These compounds are easily co-trapped with the moisture and inevitably lost, which is of course highly undesirable [20]. Purging the trap with air omits any additional adsorbing devices and is, therefore, much more attractive. Moreover, the hydrophobic and weakly retentive nature of the PDMS material is even more advantageous for use in a post-sampling purging configuration. In addition, displacement caused by co-sorption of water is unlikely to occur [21]. The only factor to consider when applying post-sampling purging is the size of the purge volume. In order to quantitatively trap and store the analytes of interest on the PDMS bed, the sum of sample and purge volume should not exceed the breakthrough volume of the most volatile compound.

In a first series of experiments, the minimal air volume required for drying the PDMS tube after sampling was determined. Therefore, the PDMS tube was saturated on-line with water by directly sampling the air coming from the humidifier for 20 min at 50 ml/min. At that stage, abundant water droplets were clearly visible at the inner wall of the sampling tube. Afterwards, the tube was purged for 1 min using different purge flow-rates, followed by the standard thermodesorption program. Flow-rates lower than 500 ml/min were far too short to achieve sufficient water removal. Regular system operation was even not possible due to ice formation inside the CIS-4 giving rise to blocking of the carrier gas flow path and complete system shutdown. At 500 ml/min injection could be carried out flawless, though the acquired chromatogram was severely disturbed by the injection of water (Fig. 2).

Based on these results, the next series of experiments were carried out with a purge time of 5 min at variable flow-rates. The effectiveness of the purge step was now verified by looking at the influence of water injection on chromatography and specifically on peak shape. Therefore, 1 μl of a plant volatile mixture (Table 2) was introduced in the system via a T-piece (Fig. 1(16)) which was kept at 250°C and placed on top of the TDS. Injections were made 4 min before the end of the sampling period (20 min in total) to achieve complete transfer from the T-piece towards the PDMS material. Repeatability of injection was determined in dry air and was excellent with a precision of ca. 5% for each compound for six replicate injections. Real recoveries, calculated by

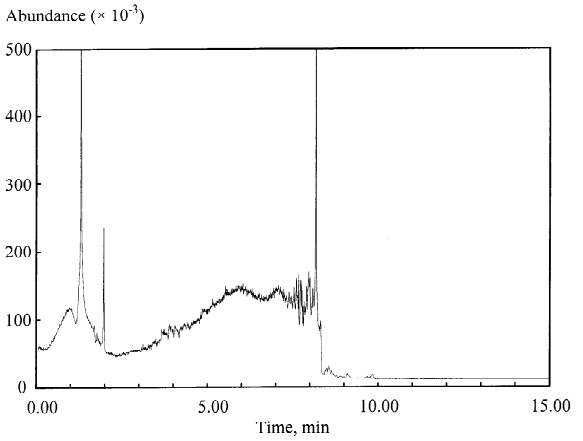

Fig. 2. Illustration of the influence of insufficient water removal on the chromatographic performance. Sampling humidified air for 20 min at 50 ml/min. Purge time 1 min at 500 ml/min. For desorption program see Experimental section.

using the area responses obtained after splitless injection in the CIS-4 injector as reference, exhibited a boiling point discrimination. The more volatile components (up to benzaldehyde) were partly lost with recoveries between 70 and 80%. For the semivolatiles (up to acetophenone) values were significantly higher (>90%). The reason behind this recovery decay is breakthrough from the PTV during thermodesorption. Prevention is possible by increasing the available trapping surface of the cryotrap, for example by packing the liner with glass beads or a small amount of PDMS material. For the purge evaluation experiments sensitivity was, however, sufficiently high.

The first set of experiments was carried out using purge flows of 200 and 300 ml/min. Both chromatograms were, however, of an unacceptable quality. Particularly the peak shape of the polar compounds eluting in the first part of the chromatogram was seriously disturbed, rendering an increased purge flow necessary. A proportional improvement in overall peak shape was observed at 400 ml/min. Higher flow-rates were also investigated, but did not induce any additional improvements. A visual representation of the influence of water injection on the response of β-pinene is given in Fig. 3. The lower response observed under incomplete purge conditions is due to inaccurate integration of the disturbed peak.

