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Journal of Chromatography A, 868 (2000) 249–259

JOURNAL OF CHROMATOGRAPHY A

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Automated capillary gas chromatographic system to monitor ethylene emitted from biological materials

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Received 6 August 1999; received in revised form 9 November 1999; accepted 22 November 1999

Abstract

An automated capillary gas chromatographic system to measure ethylene emitted from biological materials is presented. The system consists of an on-line sampling device, a thermodesorption preconcentration apparatus and a capillary gas chromatograph with a flame ionization detection system. The limit of detection achievable on the GC system alone is 5 pg ethylene. The use of the strong Carboxen 1000 adsorbent at a sampling temperature as low as −50°C allows sampling of volumes up to a few liters. Ethylene concentrations at low ppt levels can be accurately and reproducibly determined.

Keywords: Automation; Ethylene

1. Introduction

Ethylene is a plant hormone that plays an important role in the regulation of many environmentally and developmentally induced processes such as stress resistance, seed germination, fruit ripening, senescence and abscission [1]. Types of tissues and cells that can produce and emit ethylene, have been specified by Osborne et al. [2,3]. While emission of ethylene is obviously a phenomenon of virtually all types of tissue in higher plants [2], this does not happen with lower plants. The biosynthetic routes seem to be different [3]. Because of the importance of ethylene as a biomarker and the low rate of its emission, which is mostly less than 5 ng per gram fresh weight (FW) per hour [4], a sensitive and reliable analytical method for its monitoring and quantification is necessary.

Gane [5] was the first who attempted to measure chemically the amount of emitted ethylene from ripening apples. Later, various techniques such as bioassays, gravimetric analyses, manometric techniques after mercury complexation/decomposition [6] and physico–chemical colorimetric assays were applied to quantify ethylene concentrations. Detection limits down to the 10-ppb level could be achieved [7]. These methods are, however, difficult to automate.

Gas chromatography (GC) was for the first time applied in 1959 [8,9]. Due to the low sensitivity of the thermal conductivity detector initially used in these experiments, ethylene at only relatively high concentration, i.e., 10–100 ppm could be measured [8]. A common practice was to accumulate emitted ethylene for at least a few hours in small closed vials to obtain measurable quantities. Because of the

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01223-6

hormone-induction effect of ethylene, the amount measured might not reflect accurately the amount emitted by the tissue under normal physiological conditions. Ethylene detection limits in GC were improved dramatically to ppb and sub-ppb levels with the use of a flame ionization detection (FID) system and a photoionization detector [10].

In order to guarantee normal physiological conditions for analytical subjects, e.g., plants, fruits and vegetables, a flow through system with a chamber in which intact biological material can be enclosed must be used. The first attempt to realize this was described by De Greef and De Proft [11]. Although the authors reported detection limits in the range of 0.1–0.01 ppb [12], the data did not support their claim. With a lowest detectable amount of approx. 200 pg of ethylene and a sample volume of 0.5 l, the best detection limit should be 0.5 ppb only. Moreover, the system could not be automated.

Novel approaches to measure emitted ethylene are based on either laser photothermal deflection [13] or laser-driven intracavity photoacoustic spectroscopy [14,15]. The detection limits achieved for the former technique were 0.5 ppb and for the latter 10 ppt. The measurements were carried out directly and almost continuously, which proved to be highly advantageous for short-term kinetic studies. Follow-up experiments were also carried out for longer periods of time to monitor emission of ethylene during shoot growth [15]. Although these techniques are extremely sensitive and selective for ethylene, they lack the potential of widening the application range to other volatile components emitted by the selected biological materials. The number of components which can be analyzed by this technique is determined by the number of wavelengths the laser can produce and by the selectivity of the chosen laser line towards the chosen target component. The limited selectivity, together with stabilization requirements during wavelength switching render simultaneous determination of more components in the emitted mixture difficult. Other important aspects that need to be taken into consideration are the rather high instrumental and operational costs.

GC nowadays has become a routine technique that even unspecialized analysts can manage. Moreover, the instrumentation has become integrated and automated. In trace analysis, ultra-low detection limits can be achieved with high degree of reliability. The analytical procedure developed in this study for ethylene analysis is based on capillary GC technology. The application of a sensitive detection method like universal FID gives detection limits at the low ppb level for direct gas sampling. Complex biological processes, however, initiate in many instances ethylene to be emitted in much lower amounts resulting in concentrations at low ppt levels, and improvement in detectability, compared to direct sampling, must be more than 1000-fold. Sample volumes up to a few liters must be used. Reduction to volumes of a few milliliters amenable to the capillary column can be achieved by employing an adsorption–thermodesorption preconcentration device. Ethylene from a large sample volume is quantitatively trapped on to a bed of an appropriate adsorption material. The ethylene is then released into a much smaller volume of carrier gas by applying heat to the adsorbent. The sampling process can be carried out on-line to reduce the time delay between actual ethylene emission and data acquisition. The system described in this study is automated to continuously monitor the emission of ethylene as reaction of the target biological materials to environmental and/or developmental effects. Owing to the high separation power of gas chromatography and the enrichment features of the (ad)sorption–thermodesorption unit, the technique developed has also great potential in simultaneous determination of other classes of components, e.g., aldehydes, alcohols, esters, etc., emitted by biological materials with only minor adjustments.

