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INVITED REVIEW

Current methods for detecting ethylene in plants

Simona M. Cristescu[1,*], Julien Mandon[1], Denis Arslanov[1], Jérôme De Pessemier[2], Christian Hermans[2] and Frans J. M. Harren[1]

[1]*Department of Molecular and Laser Physics, Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, NL 6525 AJ Nijmegen, The Netherlands and* [2]*Laboratory of Plant Physiology and Molecular Genetics, Université Libre de Bruxelles, Campus Plaine CP 242, Bd du Triomphe, 1050 Brussels, Belgium*
** For correspondence. E-mail s.cristescu@science.ru.nl*

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• *Background* In view of ethylene's critical developmental and physiological roles the gaseous hormone remains an active research topic for plant biologists. Progress has been made to understand the ethylene biosynthesis pathway and the mechanisms of perception and action. Still numerous questions need to be answered and findings to be validated. Monitoring gas production will very often complete the picture of any ethylene research topic. Therefore the search for suitable ethylene measuring methods for various plant samples either in the field, greenhouses, laboratories or storage facilities is strongly motivated.
• *Scope* This review presents an update of the current methods for ethylene monitoring in plants. It focuses on the three most-used methods – gas chromatography detection, electrochemical sensing and optical detection – and compares them in terms of sensitivity, selectivity, time response and price. Guidelines are provided for proper selection and application of the described sensor methodologies and some specific applications are illustrated of laser-based detector for monitoring ethylene given off by *Arabidopsis thaliana* upon various nutritional treatments.
• *Conclusions* Each method has its advantages and limitations. The choice for the suitable ethylene sensor needs careful consideration and is driven by the requirements for a specific application.

Key words: Ethylene, *Arabidopsis thaliana*, gas sampling, gas chromatography, electrochemical sensing, laser-based detector.

INTRODUCTION

For a long time plants were known to emit ethylene; it is considered a gaseous phytohormone regulating various growth and development processes (Abeles *et al.*, 1992; Adams and Yang, 1979), synergistically or antagonistically with other hormones (Linkies and Leubner-Metzger, 2012; Muday *et al.*, 2012). Biotic factors can modify endogenous ethylene production, such as pathogen attack (Cristescu *et al.*, 2002), herbivorous predation (Schroder *et al.*, 2007), and abiotic environmental factors including flooding (Voesenek *et al.*, 1993), chemical exposure (Tuomainen *et al.*, 1997), O_2 or CO_2 levels (Dhawan *et al.*, 1981; Vergara *et al.*, 2012), day length and light intensity (Thain *et al.*, 2004), temperature (Wang and Adams, 1982; Orihuel-Iranzo *et al.*, 2010) or nutrient availability (Borch *et al.*, 1999; Jung *et al.*, 2009; Hermans *et al.*, 2010*a*). Moreover, the emissions may vary according to the plant species, organ type (e.g. root, leaf and flower) and developmental stage of the plant.

It is demonstrated that ethylene can stimulate fruit ripening even at levels of tens of nL L^{-1} (Pranamornkith *et al.*, 2012). Therefore, it is of paramount importance to monitor and control the ethylene emission in growth chambers, greenhouses and storage facilities to optimize fruit freshness. Controlled atmosphere was developed to allow longer storage periods and, subsequently, to provide a wide variety of fruits to the consumers over the entire year.

Hence, there is an increased demand for simple, affordable and reliable ethylene sensors that could be used in conjunction with equipment for controlling ethylene concentrations. Several sensor technologies have been proposed and their performance tested for different species in storage facilities.

Apart from the agricultural- and industrial-related involvement of ethylene in controlling fruit quality, a lot of effort has been dedicated to understanding the physiological mechanism of ethylene biosynthesis, action and perception in plants. To this end, the biosynthesis pathway has been clarified (Zarembinski and Theologis, 1994) and valuable insights into the signalling mechanisms have been achieved over the years (Chang, 1996). Also in this field of research, there is an increased demand for suitable ethylene detectors. In spite of great achievements at both the plant's molecular and physiological levels, many ethylene-related plant events remain unrevealed, mainly due to a lack of fast and sensitive ethylene detection.

