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Short communication

Does the novel fast-GC coupled with PTR-TOF-MS allow a significant advancement in detecting VOC emissions from plants?

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ABSTRACT

Most plants produce and emit a wide blend of biogenic volatile organic compounds (BVOCs). Among them, many isoprenoids exhibit a high atmospheric reactivity toward OH radicals and ozone. In the last few years, Proton Transfer Reaction–Mass Spectrometry (PTR–MS) has been widely used in both field and laboratory determination of BVOCs, complementing the traditional methods using gas chromatography–mass spectrometry (GC–MS) for their identification in air and emission sources. This technical note reports a number of experiments carried out with a PTR- (Time-of-Flight) TOF-MS equipped with a prototype fast-GC system, allowing a fast separation of those isobaric isoprenoid compounds that cannot be identified by a direct PTR-TOF-MS analysis. The potential of this fast-GC system to adequately complement the information provided by PTR-TOF-MS was investigated by using the BVOC emissions of *Quercus ilex* and *Eucalyptus camaldulensis* as reliable testing systems, due to the different blend of isoprenoid compounds emitted and the different dependence of their emission from environmental parameters. While the oak species is a strong monoterpene emitter, the eucalyptus used is one of the few plant species emitting both isoprene and monoterpenes. The performances provided by the type of fast-GC used in the new PTR-TOF-MS instrument were also compared with those afforded by conventional GC–MS methods. The results obtained in this investigation showed that this new instrument is indeed a quick and handy tool to determine the contribution of isoprene and eucalyptol to m/z 69.070 and monoterpenes and (*Z*)-3-hexenal to m/z 81.070, integrating well the on-line information provided by PTR-TOF-MS. However, some limitations emerged in the instrument as compared to traditional GC–MS, which can only be solved by implementing the injection and separation processes.

1. Introduction

Terrestrial plants produce and emit a wide variety of BVOCs, representing an important input of carbon into the atmosphere (Guenther et al., 1995). In unperturbed leaves, isoprenoids (isoprene, monoterpenes and sesquiterpenes) are the most abundant BVOCs emitted by terrestrial plants (Guenther et al., 1995). Their emission seems to have a specific ecological role. While isoprene can protect leaves from abiotic stresses (Loreto and Schnitzler, 2010; Sharkey and Loreto, 1993; Sharkey et al., 2008; Sharkey and Singsaas, 1995), and can stimulate flowering processes (Terry et al., 1995), mono- and sesquiterpenes can attract pollinators, and protect plants against insects and animals (Harborne, 1991; Brilli et al., 2009). Since many isoprenoids are much more reactive with ozone and OH radicals than the majority of VOCs released by man-made activities (AVOCs) (Fuentes et al., 2000), and their emission at a global scale largely exceeds that of AVOCs (Guenther et al., 1995, Fuentes et al., 2000), they can considerably affect the earth climate. They can produce ozone acting as a greenhouse gas, and secondary organic aerosols (SOA) altering the albedo of the earth by acting as cloud condensation nuclei (CCN) (Andreae and Crutzen, 1997). The BVOC emission from *Quercus ilex* has been extensively studied in the last three decades because it is the only abundant oak species present in the Mediterranean area (Pausas et al., 2008) emitting monoterpenes at a high rate with

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the same light and temperature dependent mechanism of isoprene emitting oaks (Staudt and Seufert, 1995; Loreto et al., 1996a,b). Some plant species of the *Eucalyptus* genus are also quite common in Mediterranean areas, and most of them display the unique feature of acting as isoprene and monoterpene emitters through two different emission mechanisms (Guenther et al., 1991; Winters et al., 2009). Due to the exponential dependence of isoprenoid emission from leaf temperature (Tingey et al., 1980; Guenther et al., 1995), it is important to assess how global warming can enhance the isoprenoid emission in the Mediterranean area (Peñuelas and Staudt, 2010), which is one of hottest spots of photochemical pollution on the Earth. Since the potential to generate ozone and secondary organic aerosols is strictly related to the amounts and type of isoprenoids released by terrestrial plants, they need to be identified and quantified on an individual basis (Fuentes et al., 2000).

In recent years, many attempts have been made to quantify the BVOC emission of terrestrial plants by PTR–MS because it allows their real-time determination at low atmospheric levels (ppbv–pttv) (Aprea et al., 2006; Lindinger et al., 1998; Kim et al., 2009; Lusini et al., 2014; Müller et al., 2006; Pallozzi et al., 2013). Due to the low resolving power afforded by quadrupole filters, PTR–MS instruments using this ion separation method (indicated here as PTR-QMS) were unable to distinguish the $(M+1)^+$ ions generated by the reaction of protons with compounds having the same nominal mass. In particular, the interferences on the isoprenoid signals generated by other compounds determined that the quantification of isoprene and total monoterpenes was affected by the content of interfering compounds present in the sample (Warneke et al., 2003; De Gouw et al., 2003). To reduce the uncertainty and extend the real-time detection to isoprenoid with a higher molecular mass (sesquiterpenes), PTR–MS instruments have been recently equipped with time-of-flight ion filters, able to separate $(M+1)^+$ ions (or their fragments) generated by compounds differing by 0.01 Da in their exact mass (Graus et al., 2010). These instruments have been used for the real-time determination of BVOCs in a number of environmental samples (Kaser et al., 2013; Brilli et al., 2014). Since many mono- and sesquiterpenes have the same exact molecular mass, there was no possibility to obtain information on their composition in plant emission by PTR-TOF-MS and conventional methods based on GC–MS were still needed to accomplish this specific task. GC–MS methods do not have, however, a real-time capability, and often require a substantial enrichment of the sample to identify and quantify all isobaric isoprenoids present in it (Ciccioli et al., 2002). Depending upon the emission rates, volumes from 0.5 to 5 L of air are often necessary to get an accurate quantification of the whole spectrum of BVOCs emitted from plant leaves. High volumes are needed because the branch enclosures containing the vegetation must be flushed with relatively high flow rates of external air to keep the leaves under the same physiological conditions experienced by those located in the ambient (Niinemets et al., 2011). The enrichment of BVOCs is usually performed on adsorption traps able to completely retain all BVOCs up to a maximum volume of ca. 5 L, and to quantitatively release them by thermal desorption (Ciccioli et al., 2002). With this sampling approach, an additional enrichment step is required to fully exploit the resolving power of capillary GC for the analysis of isoprenoids. In order to limit an excessive spreading of the sample during the injection step, compounds released from the adsorption trap need to be further concentrated in a smaller volume before the injection. This is usually done with a cryofocusing process performed on a very short capillary tube having a total volume of few μL (Ciccioli et al., 2002). If the tube is empty, the cryofocusing step is performed at temperatures equal or lower than −150 °C. The use of cryogenic liquids can be avoided if the capillary tube is filled with small amounts of solid sorbents, as the bulk of BVOCs can retained at a temperature (−30 °C) that can be easily reached by a Peltier cooler. A fast injection of BVOCs into the GC capillary column is obtained by a ballistic heating (>15 °C min^{-1}) of the cooled tube where compounds were concentrated, and by keeping the initial temperature of the column as lower as possible. To better separate low molecular weight compounds, the column can be operated at sub-ambient temperatures (Ciccioli et al., 2002). All these steps make the GC–MS determination of BVOCs rather expensive and time consuming. By considering that the maximum flow rate that can be passed through the adsorption trap is ca. 200 mL min^{-1}, the sampling time required for a complete analysis of BVOCs can span from 3 to 25 min, as a function of the emission rate of the plant investigated and the amount of biomass contained in the enclosure. The analysis time is even longer, as it can go from less than 30 min up to more than 1 h as a function of the complexity of the sample to analyze, and the amount and polarity of the liquid coating covering the internal walls of the capillary column (Ciccioli et al., 2002). Although the recent introduction of fast-GC has allowed to drastically reduce the analysis time, some limits still exist to the fastest temperature gradient that can be applied to a capillary column, because a sufficient resolution must be maintained to separate the numerous isobaric isoprenoids, such as mono- and sesquiterpenes, emitted by plants. Based on the data obtained by Jones et al. (2014), an analysis time of 13 min is still required to get enough resolution to identify and quantify most of the isobaric BVOCs by fast-GC. The complementary features of PTR–MS and GC–MS implies that both of them are needed to investigate the emission behavior of BVOCs from terrestrial plants, in the laboratory and in the field. While the former technique provides a real-time determination of the cumulative content of isobaric BVOCs in plant emission (Brilli et al., 2014; Grabmer et al., 2006), the latter allows to identify and quantify each one of them (Ciccioli et al., 2002)