Table 2
Test mixture used for purge evaluation experiments. Compounds were dissolved in dichloromethane at ca. 10 ppm each

Peak no.	Compound	Peak no.	Compound
1	*trans*-2-hexenal	9	acetophenone
2	*cis*-3-hexenol	10	linalool
3	*trans*-2-hexenol	11	methyl salicylate
4	benzaldehyde	12	carvone
5	β-pinene	13	eugenol
6	1-octen-3-ol	14	ethyl decanoate
7	*p*-cymene	15	*n*-hexadecane
8	benzyl alcohol	16	benzyl benzoate

The responses of β-pinene in dry and humidified air more or less converged at a purge volume of 2 l (5 min at 400 ml/min). This value was, therefore, taken as minimal purge volume to obtain a sufficiently dried system. It is also clear from Fig. 3 that it is to be preferred to choose a purge volume as small as possible. If taken excessively long, compound loss, due to breakthrough from the trap, becomes increasingly significant. Insert in Fig. 3 represents the experimentally determined breakthrough curve of β-pinene at 400 ml/min of air. As can be seen, the compound started to elute from the trap around 5 min (=2 l). This explains the overall decreasing trend of both curves observed in Fig. 3. The amount lost by breakthrough is, however, trivial compared to the total amount retained by the trap. The dashed line at 10 min (=4 l) indicates the 5% breakthrough level. The theoretical breakthrough volume, calculated according to the procedure proposed by Baltussen et al., gave a similar result [13]. Values were also calculated for the other sample constituents. In general, retention is adequate enough to combine purging with a sufficiently large sample volume (viz. β-pinene). Potential problems are restricted to the C_6-volatiles, which have breakthrough volumes around 600 ml. In order to include these components in an analytical cycle it is, therefore, necessary to work under relatively non-humid conditions so that the purge step becomes redundant. Eventually, equilibrium sorption can be applied to increase the amount of these components enriched on the trap [22]. Finally, it is noted that experimental breakthrough was not affected by the presence of water, not even for the polar components.

A purgeless monitoring experiment, generating

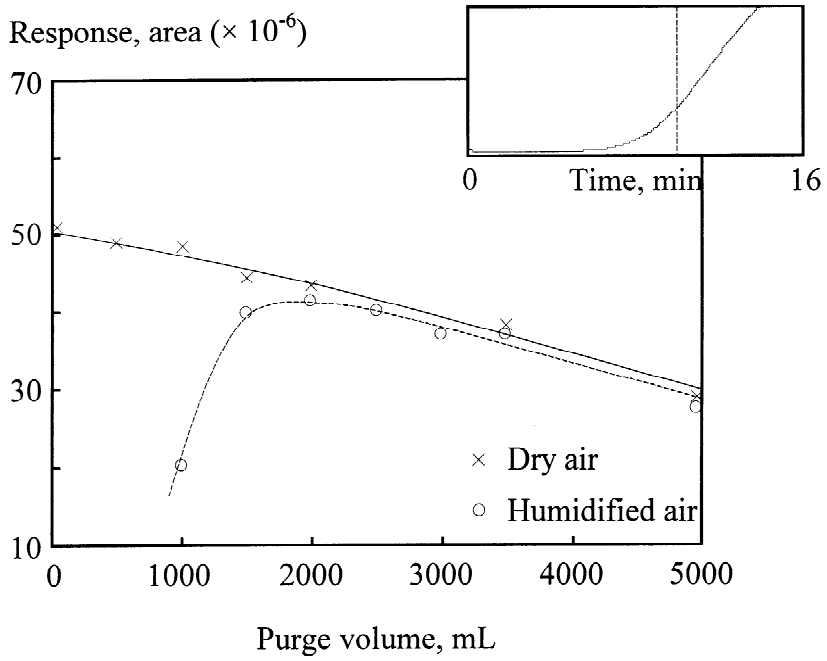

Fig. 3. Response of β-pinene as a function of purge volume. Full and dashed lines represent signal variations when sampling was performed with dry and humidified air, respectively. Insert shows the experimentally acquired breakthrough curve of β-pinene. The vertical dashed line represents 5% breakthrough of the compound.