2. Experimental

The system consists of an on-line sampling device, a thermodesorption unit, a gas chromatograph and a data system. A schematic diagram is depicted in Fig. 1. The source of ethylene emission, a fruit or a plant, is placed in a glass bulb (ca. 14 cm in diameter×21 cm in height) with a gas in- and outlet. A continuous flow of N50 clean air (Air Liquide, Schelle, Belgium) of ca. 50 ml/min is introduced into the bulb. The refreshing rate is, therefore, 1 bulb volume per hour. A simple moisture dryer (10 cm×0.9 mm I.D. glass tube) filled with $CaCl_2$ flakes (ca. 0.4 g) is

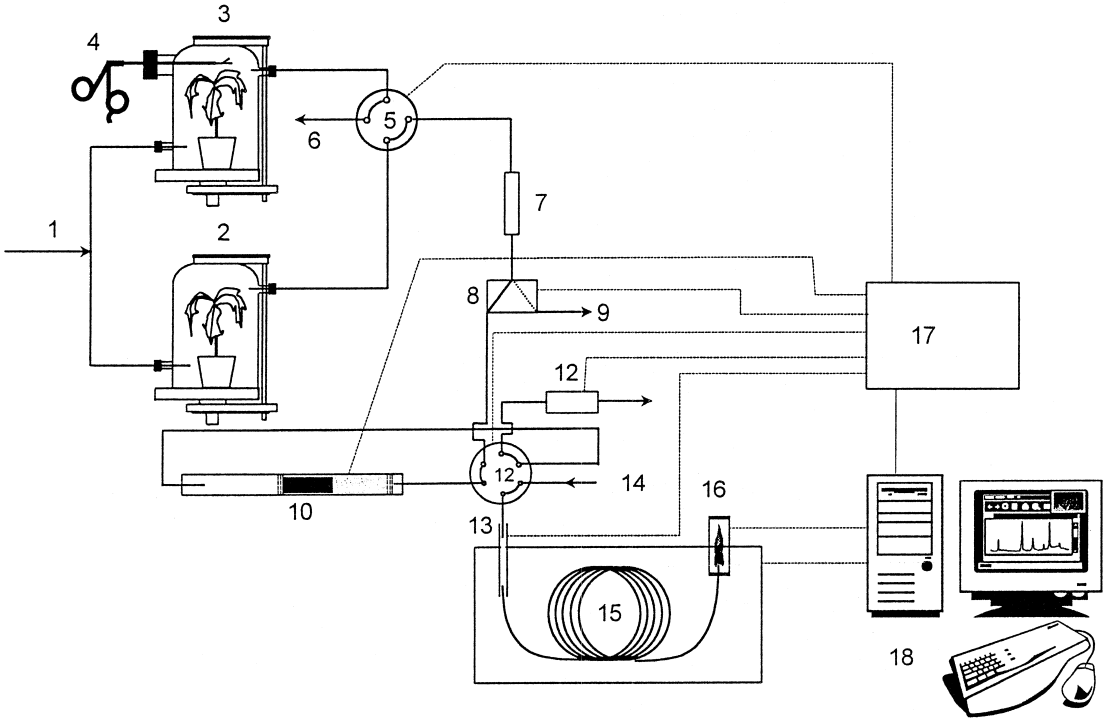

Fig. 1. Schematic diagram of the on-line dynamic automated system for ethylene analysis. (1) Clean air inlet, (2) glass bulb for reference biological material, (3) glass bulb for sample biological material, (4) in vitro wounding tool, (5) four-port switching valve, (6) vent, (7) dryer tube, (8) solenoid valve, (9) vent, (10) adsorption sampling tube, (11) six-port switching valve, (12) vacuum pump, (13) PTV refocusing device, (14) helium carrier gas inlet, (15) capillary column, (16) FID system, (17) sampling system controller, and (18) ChemStation data system.

connected in the outlet before leading the air flow to the thermodesorption device for dynamic on-line sampling. In order to minimize unexpected adsorption of ethylene in the system, PTFE tubing and PTFE-faced septa were used.

