Two Simple Methods for Measuring Isoprene Emission of Leaves by UV-Spectroscopy and GC-MS

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Two new headspace methods for the analysis of isoprene emission from detached plant leaves are described. The first method is based on the UV-absorption of isoprene emitted by leaves inside quartz cuvettes and can be used for the quantitative spectrophotometric determination of isoprene production. The second technique is a micro-extraction method of isoprene from the cuvette air for GC analysis, and is very suitable for the determination of isotope-labeled isoprene by GC-MS.

Introduction

Isoprene is a volatile hydrocarbon emitted by many plants in high amounts at high light conditions and at temperatures >30 °C (Sharkey, 1996). Its reactivity in the atmosphere makes it an important parameter in environmental research. With the discovery of the non-mevalonate 1-deoxy-D-xylulose-5-phosphate pathway (DOXP pathway) for the biosynthesis of the isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in bacteria and plants (Lichtenthaler *et al.*, 1997; Lichtenthaler, 1998; Rohmer, 1998) the isoprene biosynthesis was reinvestigated as well. In fact, deuterium labeled 1-deoxy-D-xylulose (DOX) was efficiently incorporated into isoprene demonstrating that the latter is synthesized via the DOXP pathway (Schwender *et al.*, 1997; Zeidler *et al.*, 1997). Isoprene is a very good fast model system for studying the biosynthesic steps of the DOXP pathway in the formation of the isoprenoid C_5 skeleton in plant chloroplasts, as isoprene is made at a high synthesis rate directly from DMAPP within the chloroplasts which possess a DMAPP-dependent isoprene synthase (Wildermuth and Fall, 1998). Isoprene emission can be quickly induced by heat and high-light treatment of leaves and is therefore appropriate for precursor and inhibitor screenings. Therefore we developed two easy to handle, fast screening methods for the detection of isoprene emitted by leaves.

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Materials and Methods

Plant material

Leaves of *Platanus* × *acerifolia, Quercus robur* L. and *Robinia pseudoacacia* L. were taken from plants growing on the Karlsruhe University campus.

UV test-system

Freshly cut rectangular leaf pieces are placed at one inner side of a quartz cuvette containing *ca.* 0.5 ml of either water or aqueous solutions of test substances. Depending on the type of investigation the leaf pieces are given an uptake time of the test substance of 2 h up to two days, and are then illuminated for 1 h in tightly sealed cuvettes (Teflon stopper) with a slide projector (500-1500 µmol photons m^{-2} s^{-1}) at 20 to 40 °C. Thereafter, the amount of isoprene produced is assessed by determination of isoprene UV-spectra (200–250 nm) of the cuvette air whereby the measuring beam bypasses the leaf piece through the free cuvette wall (Figs. 1 and 2).

GC preconcentration method

For GC-MS measurements of isoprene emitted from illuminated leaf pieces, as described above, a special enrichment technique was developed (Zeidler *et al.*, 1997): A leaf or a leaf piece is illuminated inside a glass vial which is hermetically sealed with a silicone septum. After the illumination a GC syringe containing 2 µl of a high boiling solvent, like decane or n-pentylbenzene, is pierced through the septum covering the vial. The solvent is pressed out as a droplet hanging at the syringe tip. After ca. 30 min exposure time, in which the volatiles of the vial air enrich in the droplet, the

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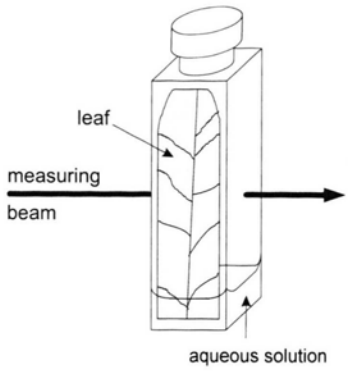

Fig. 1. Example of a sealed quartz cuvette with a cut leaf piece for registration of the high-light emitted isoprene by UV-spectroscopy. The high-light illumination of the leaf before measuring the UV-spectrum was given perpendicular to the measuring beam.