complete headspace profiles, was carried out in the first application using the ivy plants as target materials. Both plants were, therefore, watered just before starting the experiment, enclosed in the 3.5 L bulbs and supplied with dry N50 air at 50 ml/min. Headspaces were sampled 10 min at 50 ml/min and desorbed using the standard program (see Experimental section). The minor traces of water present in the headspace did not cause any freeze-related problems. The obtained headspace profiles were highly similar and mainly consisted of some mono- and sesquiterpenes. In order to allow the plants to adapt to the in vitro environment, the next set of analyses was carried out the next day. During the stabilization period, the air supply was maintained at 50 ml/min. Overnight stabilization clearly had a positive effect on plant emissions. When two new blanks were taken, monoterpene emissions had decreased significantly and sesquiterpenes were completely absent. Fig. 4 represents the ion-extracted chromatograms at m/z 93, a typical fragment ion of terpenes, before (A) and after (B) overnight stabilization.

That 'rough' handling of plant material can increase volatile terpene emissions has already been reported [23]. It is also known that plants are highly

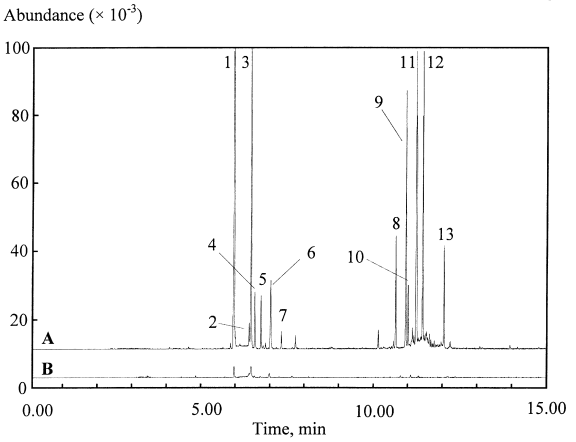

Fig. 4. Extracted ion chromatogram (m/z 93) from the headspace profiles of *Hedera helix*. (A) Profile obtained immediately after enclosing the plant in the bulb and (B) after overnight stabilization under a flow of 50 ml/min of dry air. No purge was applied. For peak identification see Table 3.

sensitive to any form of contact or movement so that pre-analysis stabilization is utmostly important, not solely when terpenes are of interest. For the same reason the applied airflow was restricted to 1 bulb volume per hour. Compounds are enlisted in Table 3 and were identified using mass spectral fragmentation in combination with retention data [24].

Since both plants seemed sufficiently stabilized, the target plant was ready to be stressed. Therefore, wounds were inflicted by means of long endoscopic pliers (Fig. 1(17)), which entered the glass bulb through a glass screw-thread connection (Duran GL 45, Schott Glas), equipped with a plastic nut and a thick PTFE lined septum (Fig. 1(18)). Each leaf was pressed several times in-between the jaws of the pliers. Care was taken not to rupture the leaf material. Target and reference ivy were sampled consecutively, starting with the target plant during wounding.

The headspace profile of the target plant (data not shown) was characterized by a huge increase in terpene emissions of ca. 10 000-fold for β-pinene compared with the blank (Fig. 4B). At the same time, a substantial amount of carry-over of sesquiterpenes was observed in the subsequent reference run, probably arising from the 1/16″ PTFE tube, which connects valves A and C (Fig. 1). When a purge time is included into the sampling cycle, this line is flushed with the purge air and cross contamination is easily avoided. Therefore, the next analyses were carried out in the split desorption mode (1:100, valve D in --- position).

After the sharp increase, terpene emissions returned to their zero levels within a period of 5 to 8 h for the mono- and sesquiterpenes, respectively. This difference obviously originated from their respective volatilities. In the first wounded plant analysis, minor amounts of the membrane-related breakdown products *trans*-2-hexenal and *cis*-3-hexen-1-ol were also detected [24].

The next experiment dealt with the response of in vitro grown tomato plants (*Lycopersicon esculentum* Mill.) to cotton leafworm (*Spodoptera littoralis*) feeding. Plants were stabilized overnight under a constant flow of 50 ml/min of humidified N50 air.