In the attempt to combine these two complementary features in one instrument, a prototype system has been developed where a fast-GC system has been coupled to a PTR-TOF-MS. In principle, this system is very promising, because the same detection system is used for real-time and discontinuous GC determinations of BVOC, by sending the sample directly to the PTR-TOF-MS or by injecting a fraction of it into a fast-GC system. By using a rather short column coated with a low polar phase, and by applying a very fast temperature gradient, the analysis of VOC mixtures can be performed in less than a minute. However, the capabilities of this system to perform an accurate determination of isoprenoids emitted from terrestrial plants needs still to be tested. Since no sample enrichment is performed, the sensitivity can be lower than that of conventional GC–MS systems used for BVOC determination. It is not clear also if and how the drastic reduction in the sampling and analysis time compromises the column resolution.

To clarify these aspects, the performances of the prototype system combining on-line PTR-TOF-MS detection with a fast-GC were evaluated by using *Q. ilex* L. and *Eucalyptus camaldulensis* as testing systems. These species were selected because they show not only a marked difference in the amount and composition of emitted isoprenoids, especially the isobaric ones, but are also characterized by a different dependence of their emission from temperature.

2. Materials and methods

2.1. Plant material

Three-year-old *Q. ilex* saplings were potted into 10 dm^3 pots containing commercial soil. All saplings were grown outdoor in Monterotondo Scalo (RM), Italy (42°06′27.9″N 12°38′17.6″E) under natural sunlight conditions, regularly watered to pot water capacity and fertilized once a week. Two-year-old *E. camaldulensis* saplings

were instead propagated from mature trees and grown in 10 dm^3 pots, filled with a mixture of 50% commercial soil and 50% sand.

2.2. Branch enclosure

Isoprenoids emissions were assessed using a branch enclosure of 1.7 L equipped with light, humidity and temperature sensors. The vegetation in the enclosure included always at least 10 fully developed leaves. The internal temperature of the enclosure, made by transparent glass, was controlled by circulating water coming from a thermostatic bath in a chamber surrounding the enclosure. The branch enclosure was flushed with 1.5 L min^{-1} of VOC-free, artificial air containing 400 ppm of CO_2. The constancy of the air flow and air composition was obtained using Brooks 5850 series E mass flow controllers supplied by Brooks Instrument (Veenendaal, Netherlands). The emission was measured under an incident flux of the photosynthetic active radiation (PAR) of 800 μmol m^{-2} s^{-1} generated by an OSRAM Power Star lamp (OSRAM, Milano, Italy). The desired PAR flux was reached by regulating the distance between the lamp and the branch enclosure. During the experiments, the relative humidity was maintained between 45 and 60% by condensing the excess of water vapor in a glass coil immerged in a water bath. The gas exchange rates of the enclosed biomass were controlled with a LI-7000 IRGA (LI-COR, Lincoln, NE, USA) to assess the physiological status of plant leaves. Their values were stored on a CR23X Micrologger (Campbell Scientific Inc.) with a frequency of 1 Hz. After the experiments, the leaves were detached from the branch, dried at 70 °C for 48 h and their dry weight was measured to get normalized values of the emission from the different experiments performed.