All the adsorption materials used in these experiments except Lichrolut EN (Merck, Darmstadt, Germany) were purchased from Supelco (Bellefonte, PA, USA). Physical characteristics of these adsorbents are listed in Table 1. The on-line adsorption–thermodesorption device TDS-G was purchased from Gerstel (Mülheim a/d Ruhr, Germany). During the

Table 1
Characteristics of adsorbents used

Adsorbent	Structure	Surface area (m^2/g)	Mesh	MAOT[a] (°C)
Carbosieve SIII	Spherical carbon molecular sieve	820	60–80	400
Carboxen 1000	Carbon molecular sieve	1200	45–60	225
Tenax TA	2,6-Diphenyl-*p*-phenylene oxide polymer	35	60–80	350
Porapak Q	Ethylvinylbenzene–divinylbenzene copolymer	500–600	60–80	250
Chromosorb 101	Polyaromatic cross-linked	350	60–80	250
Lichrolut EN	High purity divinylbenzene polymer	na[b]	120–400	250
HayeSep D	High purity divinylbenzene polymer	800	60–80	290

[a] Maximum allowable operation temperature.
[b] Not available.

sampling step the air sample flow is pulled through a glass tube (178 mm×6 mm O.D.×4 mm I.D.) packed with a combined adsorbent bed of Tenax TA (ca. 0.1 g, 3 cm long bed) and Carboxen 1000 (ca. 0.2 g, 4 cm long bed). The adsorbent beds are secured in place and separated from each other by plugs of silanized glass wool (Supelco). The tube is held at −50°C using liquid nitrogen. Tenax is used to prevent high boiling contaminants from reaching the Carboxen part of the sampling tube, where ethylene is trapped. A typical sample volume is 1 l at a flow of 50 ml/min, although the adsorption tube allows a much larger sample volume. During the desorption step, the adsorption tube is heated up to 225°C at a rate of 60°C/min. A helium carrier gas flow of 100 ml/min was used to sweep the desorbed ethylene from the sampling tube to a cooled program temperature vaporizer (PTV) liner to refocus ethylene before injection into the GC analytical column. The PTV (CIS 4, Gerstel) liner was packed with the HayeSep D adsorbent (mesh 60–80) which was held at −120°C with liquid nitrogen. After completing desorption, ethylene is injected into the GC column by rapidly heating up the PTV. A two-stage PTV temperature program was used to avoid introducing contaminants into the column: −120°C (0.5 min) to 50°C (5 min) at 12°C/s in the splitless mode and then to 250°C (5 min) at 12°C/s in the split mode.

Ethylene analysis was carried out on a HP 6890 GC instrument (Hewlett-Packard, Little Falls, DE, USA) equipped with an FID system. A column: 50 m×0.53 mm I.D., 5 μm d_f CarboBOND capillary column was purchased from Chrompack (Middelburg, The Netherlands). The column head pressure was set at 10 p.s.i. for helium as carrier gas (1 p.s.i.=6894.76 Pa). The oven temperature program was 80°C (7 min) to 300°C at 10°C/min. Ethylene was injected splitlessly from the PTV to the column in 2 min. Detector gases were 45 ml/min of hydrogen and 300 ml/min of air. Detector temperature was set at 300°C.

A gas standard cylinder containing 100 ppm of ethylene in air was purchased from Air Liquide. Gas standard mixtures with different concentrations were made by diluting the gas standard from the cylinder with clean air in 10-l Tedlar sample bags (Chrompack). First, the 100 ppm gas standard was filled into a carefully pre-flushed sampling glass bulb. Corresponding volumes of this gas standard were then injected into the Tedlar bags, also pre-flushed and half-filled with N50 air. Finally the bags were filled to 10 l with N50 air giving ethylene standards with desired concentrations. During this process utmost care must be taken in order to minimize possible contamination from laboratory air.

The GC control, data acquisition and processing were done with ChemStation software (Hewlett-Packard). The thermodesorption device was controlled by Master software (Gerstel). All softwares were installed on a Vectra XA computer (Hewlett-Packard).

3. Results and discussion

3.1. Selection of adsorption material

A proper adsorption material must be strong enough to quantitatively trap the component(s) of interest but also weak enough to release them easily during desorption. The extremely high volatility of the target component, i.e., ethylene with a boiling point of −100°C, indicates that a very strong adsorbent is required. The search, therefore, was focused on carbon-based materials. Two adsorbents, Carbosieve SIII and Carboxen 1000, were evaluated. The Carbosieve SIII proved to be too weak to trap ethylene. At −50°C the breakthrough volume of ethylene was less than 200 ml, which is too small a sample volume for the experiment. Carboxen 1000, although it has a much lower maximum operation temperature, namely 225°C compared to 300°C for Carbosieve SIII, is much stronger. The breakthrough curves of ethylene on Carboxen 1000 adsorbent bed at different sampling temperatures are shown in Fig. 2. At −50°C a breakthrough volume of ethylene achieved on this sampling tube, which contains approximately 0.2 g Carboxen 1000, was larger than 1.8 l. This curve can also be used as calibration line for quantification. The concentration of ethylene in the Tedlar sample bag used in this experiment was 0.25 ppb.