One caterpillar was introduced into the target unit after three analyses of both containers and closely before the next analysis of the target unit. The animal was carefully taken from the petri dish by means of tweezers and placed onto a leaf. Both tweezers ends were covered with cotton plugs to avoid wound infliction. Due to overnight starvation the animal started feeding instantly. The headspace profile of both plants, consecutively obtained during the monitoring experiment, is given in Fig. 5. Headspaces were sampled 20 min at 50 ml/min. Compounds are listed in Table 4 and were identified using mass spectrometry and GC retention data [24].

Mono- and sesquiterpenes were the main components present in the headspace of both tomato

Table 3
Components identified in the headspace of *Hedera helix*

Peak no.	Compound	Peak no.	Compound
1	α-pinene	8	β-elemene
2	sabinene	9	*trans*-caryophyllene
3	β-pinene	10	γ-elemene
4	β-myrcene	11	α-humulene
5	α-phellandrene	12	germacrene D
6	β-phellandrene	13	germacrene B
7	γ-terpinene		

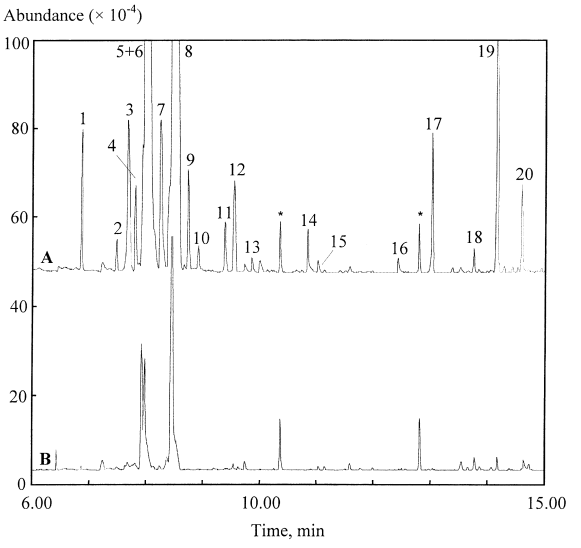

Fig. 5. Total ion chromatograms (TICs) of the headspace of *Lycopersicon esculentum* Mill. (A) Plant exposed to caterpillar (*Spodoptera littoralis*) and (B) reference plant. For peak identification see Table 4. For situating the selected TICs in the total monitoring experiment see bold data points in Fig. 6. Headspaces were sampled 20 min at 50 ml/min. Purge time was 5 min at 400 ml/min.

Table 4
Compounds identified in the headspace of *Lycopersicon esculentum* Mill

Peak no.	Compound	Peak no.	Compound
1	α-pinene	12	linalool[a]
2	*m*-cymene	13	terpinolene 2
3	*trans*-isolimonene	14	*cis*-3-hexenyl butyrate
4	β-myrcene	15	methyl salicylate
5	2-carene	16	indole[a]
6	α-phellandrene	17	δ-elemene
7	α-terpinene	18	α-copaene
8	β-phellandrene	19	*trans*-caryophyllene
9	*trans*-ocimene	20	α-humulene
10	γ-terpinene	*	cyclic siloxane degradation
11	terpinolene 1		

[a] Not detected in wounding experiment.

plants. Similar to the ivy wounding experiment, caterpillar feeding initiated the release of terpenes. A number of more specific compounds were also identified in the headspace of the stressed plant (Fig. 5A). The emission of linalool (peak 12) and indole (peak 16) is known to be directly related to insect feeding [4,26]. Methyl salicylate (peak 15) is another stress-related volatile. This widespread compound, also known as 'Oil of Wintergreen', is synthesized from salicylic acid, a non-volatile substance important for establishing local (disease) resistance. Methyl salicylate acts as an airborne defense signal to activate this resistance in healthy tissues of the plant by converting to salicylic acid upon absorption [3]. *Cis*-3-hexenyl butyrate originates from the same biosynthetic pathway as the previously mentioned membrane-related breakdown products *trans*-2-hexenal and *cis*-3-hexen-1-ol [25]. Samples of caterpillar- and excrement emissions were also taken to verify the specificity of the detected volatiles. The headspace profile of the caterpillar was free of any compound, while the headspace of caterpillar excrements mainly consisted of phenol and 2-methoxyphenol (guaiacol). Minor traces of linalool and methyl salicylate were also present, but in much lower quantities compared to the plant samples. Phenol and 2-methoxyphenol were not detected in the monitoring experiments.