2.3. GC–MS determinations

BVOCs were sampled by deviating a part of the gas flow from the outlet of the branch enclosure into an adsorption trap made of a coated steel tube 8.9 cm long, and with an external diameter of 5 mm, filled with 200 mg of Tenax GC®. Traps were supplied by Markes International Ltd (Llantrisant, UK). Sampling was performed with a VSS-1 aspirating pump (A.P. Buck Inc., Orlando, FL, USA) ensuring a constant flow of 200 mL min^{-1} through the trap during sampling. Volumes of 5 and 3 L of the emission produced by oak and eucalyptus species, respectively, were collected. In all cases, sampling started when the vegetation was fully adapted to the enclosure conditions. This usually happened after 20 min the branch was placed inside the enclosure. Adsorption traps collected during the various experiments were thermally desorbed on a Unity desorption system from Markes International Ltd. (Llantrisant, UK), equipped with a cryofocusing unit using a Peltier system to cool at −30 °C the capillary tube, filled with small amounts of Tenax GC®, to retain BVOCs released from the adsorption traps. The fast injection of BVOCs into the capillary column was performed by a ballistic heating of the capillary tube. The GC–MS analysis was performed on a GCMS MSD 5975C system supplied by Agilent Technologies (Wilmington, USA), equipped with a 30 m long MS-5HP capillary column (J&W Scientific USA, Agilent Technologies, Palo Alto, CA, USA) with an inner diameter of 0.25 mm. The initial column temperature was maintained at 35 °C for 5 min after the sample injection, and then raised at a rate of 4 °C min^{-1} up to 250 °C. The calibration of the GC–MS system was performed by injecting 1 μL of methanol solutions of BVOCs into the adsorption traps kept under a flow rate He of 200 mL min^{-1}. The bulk of the solvent was eliminated by keeping the trap under the same flow rate of He for 1–2 min after the injection. Highly pure α-pinene, β-pinene, sabinene, myrcene, D-limonene and β-ocimene were used to make the liquid standard solutions, and they were all supplied by Sigma Aldrich (St. Louis, MI, USA). GC–MS chromatograms collected in the total-ion mode from m/z 20 to 250 were stored on a computer and later processed with the Enhanced ChemStation (Agilent Technologies Inc.,Wilmington, USA) supplied with the GC–MS system. Before any experiment, the content of VOCs in the empty enclosure was determined by collecting 5 L of air to detect any possible contamination by the sampling system.

2.4. PTR-TOF-MS determinations

The real time detection of isoprenoids emitted by *Q. ilex* and *E. camaldulensis* leaves was accomplished by sending 100 mL min^{-1} of the total outflow from the branch enclosure to the heated inlet (50 °C) of the PTR-TOF system. The apparatus used was a PTR-TOF 8000 model supplied by Ionicon Analytik (Innsbruck, Austria). The instruments was able to distinguish ions with a difference of m/z of 0.01 (Jordan et al., 2009). All determinations were performed by keeping the drifting tube at a pressure of 2.22 mbar, the voltage at 600 V and the temperature at 60 °C. Under these conditions a ionization energy (E/N) of ~1.34×10^{-15} cm^2 V^{-1} s^{-1} was generated. All protonated ions, that were extracted from the drifting tube every 30 μs, were separated in the TOF region accordingly and detected on a multi-channel-plate (MCP, Burle Industries Inc., Lancaster, PA, USA). Raw data acquired at a frequency of 2 Hz by the TofDaq software (Tofwerk AG, Switzerland) were first merged in 10 min files, and then re-processed according to the procedure proposed by Müller et al. (2013) for the ion counting correction, the accurate mass scale calibration, the peak identification and the peak area quantification. This was done on the 4.30 version of the PTR-TOF Data Analyzer software. In the low mass range the accuracy of the scale was checked using three ions always present in the background of PTR-TOF-MS spectra. Generated by the H_3O^{18+}, NO^+ and H_2O–H_3O^{18+} ions, they have a m/z value of 21.022, 29.998 and 39.033, respectively. In the high mass range, the $(M+1)^+$ ion with m/z = 330.848 generated by 1,3-diiodo-benzene, and by its most intense fragment with m/z = 203.944 were used for calibrating the ion masses. This compound was continuously sent to the PTR-TOF-MS by a diffusion system. The quantification of isoprenoids was performed by using a calibrated gas cylinder containing known levels of different isoprenoids. In the calibrated cylinder, that was provided by Apel Riemer Environmental Inc. (Broomfield, CO, USA), the compounds were diluted into VOC-free air.

2.5. Design and features of the fast-GC system coupled with PTR-TOF-MS

The built-in, fast-GC developed by Ionicon Analytik (Innsbruck, Austria) was coupled to a PTR-TOF-MS 8000 system with the specific aim to get information on the organic components producing protonated ions that were not separated by the ion filter. In this study, specific focus was addressed to assess the potentiality of the fast-GC system to provide information on the individual content of monoterpenes with an elemental composition of $C_{10}H_{16}$, because they are by far the most abundant compounds emitted by many plant species, and produce the same protonated molecular ion at m/z = 137.133 by reaction with protons. The sampling unit of the fast-GC system was composed by a calibrated loop made by a 55 cm PEEK capillary tube having an internal diameter of 1 mm, and a total volume of ca. 0.45 μL. When the prototype system was used for real-time determinations of BVOCs, the sampling unit of the fast-GC system was isolated from the fast-GC column and the PTR-TOF-MS detection system. The capillary column was maintained under a flow of 2 mL min^{-1} of pure N_2, and the exiting gas mixed with the air entering the PTR-TOF-MS. The first phase of the fast-GC determination, was to fill the sample loop with the air sample to analyze. This task was accomplished by switching a set of three-way valves

inserted in the gas lines of the fast-GC and PTR-TOF-MS detection units. Once the sample was equilibrated, valves were switched in order to connect the sample loop in series with the fast-GC column, and inject the sample into it. In this phase only N_2 was sent to the PTR-TOF-MS detector and separated compounds emerging from the column were detected as peaks. Details on the way injection and detection of VOCs was performed in the fast-GC connected to the PTR-TOF-MS system can be found in Romano et al. (2014). The separation of VOCs was performed on a 6 m Silcosteel®-lined capillary column (0.25 mm I.D.) internally coated with MXT®-1, supplied by Restek Corporation (Bellefonte, PA, USA). The liquid phase, consisting of a 100% dimethyl polysiloxane, was specifically selected to perform fast-GC analysis in combination with MS detection, because it produces a rather low bleeding of the liquid phase up to a temperature of 350 °C. Although the non-polar nature of the liquid phase was particularly suitable to separate non-polar isobaric compounds such as monoterpenes, the column resolution R afforded by the fast-GC was strongly limited by the short length of the column and by the way the sample was injected into the column. This parameter, that measures the ability of a GC column to separate two adjacent peaks (indicated here as *A* and *B*) under isothermal conditions, can be determined with the following equation:

$$R = \frac{2\,[\mathrm{d}R(B) - \mathrm{d}R(A)]}{[w(B) + w(A)]]}$$

where R is the column resolution, $\mathrm{d}R(A)$ and $\mathrm{d}R(B)$ are the differences in retention between A and B, and $w(A)$ and $w(B)$ are the relative widths of each peak at its base. In addition to the polarity of the liquid phase and a factor depending on the retention, the other determining factor affecting the value of R in a GC column is the number of theoretical plates, N, which is proportional to the column length in columns having the same internal diameter. Since the resolution increases with the square root of N (Ciccioli et al., 2002), the resolution of the fast-GC column was estimated to be ca. 0.45 times lower than that afforded by the 30 m capillary column used for the analysis of BVOCs by GC–MS. By considering that the sample injected (0.47 mL) was ca. 10% of the void volume of the fast-GC column and no cryofocusing was performed, an additional reduction of R was expected to come from the broadening of the GC band during the injection step. This would have hindered the separation of those monoterpenes that are only partly separated on the 30 m capillary column used in GC–MS determinations. In this respect, the BVOC emission of *Q. ilex* represented a very good test to assess the suitability of the fast-GC system to provide speciated values of monoterpenes generating ions with $m/z = 137.133$ by proton transfer, because it contains sabinene and β-pinene, that are only partly resolved by high efficient capillary columns (Ciccioli et al., 2002). To get the maximum resolution, the column was operated at the lowest initial temperature afforded by the fast-GC system (35 °C) and maintained in these conditions for 30 s. After that, the column temperature was increased first up to 70 °C at a rather slow rate of 15 °C min^{-1}, and then up to 150 °C at a rate of 250 °C. This temperature program provided the best resolution for the separation of mixtures containing α-pinene, sabinene, β-pinene, myrcene, D-limonene and *cis*-β-ocimene, that are the among the most abundant monoterpenes released by many plant species present in the Mediterranean area (Kemper Pacheco et al., 2014). The results displayed in Fig. 1 were obtained by minimizing the effect caused by an excessive spreading of the GC band during the injection. After various attempts, an injection time of 2 s was found to give the highest intensity of the GC signal in the PTR–MS-TOF detector, with the lowest values of the band width measured at half height. PTR-TOF-MS spectra of all ions from $m/z = 1$ to 340 were collected at a frequency of 2 Hz to detect all peaks eluted by the fast-GC column. The system was calibrated by a dynamic

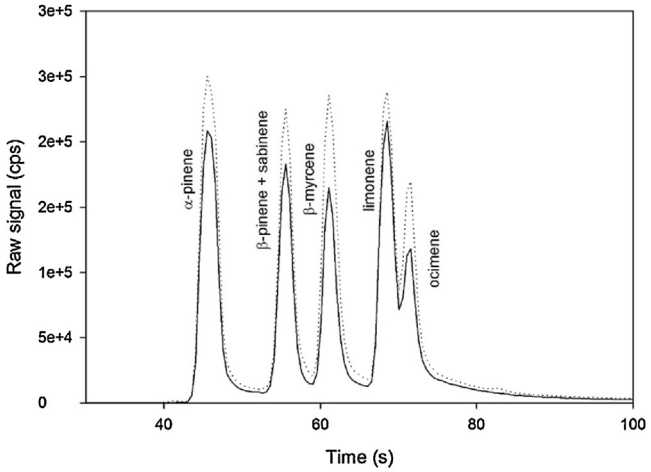

Fig. 1. Mixture of monoterpenes analyzed by fast-GC-PTR-TOF-MS. Dotted line represents m/z 137.133 (monoterpene parent ion), continuous line represents m/z 81.070 (monoterpene fragment). This graph represents one out of five replicates performed with the same mixture.

dilution of the same standard mixture contained in the gas cylinder described in Section 2.3. As shown in Fig. 1, the selective detection of monoterpenes having an exact mass of 136.125201 g mol^{-1} with PTR-TOF-MS was achieved by selecting the two ions produced by the reaction of these compounds with protons. The first was the protonated molecular ion recorded at m/z values of 137.133, the second was the ion fragment at m/z 81.070 (Tani et al., 2003). This fragment, corresponding to a $C_6H_9^+$ ion (Lee et al., 2006; Kaser et al., 2013), is so specific of $C_{10}H_{16}$ monoterpenes, that has been often used to identify these compounds with PTR-QMS.

2.6. Experimental setup

The temperature dependence of BVOC emission from *Q. ilex* and *E. camaldulensis* was determined by setting the temperature of the leaves in the branch enclosure at 25, 30, 35 and 40 °C. At each temperature step, an adaptation period of 20 min was used to allow the leaves to adjust to the new conditions. A further experiment was performed at 60 °C on both species. BVOC determinations by PTR-TOF-MS started 10 min after the steady-state in the physiological activity was reached, and averaged values of the emission were determined. While several fast-GC runs were performed after a series of real time determinations, adsorption traps for GC–MS determinations were collected only at the end of each temperature step. The whole experiment was performed in middle September.

3. Results and discussion

The ion profiles shown in Fig. 1 were quite consistent in suggesting the co-elution of β-pinene and sabinene on the fast-GC column. This was confirmed by the analysis performed with different monoterpene mixtures. While data displayed in Fig. 2a and b show that mixtures containing α-pinene (used as reference peak) with sabinene or with β-pinene produced both two distinct peaks (Fig. 2a), the one obtained by mixing β-pinene and sabinene produced only one broad peak (Fig. 2c). These experiments indicated that the short length of the fast-GC column, combined with its high initial temperature and the lack of a cryofocusing unit to limit an excessive spreading of the injection band, caused a drastic reduction in the maximum resolution reached by the fast-GC system. This is quite evident when compared to that of 30 m capillary columns normally used for GC–MS determinations, where these compounds originate two distinct peaks with a 50% overlap