A larger breakthrough volume allows a higher degree of flexibility in the analysis of ethylene with a wide range of concentrations. Ethylene at relatively high concentration can be trapped from smaller

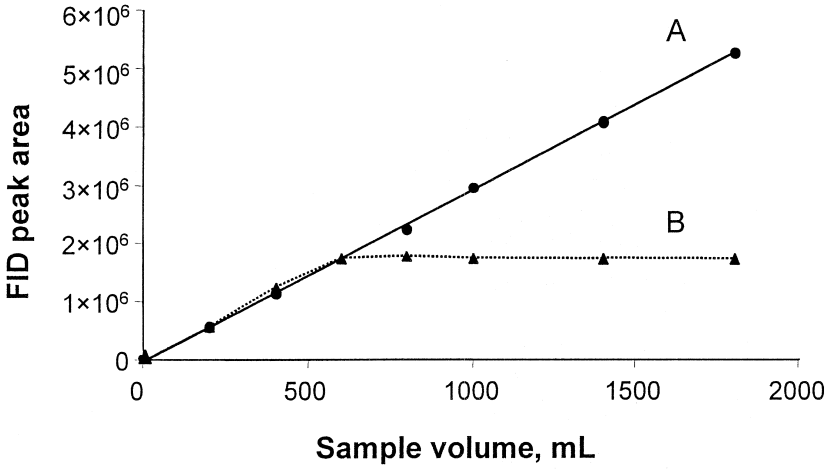

Fig. 2. Breakthrough curves of ethylene on the Carboxen 1000 adsorbent bed (ca. 0.2 g) at (A) −50°C and (B) 0°C. The ethylene concentration in the sample was 0.25 ppb.

sample volumes. Lower concentrations necessitate larger sample volumes to be used. The sampling time is then governed by the maximum allowable flow of air put through the sampling system. Due to the fact that a higher air flow-rate can unnecessarily induce a stress situation to the target biological material with the result of false positive ethylene emissions, a typical flow-rate of air was set at 50 ml/min.

A disadvantage accompanied with the use of a strong adsorbent is the risk of irreversibly adsorbing other high boiling contaminants, which eventually occupy the surface available for ethylene adsorption. In order to avoid this risk a bed of a weaker adsorbent, namely ca. 0.1 g of Tenax TA, was placed in front of the Carboxen 1000 bed. Tenax traps the higher boiling components present in the sample, preventing them from reaching the Carboxen 1000 adsorbent. Due to its weak adsorption strength the trapped components are completely released during the desorption step.

Another serious problem we observed and arising when sampling at sub-ambient temperature is the presence of water vapor in the sample. At −50°C even a minute amount of water vapor can freeze on the adsorption material and eventually block the flow path. In order to remove the water vapor from the sample gas flow before entering the adsorption tube, a glass tube filled with dried calcium chloride flakes (ca. 0.4 g) was incorporated in the sampling line (see Fig. 1). This proved to be effective enough to reduce the humidity level of the sample down to the level where no blockage related to water vapor condensation occurred, even when the sample volume was as large as a few liters. Calcium chloride showed no adsorption towards ethylene. This was proved by comparing the signal of ethylene in a standard sample with and without the calcium chloride incorporated. Different levels of humidity were generated by passing the dry standard gas flow through water before feeding it into the on-line sampling system. Calcium chloride effectively reduced water vapor while the ethylene signal remained at the same level as without the drying device. Calcium chloride flakes were replaced with new dry ones when one third of the tube was visibly moistened.

3.2. Selection of the refocusing liner filling

After passing through the adsorption–thermodesorption device, the sample volume is reduced from a few liters to a few milliliters. Desorption is, however, a slow process for the strong Carboxen 1000 adsorbent used. Excessive peak broadening was noted for a direct transfer onto the chromatographic column. Refocusing was therefore mandatory. Due to the much smaller volume of the liner and much faster heating rate of the PTV device (12°C/s), the re-trapped ethylene is rapidly released and injected

onto the column in a matter of seconds. The adsorbent packed in the PTV liner must be strong enough to re-trap ethylene released from the Carboxen 1000 adsorbent but also weak enough to rapidly release it during flash heating. Moreover, it should be thermally stable and not to decompose during flash heating to avoid contamination of the analytical column. Carbon-based adsorbents were eliminated in this stage because of their excessive strength. The lower temperature achievable with the PTV, namely −150°C, allows the use of much weaker adsorbents also guaranteeing rapid release of ethylene. Tenax TA, Porapak Q, Chromosorb 101, Lichrolut EN and HayeSep D were evaluated. Their basic characteristics are shown in Table 1.

Tenax TA, although having great advantages in thermal stability, has a rather small surface. It is easily saturated by other components also trapped at −150°C, for example carbon dioxide, which is present abundantly in the sample. Its trapping efficiency towards ethylene is then reduced by displacement effects. Porapak Q has a higher adsorption power but is rather thermally unstable. Degradation products of this adsorbent can be seen in the blank run depicted in Fig. 3. Chromosorb 101 had a similar problem. Lichrolut EN, which is a very pure divinyl benzene polymer, had no problem related to degradation. The thermal expansion coefficient of this adsorbent is, however, very large, resulting in blockage of the flow path of the carrier gas during heating. HayeSep D showed the best compromise between thermal stability, purity and adsorption strength.