Continuous sampling combined with the high separation power of GC, generated a large amount of data points. This is, however, one of the strengths of the described set-up since it allows construction of compound-specific emission profiles in function of time, as illustrated in Fig. 6. Visual representation and comparison of different emission profiles allows determination of the point in time when a certain stress response is initiated and its relation to the emission of other compounds.

Fig. 6 represents the emission profiles of some selected compounds detected in both headspaces. The profiles of indole and methyl salicylate are quite comparable. An initial increase, shortly after introducing the cotton leafworm into the system was followed by a five-fold rise ca. 10 h later. Afterwards, the methyl salicylate profile was characterized by a somewhat steady state, while the amount of indole in the headspace continuously decreased. The emission profile of linalool greatly resembled the indole profile (Fig. 6B), but without the initial ascent. α-Pinene and *trans*-caryophyllene emissions were chosen as representative for mono- and sesquiterpene emissions. Both profiles for the target plant were characterized by a number of emission maximums. The increase in the α-pinene profile observed in the reference unit is most probably related to the day/night cycle and was also observed for other monoterpenes.

The specificity of the volatile emissions towards cotton leafworm feeding was determined by carrying out an in vitro wounding experiment (see ivy experiment for details). All terpenes had emission profiles comparable to the ivy experiment. A 150 and 400-fold increase for α-pinene and *trans*-caryophyllene, respectively, during wounding, followed by a steady decrease. Consequently, the different maximums obtained in both terpene profiles in Fig. 6 (C and D)

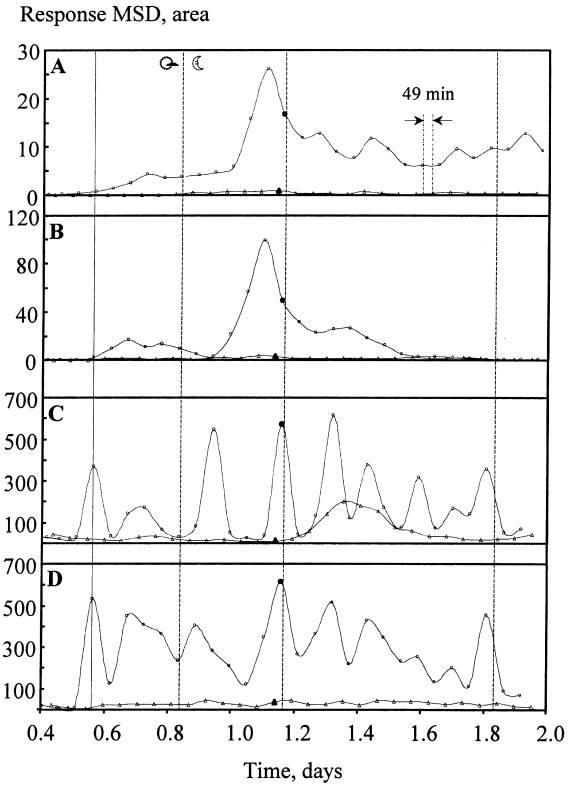

Fig. 6. Emission profiles of (A) methyl salicylate at *m*/*z* 120, (B) indole at *m*/*z* 117, (C) α-pinene and (D) *trans*-caryophyllene, both at *m*/*z* 93. Full line denotes the introduction of *Spodoptera littoralis*, dashed lines indicate the light/dark transition. Bold data points refer to the TICs in Fig. 5.

can be related to the caterpillar feeding cycle. With the exception of linalool and indole all other volatiles were also detected in this wounding experiment.

Total cycle time, i.e. from analysis to analysis, was in this application 49 min. This time consisted of 20 min sampling and 5 min purge. Thermodesorption itself takes approx. 9 min to complete. At the GC start signal, the bulb selection valve (Fig. 1, valve B) is turned into the opposite position. To avoid carry-over, the PTFE line, connecting valves B and C, is flushed with headspace of the new bulb for an additional 15 min.