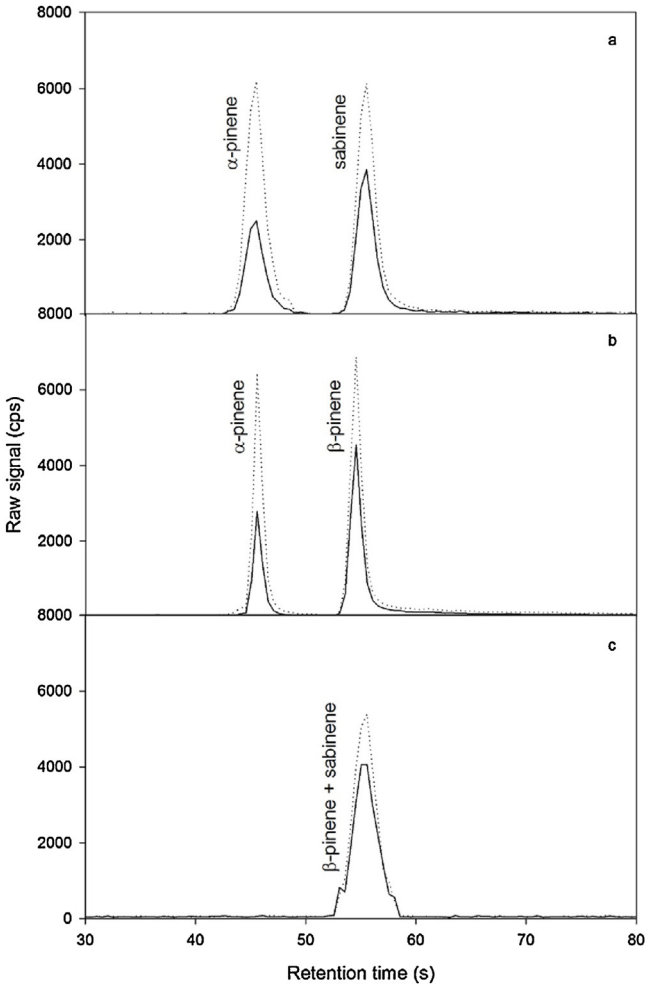

Fig. 2. Fast-GC-PTR-TOF-MS chromatograph of different calibration mixture containing: α-pinene + sabinene (a), α-pinene + β-pinene (b) and β-pinene + sabinene (c). This graph represents one out of five replicates performed with the same mixtures. Dotted line represents *m/z* 137.133 (monoterpene parent ion), continuous line represents *m/z* 81.070 (monoterpene fragment).

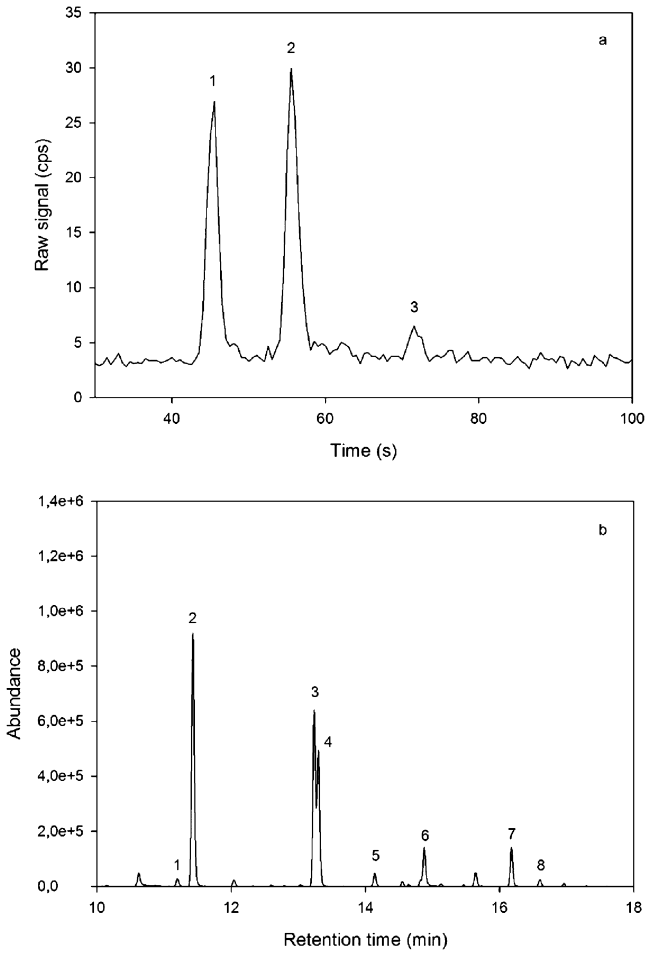

Fig. 3. Fast-GC/PTR-TOF-MS (a) and GC–MS (b) 25 °C chromatogram of monoterpene emissions in *Q. ilex*. Numbers are referred to: (1) α-pinene, (2) sabinene + β-pinene, (3) *cis* + *trans*-β-ocimene for fast-GC/PTR-TOF-MS (a) and (1) α-thujene, (2) α-pinene, (3) sabinene, (4) β-pinene, (5) β-myrcene, (6) limonene, (7) *cis*-β-ocimene, (8) *trans*-β-ocimene for GC–MS (b). Only the portion of the chromatograms with the compounds of interest has been reported. Peaks not numbered are related to compounds released by the column or the Tenax in the adsorbing cartridges. The graphs represent one out of five different experiments performed.

(Ciccioli et al., 2002). The limited resolution reached by the column was also confirmed by the partial separation of D-limonene and *cis*-β-ocimene (30% overlap) that are usually clearly separated on longer capillary columns.

In spite of these limitations, the whole GC run was, however, extremely fast, even when compared to the data reported in the recent literature (Jones et al., 2014). By considering the small volume injected and the low resolution reached by the column, the sensitivity was satisfactory, as it allowed to produce a well detectable monoterpene peak with $S/N = 4$ at concentrations of 68.15 $\mu g\,m^{-3}$ (corresponding to 12.2 ppbv) in the scan mode. The minimum detected amount was thus 32 pg of monoterpene, against a value of 2–3 ng that is usually seen by a GC–MS system operated in the same scan mode (Ciccioli et al., 2002). According to the values reported by Kemper Pacheco et al. (2014), the basal monoterpene emission (E°) of *Q. ilex* at 30 °C and 1000 $\mu mol\,m^{-2}\,s^{-1}$ of PAR is 20 $\mu g\,g_{DW}\,h^{-1}$, this means that 1 g of biomass enclosed in a branch enclosure of 1 L flushed with a 1.5 $L\,min^{-1}$ of air should generate a total monoterpene concentration of 222 $\mu g\,m^{-3}$. Since the mean relative content of α-pinene in the emission is 32.3%, the concentration produced (72 $\mu g\,m^{-3}$) was too close to the detection limit for a reliable testing. By considering that, the E° value of this evergreen oak undergoes very strong seasonal variations and reaches its maximum value only in July and August (Kemper Pacheco et al., 2014), while our measurements were performed in middle September, starting at 25 °C and with a PAR of 800 $\mu mol\,m^{-2}\,s^{-1}$, using about 10 g of leaf biomass. The GC profiles of $C_{10}H_{16}$ monoterpenes obtained at 25 °C with the two independent methods are displayed in Fig. 3a and b, and the emission values observed by GC–MS, fast-GC and real-time PTR-TOF-MS are reported in Table 1.