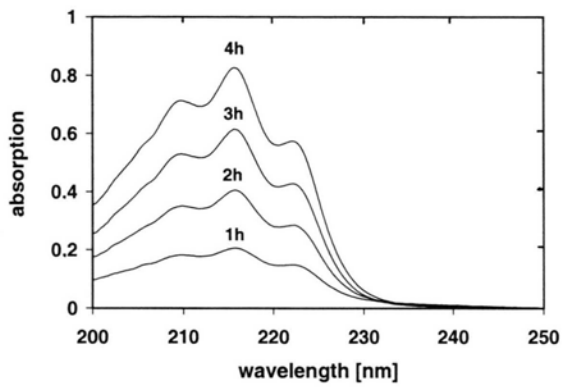

Fig. 2. Accumulation of isoprene emitted from a *Platanus* leaf piece during a 4 h illumination period as measured via the UV-spectra of isoprene.

latter is pulled back into the syringe and from there it is directly injected into a GC. In our case a Hewlett-Packard 5890 II GC was used together with a Hewlett-Packard 5971A Mass Selective Detector. The GC-method can be combined with the UV-method by closing the quartz cuvette with a septum instead of a teflon stopper.

Results and Discussion

As a first method we developed an *in vivo* test sytem for isoprene emission from plant leaves using a UV/VIS spectrometer and quartz cuvettes. Since isoprene as a conjugated diene has a characteristic UV absorption spectrum (Fig. 2), it is possible to identify and quantify isoprene via this method. One can easily measure the time-dependent emission of isoprene as shown in Fig. 2. Furthermore, one can also determine the temperature and irradiance dependencies of isoprene emission. This UV-test also allows the evaluation of the differential capacity of different plants for isoprene emission. UV absorption as a detection principle for isoprene was described for GC (Jones *et al.*, 1995). In contrast to a GC with a UV-detector, all the materials needed for our cuvette test are available in most laboratories.

The development of our second technique, the GC-MS method, for the determination of isoprene emission was inspired by the solid phase microextraction method (SPME) from Supelco. In contrast, our method could be called a liquid phase micro-extraction. One big advantage of this method is the much lower price as compared to the SPME equipment. The organic solvents used can be varied and adapted to other analytical problems. The only criteria are 1) they must be totally evaporable at the highest temperature allowed for the GC-column and 2) they should not be too volatile at the temperature during the exposure time in the cuvette.

The GC-MS method can be applied to study the incorporation of deuterium or ^{13}C-labeled precursors of the DOXP pathway. Thus, isoprene emitted from leaf pieces of *Platanus* × *acerifolia, Quercus robur* L. and *Robinia pseudoacacia* L. was labeled from applied [1-$^{2}H_1$]-1-deoxy-D-xylulose to ca. 80% (Fig. 3), 40% and 35%, respectively. By combining the GC with a radioactivity detector one can also follow the incorporation of ^{14}C-labeled

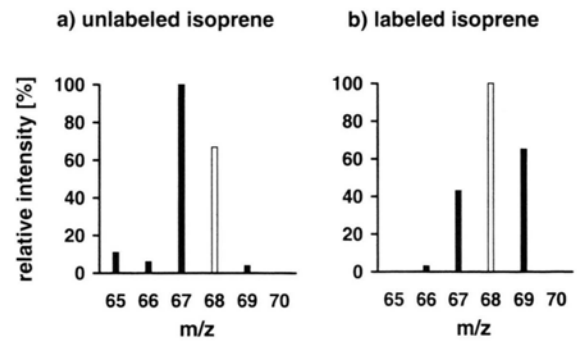

Fig. 3. Mass spectra of the molecular ion region of a) unlabeled isoprene and b) of deuterium-labeled isoprene emitted from a *Platanus* leaf 21 h after administration of a [1-$^{2}H_1$]-1-deoxy-D-xylulose solution. From the mass spectrum a labeling degree of the emitted isoprene of ca. 80% was estimated.

precursors into isoprene. Also, the effect of potential inhibitors of the plastidic DOXP pathway can be studied via the UV-test and the GC-MS test of isoprene emission. The GC-MS method is also applicable to other volatile compounds emitted by plants or other samples.

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