Many other components are re-trapped together with ethylene on the HayeSep D liner and some degradation products are also formed. They are, however, released at different temperatures, mostly higher than ethylene, and a major part of these unwanted ''contaminants'' can be flushed away through the split vent by modifying the temperature program of the PTV (Fig. 4). If the PTV liner was heated up directly from −120°C to 250°C, many of these ''contaminants'' were transferred to the GC column while the split valve was still closed (Fig. 4A). If the PTV temperature program was paused at 50°C for 5 min, ethylene was completely released but not the major part of contaminants which were desorbed at a subsequent heating up to 250°C. During this step, the split valve was open allowing most of the contaminants to be flushed out of the system (Fig. 4B).

3.3. Selection of the analytical column

The analytical column must provide on the one hand sufficient retention and on the other hand adequate separation of ethylene from other potentially interfering components such as ethane and

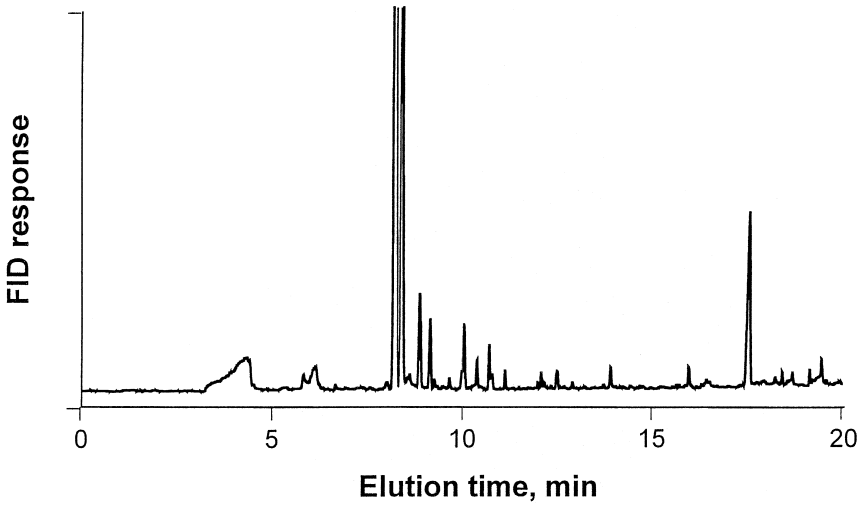

Fig. 3. Degradation products of Porapak Q.

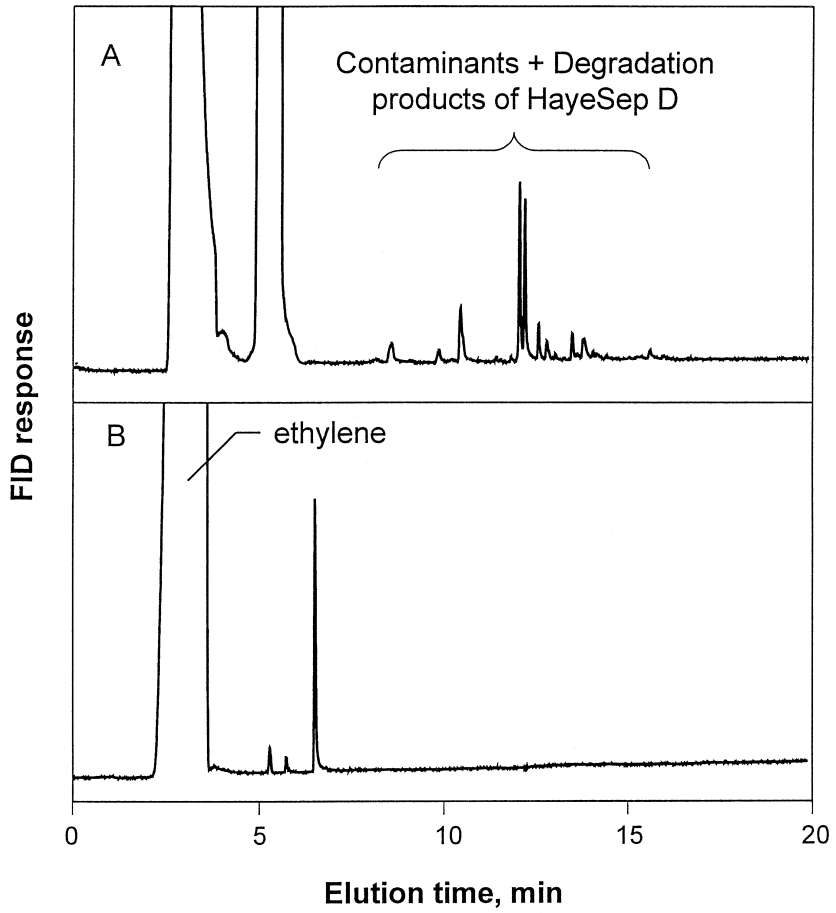

Fig. 4. Effect of the PTV temperature program on the injection into the GC column. (A) −120°C (0.5 min) to 250°C (5 min) at 12°C/s; (B) −120°C (0.5 min) to 50°C (5 min) at 12°C/s then to 250°C (5 min) at 12°C/s. Splitless time was 2 min.

acetylene. The high volatility and apolar nature of these solutes dictate the use of porous-layer open tubular (PLOT) columns. Three columns that came into consideration were PoraBOND Q, Al_2O_3 PLOT and CarboBOND.