A comparison between the data shows that total monoterpene concentrations obtained by real-time PTR-TOF-MS are fully consistent with those obtained by GC–MS. Also consistent with GC–MS are the concentrations of α-pinene and the sum of co-eluted sabinene and β-pinene determined by fast-GC. The difference in the concentrations measured by fast-GC and real-time determinations can be due to the minor compounds which were indeed determined by GC–MS. Both methods indicate a mean monoterpenes emission rate for *Q. ilex* considerably lower than the maximum basal one for this species, which can be mainly attributed to the seasonality effect (Kemper Pacheco et al., 2014). Seasonality effects also accounted for the slightly different composition determined in our experiments as compared to those reported in the literature (Kemper Pacheco et al., 2014). This effect is particularly evident in the case of sabinene that accounts for a fraction of total monoterpenes three times higher than that measured in the hottest months of the year (Kemper Pacheco et al., 2014).

Table 1
Concentration in $\mu g\,m^{-3}$ produced by 1 g (dry weight) of *Q. ilex* leaves measured by GC–MS and fast-GC/PTR-TOF-MS in a branch enclosure kept at 25 °C and with PAR of 800 $\mu mol\,m^{-2}\,s^{-1}$. Averages and standard errors were calculated on 5 replicates.

Enclosure temperature	$T = 25$ °C		
Type of analysis	GC–MS		PTR-TOF-MS
Compounds determined	($\mu g\,m^{-3}\,g_{DW}^{-1}$)	Fast-GC ($\mu g\,m^{-3}\,g_{DW}^{-1}$)	Real-time detection ($\mu g\,m^{-3}\,g_{DW}^{-1}$)
α-Pinene	35.11 ± 2.06	34.07 ± 1.90	
Sabinene	24.61 ± 2.22		
β-pinene	19.32 ± 1.12		
Myrcene	1.68 ± 0.12		
D-limonene	2.47 ± 0.17		
Cis-β-ocimene	5.03 ± 0.32		
Trans-β-ocimene	0.05 ± 0.01		
Sabinene + β-pinene	43.93 ± 3.34	42.60 ± 2.10	
Total of $C_{10}H_{16}$ Monoterpenes	88.27 ± 6.01	76.67 ± 4.00	85.15 ± 4.51
Total of components with $C > 10$ $\mu g\,m^{-3}\,g_{DW}^{-1}$	79.04 ± 5.39	76.67 ± 4.00	
Total of components with $C < 10$ $\mu g\,m^{-3}\,g_{DW}^{-1}$	9.23 ± 0.62		
Total emission of $C_{10}H_{16}$ monoterpenes ($\mu g\,g_{DW}^{-1}\,h^{-1}$)	7.94 ± 0.54	6.90 ± 0.36	7.66 ± 0.14

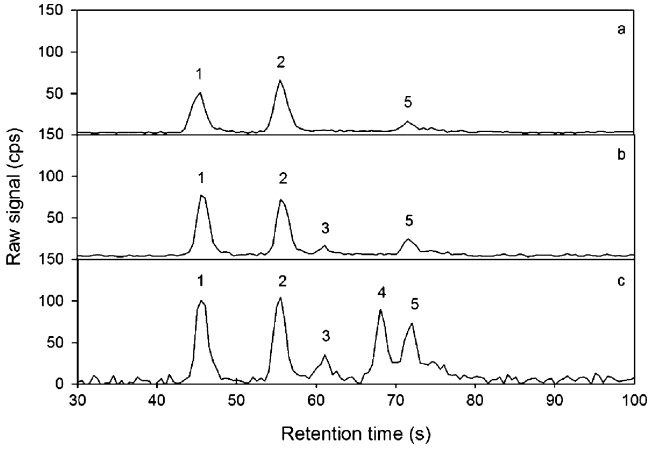

Fig. 4. Fast-GC-PTR-TOF-MS chromatograms of monoterpene emissions in *Q. ilex* at 30, 35 and 40 °C. Numbers are referred to: (1) α-pinene, (2) sabinene + β-pinene, (3) myrcene, (4) limonene, (5) *cis* + *trans*-β-ocimene. Only the portion of the chromatograms with the compounds of interest has been reported. The graphs represent one out of five different experiments performed.

After having assessed the suitability of fast-GC/PTR-TOF-MS to provide quite accurate information on monoterpenes emitted at 25 °C, the system was used to detect the effect produced by rising the temperature of *Q. ilex* leaves in the enclosure. Data in Fig. 4a–c, reports the fast-GC/PTR-TOF-MS recorded at 30, 35 and 40 °C, whereas Table 2 summarizes the results obtained by real-time detection, fast-GC and GC–MS. This comparison shows that data obtained by the two independent methods provided quite consistent results up to 35 °C, with a similar increase in the emission with the increase in temperature. Data obtained at 30 and 35 °C showed the ability of the fast-GC to detect the emission of myrcene, *cis*-β-ocimene and *trans*-β-ocimene, although the peaks generated by the last two compounds showed some overlap. The results obtained at 40 °C with the two techniques followed an analogous exponential increase with the temperature, and basically confirmed the ability of the fast-GC to separate most of the monoterpenes in the emission, although the values obtained by the fast-GC and PTR-TOF-MS were ca. 10% higher than those measured by GC–MS. As shown in Table 2, this difference was mainly related to the larger amount of D-limonene in the samples measured by PTR-TOF-MS and fast-GC. Several hypotheses can be formulated to explain the observed bias. The most reasonable one is that a burst in the emission of D-limonene occurred in the first phase of the experiment, when only the fast-GC and PTR-TOF-MS were connected to the enclosure, and it decreased later when sampling was performed with the adsorption trap. We think that the emission of a compound simulating the presence of D-limonene in the fast-GC was unlikely to occur because the ratio between the two ions used for monoterpene detection was basically the same as that measured with the standard mixture. In general, the experiments performed with *Q. ilex* indicated that the fast-GC technique coupled with PTR-TOF-MS was sensitive enough to allow the detection of the main $C_{10}H_{16}$ monoterpene components present in the emission, but the column resolution was not sufficient to get the individual content of each one of them.