In the final experiment, two jasmine plants were enclosed inside the 9.4 L bulbs and flushed overnight with humidified air at 100 ml/min (=1 bulb volume/h). The next day, two 500 ml samples were taken from each plant. The headspace composition was similar as described in previous work with benzyl acetate as main component [15]. Afterwards, one bulb was completely covered with aluminum foil to prevent light from reaching the plant. The benzyl acetate emission of both plants was monitored every 6 h during a total period of 4 days. The emission trend is shown in Fig. 7.

The emission profile for both jasmine plants is characterized by an overall decreasing trend that is independent of the day/night regime and proved as such the usefulness of incorporating a reference unit. This decreasing trend is probably caused by a reduced number of emitting flowers as function of time. During the light period, the reference plant showed, however, a small but clear increase in emission, which is absent in the dark-held plant. This deviation from the declining trend is also observed the next day as well as the last day, though to a lesser extent. All increases are marked in Fig. 7 by vertical arrows. Other emittants were characterized by an overall decreasing trend in both target and reference plant. Emissions were quantified by injection of a hexane solution of benzyl acetate directly onto the PDMS material, followed by the usual desorption procedure.

In a final experiment, the adsorptive properties of the sampling bulb were evaluated. Therefore, gaseous benzyl acetate, spiked into the N50 air, was fed towards the 3.5 L bulb. The spiked airflow was generated in situ using a simple procedure described previously [27]. Headspace samples (10 min at 50 ml/min) were taken automatically every 30 min. System equilibration took approx. 3 h to complete. Afterwards, the bulb was removed from the sampling line and analyses restarted. In these experiments, the response of benzyl acetate did not change significantly compared to sampling with the bulb included. Therefore, the influence of bulb adsorption on benzyl acetate recovery was considered negligible.

4. Conclusion

The instrumental set-up for automated on-line monitoring of biogenic emissions presented in this paper has proven to be very flexible and versatile. The use of a reference unit can compensate for external parameters such as temperature, relative

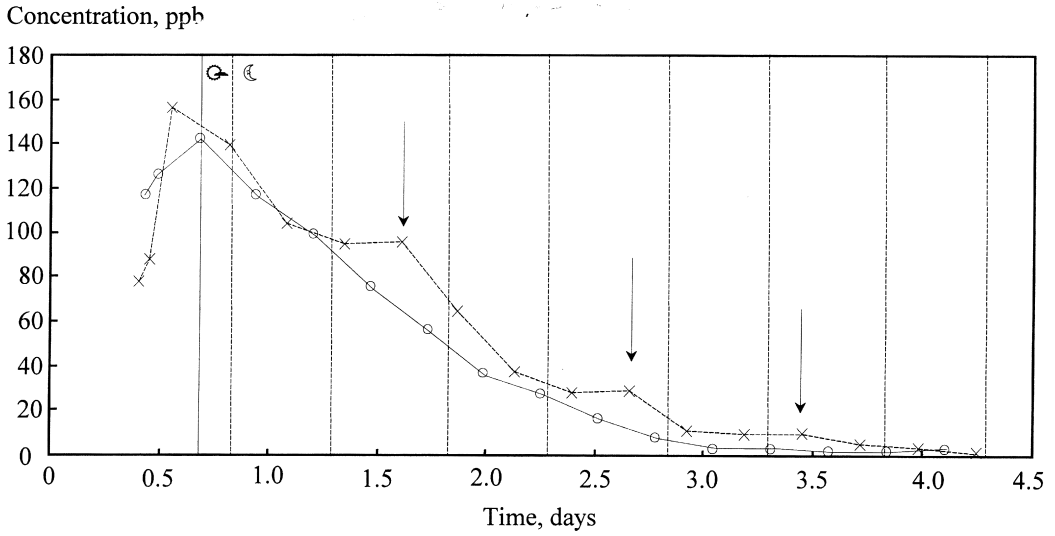

Fig. 7. Benzyl acetate emission from *Jasminum polyanthum*. The full curve represents plant kept in dark. The full line indicates the moment of covering of target plant with aluminum foil. The dashed lines indicate the light–dark transition. For quantitation details see text.

humidity and light strength. System operation and chromatographic performance were strongly influenced by the unavoidable presence of water, which demanded the use of post-sampling purging with dry air. The use of sorption-based enrichment and the hydrophobic PDMS was especially useful in this respect. Three applications have been shown that fully demonstrate the possibilities of the presented set-up.

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