On a PoraBOND Q column, the critical peak pair is acetylene/ethylene. Under the best circumstances, i.e., split injection and lowest ambient oven temperature (30°C), a barely baseline separation between these two solutes was achieved. Moreover, acetylene and ethylene eluted quite close to the CO_2 peak. Although CO_2 is not visible on the FID signal, the high amount of CO_2 present in the sample could effect the chromatographic performance of the target component. Better separation could be achieved with sub-ambient oven temperature. This, however, leads to a high consumption of liquid nitrogen as oven coolant.

Focusing on the acetylene/ethylene peak pair, an Al_2O_3 PLOT column was evaluated. Owing to the much higher polarity of the Al_2O_3 layer, ethylene was well separated from acetylene. The elution order is now reversed, i.e., ethane/ethylene/acetylene instead of acetylene/ethylene/ethane in case of the PoraBOND Q column. There is, however, a problem with separation between ethane and ethylene on the Al_2O_3 column.

The best solution was found in a CarboBOND column. Excellent separation of all three C_2 hydrocarbons was achieved at an oven temperature as high as 80°C (Fig. 5). The newly developed column technology of in situ bonding gives the column a

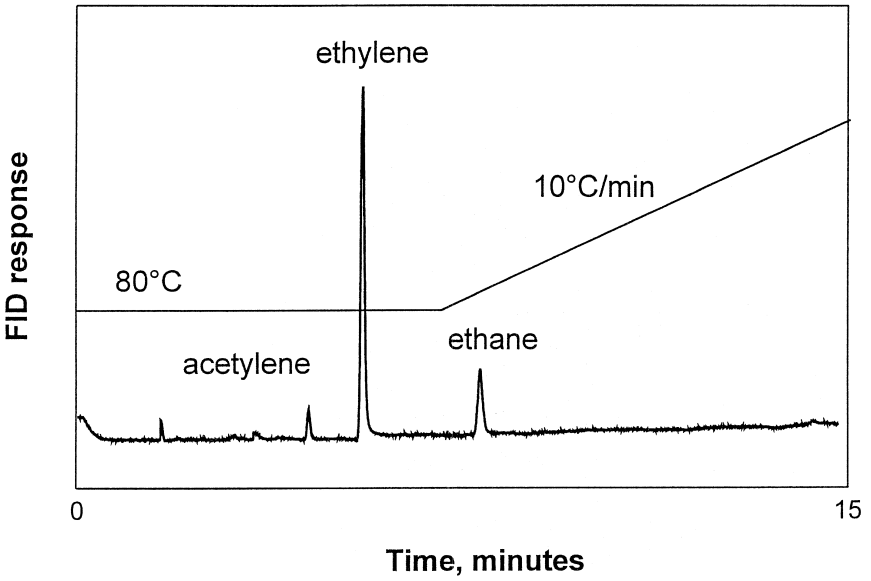

Fig. 5. Separation of C_2 hydrocarbons on the 50 m×530 μm I.D., 5 μm d_f CarboBOND PLOT column. Injection technique: TDS-G+PTV.

very high thermal stability, a characteristic not available to this type of Carbon-based PLOT columns in the past. The maximum operation temperature of 300°C allows higher boiling contaminants to be purged from the column. The large inner diameter of the column renders the use of high column flows possible. This feature is especially useful when working with the PTV as a refocusing device. The components are transferred to the column as narrow bands without the need of an extra on-column cryofocusing step or a pressure pulse program.

3.4. Calibration curves and detection limit

The calibration graph ($y=748.97x+14437$, $r^2=0.9978$) was constructed by injecting different amounts, up to 1 ml, of a 0.5-ppm ethylene standard gas in air to the clean air flow fed into the automatic sampling device. Sampling and analysis procedures were repeated exactly the same as would be applied for real samples. The limit of detection, calculated as the amount of ethylene giving a signal with signal-to-noise ratio of 3, is 5 pg ethylene. If the sample volume was 1 l as usually set during real-time sampling, the detection limit is approximately 4 ppt.

The calibration curve was measured several times with different concentrations of the standard gas. The slopes of these curves stay within 10% relative error boundary. The highest amount of ethylene injected in this way was 50 ng. This indicates that the linear dynamic range of the method is at least four-orders of magnitude, i.e., from 4 pg to 50 ng. In case a larger amount of ethylene is expected to emit from the sample, the sample volume should be reduced.