To further explore the capabilities afforded by the prototype system in the determination of isoprenoids emitted by terrestrial plants, the same experiments were repeated by inserting a branch of *E. camaldulensis* in the enclosure**.** The fast-GC profiles displayed in Table 3 show that a progressive increase in the $C_{10}H_{16}$ monoterpene emission occurred when the leaf temperature exceeded 35 °C, and was mainly due to α-pinene. Myrcene was also present in the emission, but its content was so small to be detected by fast-GC. Two other isoprenoid compounds, eucalyptol and isoprene, were highly emitted by this species. While the former is a monoterpene compound generating a protonated molecular ion at $m/z = 155.144$ because it has an elemental composition of $C_{10}H_{18}O$, the latter is a C_5H_8 hemiterpene compound producing a protonated molecular ion at 69.070. These two compounds were clearly detected by fast the fast-GC system coupled with PTR-TOF-MS, and their levels were also consistent with those obtained by GC–MS.

Since in real-time determinations of BVOCs by PTR-TOF-MS the amount of BVOCs emitted by plants is solely derived from the intensity of the selective ions generated by a specific compound, it is possible that other compounds present in the emission blend can somehow contribute to the measured signals, producing an error in their quantitative evaluation. To detect if fast-GC was of some help in assessing the possible interferences that could have affected the real-time signal, additional tests were performed with both plants by rising their leaf temperature up to a level (60 °C) where degradation processes start to occur. Data displayed in Fig. 5a show that when the membranes retaining eucalyptol are partly destroyed and huge amounts of this compound emitted, the fragmentation of the protonated molecular ion at $m/z = 155.144$ can be so intense to simulate the presence of isoprene in in the real-time determinations performed with PTR-TOF-MS. This is due to an ion with m/z 69.070 highly abundant during fragmentation of eucalyptol. As it can be seen from the figure, both the fast-GC peaks corresponding to isoprene and eucalyptol generate an ion at m/z 69.070. However, the one produced by the fragmentation of the protonated molecular ion of the eucalyptol is so intense that a 61% correction must be performed on the real-time PTR-TOF-MS signal recorded at m/z

Table 2
Concentration in $\mu g\, m^{-3}$ produced by 1 g (dry weight) of *Q. ilex* leaves measured GC–MS and fast-GC/PTR-TOF-MS in a branch enclosure kept at 30, 35 and 40 °C and with a PAR of 800 $\mu mol\, m^{-2}\, s^{-1}$. Averages and standard errors were calculated on 5 replicates.

Enclosure temperature	$T = 30\,°C$			$T = 35\,°C$			$T = 40\,°C$		
Type of analysis	GC–MS	PTR-TOF-MS		GC–MS	PTR-TOF-MS		GC–MS	PTR-TOF-MS	
Compounds determined	($\mu g\, m^{-3}\, g_{DW}^{-1}$)	Fast-GC ($\mu g\, m^{-3}\, g_{DW}^{-1}$)	Real-time detection ($\mu g\, m^{-3}\, g_{DW}^{-1}$)	($\mu g\, m^{-3}\, g_{DW}^{-1}$)	Fast-GC ($\mu g\, m^{-3}\, g_{DW}^{-1}$)	Real-time detection ($\mu g\, m^{-3}\, g_{DW}^{-1}$)	($\mu g\, m^{-3}\, g_{DW}^{-1}$)	Fast-GC ($\mu g\, m^{-3}\, g_{DW}^{-1}$)	Real-time detection ($\mu g\, m^{-3}\, g_{DW}^{-1}$)
α-Pinene	55.23 ± 3.71	50.20 ± 4.30		60.44 ± 5.07	72.17 ± 5.73		107.32 ± 6.56	98.50 ± 8.47	
Sabinene	41.53 ± 3.06			58.10 ± 5.58			95.50 ± 5.71		
β-pinene	33.59 ± 2.66			28.64 ± 2.71			50.50 ± 3.45		
Myrcene	3.12 ± 0.27			5.12 ± 0.54	7.20 ± 0.72		11.09 ± 0.93	28.50 ± 2.54	
D-limonene	3.91 ± 0.20			4.99 ± 0.42			7.69 ± 0.63	69.55 ± 4.72	
Cis-β-ocimene	9.21 ± 0.71	10.04 ± 0.93		29.34 ± 2.58	21.30 ± 1.95		61.75 ± 4.09	58.20 ± 3.30	
Trans-β-ocimene	1.97 ± 0.13	2.51 ± 0.27		6.35 ± 0.60	7.10 ± 0.54		14.87 ± 1.31	16.63 ± 1.44	
Sabinene + β-pinene	75.13 ± 5.72	71.10 ± 5.34		86.74 ± 8.29	64.95 ± 4.31		146.00 ± 9.16	108.35 ± 6.20	
Total of $C_{10}H_{16}$ Monoterpenes	148.56 ± 10.74	133.85 ± 10.83	139.47 ± 6.82	192.99 ± 17.49	172.72 ± 13.25	180.43 ± 10.02	348.72 ± 22.68	379.73 ± 26.67	389.87 ± 16.60
Total of components with $C > 10\, \mu g\, m^{-3}\, g_{DW}^{-1}$	130.35 ± 9.43	131.34 ± 10.56		176.52 ± 15.94	158.42 ± 12.00		341.03 ± 22.05	379.73 ± 26.67	
Total of components with $C < 10\, \mu g\, m^{-3}\, g_{DW}^{-1}$	18.21 ± 1.31	2.51 ± 0.27		16.46 ± 1.55	14.30 ± 1.26		7.69 ± 0.63		
Total emission of $C_{10}H_{16}$ Monoterpenes ($\mu g\, g_{DW}^{-1}\, h^{-1}$)	13.37 ± 0.97	12.05 ± 0.97	12.55 ± 0.61	17.37 ± 1.57	15.55 ± 1.19	16.24 ± 0.90	31.38 ± 2.04	34.18 ± 2.40	35.09 ± 1.49

Table 3
Concentration in $\mu g\,m^{-3}$ produced by 1 g (dry weight) of *E. camaldulensis* leaves measured GC–MS and fast-GC/PTR-TOF-MS in a branch enclosure kept at 25, 30, 35 and 40 °C and with a PAR of 800 $\mu mol\,m^{-2}\,s^{-1}$. Averages and standard errors were calculated on 5 replicates.

Enclosure temperature	T = 25 °C		T = 30 °C	
Type of analysis	GC–MS	PTR-TOF-MS	GC–MS	PTR-TOF-MS
Compounds determined	($\mu g\,m^{-3}\,g_{DW}^{-1}$)	Real-time detection ($\mu g\,m^{-3}\,g_{DW}^{-1}$)	($\mu g\,m^{-3}\,g_{DW}^{-1}$)	Real-time detection ($\mu g\,m^{-3}\,g_{DW}^{-1}$)
Isoprene	225.93 ± 24.63	204.98 ± 16.36	317.13 ± 28.20	305.02 ± 22.29
α-pinene	83.67 ± 7.86	80.42 ± 6.43	128.89 ± 16.16	123.82 ± 10.55
Myrcene	1.87 ± 0.31	1.69 ± 0.31	2.80 ± 0.63	2.64 ± 0.34
1,8 Cineole	9.60 ± 1.03	9.76 ± 0.63	16.42 ± 1.39	16.22 ± 1.58
Enclosure temperature	T = 35 °C		T = 40 °C	
Type of analysis	GC–MS	PTR-TOF-MS	GC–MS	PTR-TOF-MS
Compounds determined	($\mu g\,m^{-3}\,g_{DW}^{-1}$)	Real-time detection ($\mu g\,m^{-3}\,g_{DW}^{-1}$)	($\mu g\,m^{-3}\,g_{DW}^{-1}$)	Real-time detection ($\mu g\,m^{-3}\,g_{DW}^{-1}$)
Isoprene	442.67 ± 21.61	449.33 ± 14.91	562.53 ± 28.64	597.56 ± 23.82
α-pinene	176.49 ± 16.88	173.84 ± 15.04	219.71 ± 20.10	226.04 ± 20.94
Myrcene	4.27 ± 0.63	4.33 ± 0.62	6.78 ± 0.72	6.73 ± 0.63
1,8 Cineole	26.84 ± 3.06	22.42 ± 2.72	44.49 ± 3.14	36.53 ± 3.57

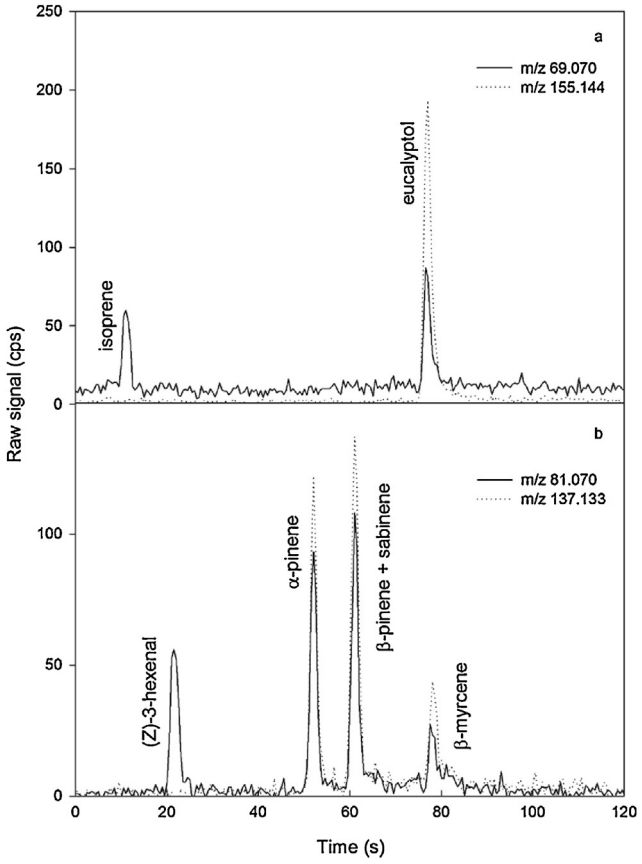

Fig. 5. Fast-GC-PTR-TOF-MS analysis of (a) *E. camaldulensis* emissions (continuous line represents *m/z* 69.070, dotted line represents *m/z* 155.144) and (b) *Q. ilex* emissions (continuous line represents *m/z* 81.070, dotted line represents *m/z* 137.133). The graphs represent one out of five different experiments performed.

69.070 in order to get the real concentration of isoprene in the emission recorded at 60 °C. In the case of *Q. ilex*, the increase in the leaf temperature up to 60 °C allowed to identify (*Z*)-3-hexenal as potential interfering compound in the real-time detection of $C_{10}H_{16}$ monoterpenes by PTR-TOF-MS. The presence of this compound in the emission is not surprising because it is known to be formed when leaves get seriously damaged (Fall et al., 1999; Brilli et al., 2012). As shown in Fig. 5b, the detection of this compound by fast-GC was possible because it generated a peak at *m/z* 81.070 characterized by a much shorter retention time (24 s) than all other $C_{10}H_{16}$ monoterpenes having a retention time higher than 50 s, although it did not produce any ion at *m/z* 137.133. While no correction is required for the PTR-TOF-MS signal if only the ion at *m/z* 137.133 is used to assess the total amount of $C_{10}H_{16}$ monoterpenes in the mixture, a correction is needed in the case the sum of the ions with *m/z* 137.133 and *m/z* 81.070 is used for their selective detection.

4. Conclusions

The fast-GC module integrated in a PTR-TOF Mass Spectrometer is certainly an important advancement in the field of isoprenoid emission from terrestrial plants as it provides, in an extremely short time, important information on the isobaric monoterpene components that generate the selected ions signal used for real-time PTR-TOF-MS detection. Moreover, it also allows to assess the potential interferences generated on the isoprene signal by other BVOCs, such as those naturally emitted by some species or induced by abiotic and biotic stresses. Our results show that the system appears to be quite sensitive but not sufficiently selective to provide an accurate information on some important monoterpene components (such as sabinene and β-pinene) present at high levels in the emission of many terrestrial plants. We believe that with small modifications the system could be considerably improved without increasing drastically the time required for the fast-GC analysis. In our opinion, this can be done by replacing the original column, with a much longer one. For instance, the use of a 60 m column would certainly provide a sufficient resolution to separate the majority of isobaric monoterpene compounds that were not separated by the 6 m column used in the prototype system. This would also improve the sensitivity of the fast-GC system: in fact the same amount injected would produce a higher GC peak, due to the strong reduction in the width at half-height consequent to the much higher number of theoretical plates afforded by the column, and the more favorable ratio between the sample volume injected and that of the void volume of the column. The side effect in terms of analysis time will be minimal because the most important term determining the retention of organic compounds in a GC column is the temperature and not so much the flow rate of the carrier gas, that must be kept at a certain value to obtain the maximum resolution. By considering that temperature gradient that was used to separate the various monoterpenes (15 °C min^{-1}) on the 6 m column was not really a very fast one, it is conceivable to think that using faster gradient (such as 25 °C min^{-1})

would be sufficient to maintain the analysis time in the same range.

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