The calibration graphs are, however, not going through zero due to the fact that the clean air used in this experiment still contains a minute amount of ethylene. The background level of ethylene in the N50 clean air used in this series of experiments is about 10 ppt as calculated from a series of blank runs. The calibration graphs themselves have positive intercepts (see equation above) indicating a background level of approx. 20 pg ethylene. This can be explained by contamination during preparation of the gas standards with ethylene which is always present in laboratory air. Despite the fact that an utmost precaution has been taken, this background value could not be improved. Analysis of laboratory air gave a background level of approximately 3.6 ppb of ethylene.

3.5. Monitoring the ethylene emission from biological materials

The described analytical procedure was applied to monitor the emission of ethylene from a tomato

during its ripening process and from a fern plant with and without wounding. Before starting sampling, the target material was placed in the sample bulb (see Fig. 1), which was placed on a glass plate and tightly secured with a clamp to prevent contamination from outside air. The ambient-air tightness of the system was carefully checked before putting in the plants. Ethylene levels in the empty bulbs, flushed with N50 synthetic air at 50 ml/min each, were monitored continuously from the moment they were closed. About 4–5 h were needed to get the ethylene level in both bulbs down to an acceptable level, significantly lower than the level of ethylene that the reference plant would emit. It also minimizes any other stress effects that might be inflicted on sample materials before starting the measurements.

Starting with a green tomato the system was programmed to take samples automatically for almost 2 weeks. The amount of emitted ethylene was calculated per gram fresh mass of tomato. The tomato was weighed before and after the experiment, i.e., when it was still green and when it turned totally red. A very small difference in its mass was observed. The average value was taken as FW. The amount of ethylene emitted reached a maximum closely before the green tomato turned red (Fig. 6).

To monitor the reaction of the fern plant to wounding, a reference plant was placed into the second glass bulb (Figs. 1 and 7). After overnight stabilization, the leaves of the sample plant were wounded using a pair of special long pliers. The pliers enter the bulb through a thick PTFE septum preventing contamination from the outside air during the wounding process. The instrument was programmed to switch regularly from the sample plant to the reference plant. Ethylene emissions from these plants are compared in Fig. 8.

The sample fern plant was wounded in vitro at 14:45. This is indicated in Fig. 8 by a vertical line. Before this point the ethylene emission level of both plants were comparable. After wounding the sample plant reacted almost immediately by emitting much more ethylene compared to the intact reference plant. From this point on, the ethylene emission level of the sample plant was always higher than of the reference plant, although the difference seems to diminish at the later stage. The reference plant seemed to rise the ethylene emission in the afternoon and to decrease it after midnight.

With the valve system build in the sampling line, there is no possibility for the air from one bulb to get to the other. Air in both bulbs is in fact slightly over-pressurized due to a small restriction over the connecting tubing. Carry-over/contamination might occur only in the tubing, shared by both bulbs during sampling, from valve 5 to valve 8 and then to valve 11 (Fig. 1). The tubing from valve 5 to valve 8 was flushed by the air content from the bulb that is to be

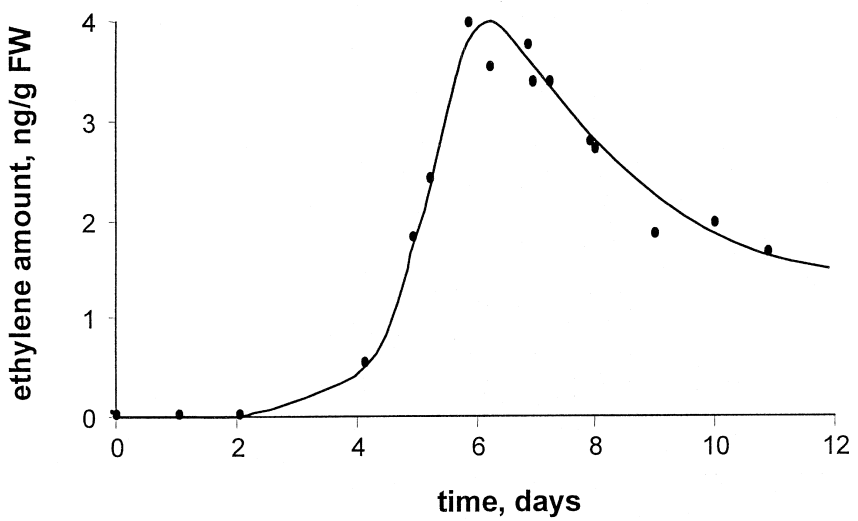

Fig. 6. Emission of ethylene from a tomato during its ripening process.

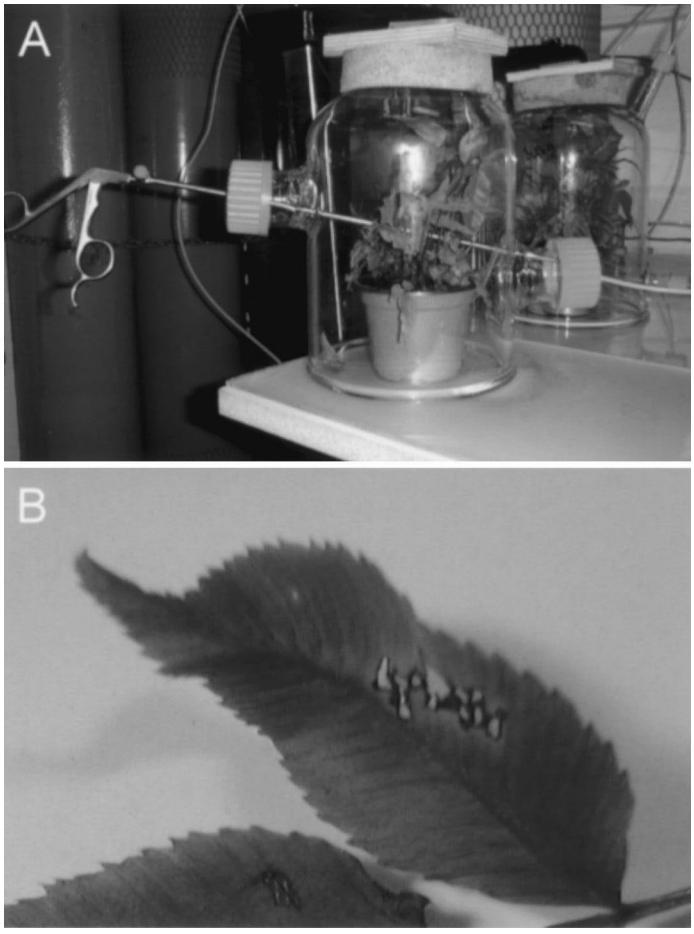

Fig. 7. Experimental set-up to monitor ethylene emission from a wounded fern plant. (A) Picture of the sample plant with pliers for in vitro wounding and the reference plant, (B) wound inflicted on a leaf of the sample plant.

sampled, at least 5 min before the actual sampling starts. Only the volume of tubing from valve 8 to valve 11 cannot be refreshed between sampling, but it was minimized by placing valve 8 as close as possible to valve 11. This volume was less than 1 ml and was negligible compared to the sample volume of 1 l. Due to the regular switching from one bulb to the other, any contamination/carry-over between the two bulbs should be reflected in an immediate rising of the ethylene signal from the reference plant after the increase in ethylene signal from the wounded plant acquired in the previous analysis. Independence in signal profiles from both plants showed that no contamination/carry-over occurred.

A number of factors, such as temperature, humidity, flow-rate etc., can have an influence on the plant ethylene production. The present study has proved that we can measure the reaction, reflected by the change in ethylene production, of the plant upon wounding, which is one of the stress conditions. Other factors are compensated by using the reference plant. A systematic study to evaluate the effects of different stress conditions will be carried out in the near future.

4. Conclusions

By using state-of-the-art gas chromatographic instrumentation the border of ethylene detection limit

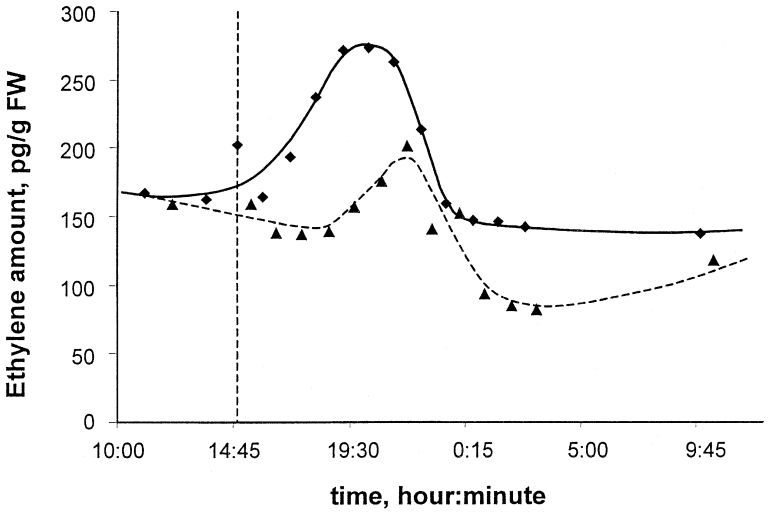

Fig. 8. Ethylene emission of fern plants. Solid line: plant with leaves wounded. Wound inflicted after 28 h of stabilization. Dashed line: reference plant.

and its analytical reliability have been pushed a great step forwards. The system is totally automated. Near real-time response at ppt level is achieved for ethylene. The instrumental set-up and experimental procedure can without major modifications be used to monitor other volatile components emitted by biological materials under different stress conditions.

Acknowledgements

We thank the University of Ghent for supporting this work through grant GOA 12051897. J.V. thanks the Flemish Institute for the Promotion of Scientific and Technological Research in the Industry (IWT), Flanders, Belgium for a study grant.

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