Here, an overview is presented on the current methods used to sample and detect ethylene in plants. In particular, three categories are discussed: gas chromatography (GC) detection, electrochemical sensing and optical sensing. The working principle for each type of sensor is briefly described, with emphasis on advantages and disadvantages. Application of a laser-based detector used to monitor ethylene given off by the model species *Arabidopsis thaliana* under various nutrient conditions is used to illustrate the suitability of such a sensor in

plant-physiology research. Finally, the sensors are compared in terms of sensitivity, response time and price.

METHODS FOR ETHYLENE DETECTION IN PLANTS

This review plans to cover the three main categories of methods commonly used (or having the potential to be used) for ethylene detection in plants: (1) GC detection, (2) electrochemical sensing, and (3) optical detection.

GC detection

GC is a common detection technique for separation and analysis of volatile compounds in many research and industrial laboratories (James and Martin, 1952). Fundamentally, GC can separate components from complex mixtures by using a specific column and then analyse and quantify them individually (Schomburg, 1990). The gas sample is carried through a stationary phase of the column by a carrier gas (known as the mobile phase) which is usually an inert/non-reactive gas such as helium, argon, nitrogen, etc. The stationary phase is a microscopic layer of liquid or polymer on an inert solid support which interacts with the components of the sample and is located inside the column, e.g. a film coated on the inner wall of the column. The speed with which the molecules progress along the column is influenced by the strength of adsorption on the surface of the stationary phase. Compounds that are strongly retained remain attached to the stationary phase longer and take more time to go through the column, i.e. they have a longer retention time (RT) (Phillips, 1949). A detector placed at the end of the column measures the resultant signal which produces a series of peaks forming a chromatogram. Each individual compound can be identified based on its arrival time (the RT) at the detector. Furthermore, the area under the resultant peak represents a quantitative measure of that compound's concentration.

The separation of different components and the RT are influenced by several factors such as the polarity of the stationary phase, the temperature and length of the GC column, the flow of the carrier gas, etc. The polarity of the stationary phase determines the RT; polar compounds have a longer RT due to their strong adsorption on the stationary phase. The speed the sample passes through the column is dependent on the temperature of the GC column and the flow of the carrier gas (Bernhard, 1960; Blumberg and Klee, 2000). The higher the temperature or the carrier gas flow, the faster the sample moves through the column and the less interaction it with have with the stationary phase. This leads to a shorter RT, but fewer separated components. Therefore, the column temperature is selected to compromise between the length of the analysis and the resolution of the separation. Also important, is the length of the GC column; the longer the column is, the better the separation, although this leads to a longer RT (Gupta, 1969).

In 1959, the first application of GC was made to measure ethylene from apples (Burg and Stolwijk, 1959; Huelin and Kennett, 1959). The major drawback of GC was a relatively poor detection limit, i.e. 10–100 μL L^{-1}, determined by the available detection system: a thermal conductivity detector (TCD) (Lawson and Miller, 1966). The TCD measures the difference in thermal conductivity between the sample components in the carrier gas and the pure carrier gas alone (as reference). This difference generates a voltage signal proportional to the concentration of the sample components. Currently, TCDs are considered universal detectors and, in spite of lack of sensitivity, TCDs are non-specific and non-destructive detectors. To overcome the GC's lack of sensitivity, plants were enclosed into a sealed cuvette, allowing ethylene to accumulate for several hours, sometimes as long as 1 d (Freebairn and Buddenhagen, 1964; Abeles, 1972). It was a compulsory step, necessary to generate a detectable GC signal, in spite of the risk that the plant was experiencing several environmental stress factors influencing the ethylene production (e.g. lack of CO_2).

A major breakthrough was achieved in the early 1960s, when novel detector technologies became available (Lovelock, 1960; McWilliam, 1983). The progress of flame ionization detection (FID) and the photoionization detector (PID) significantly improved the detection limit of ethylene to tens of nL L^{-1} levels (Bassi and Spencer, 1989). Flame ionization detectors became the first standard practice in plant-physiology laboratories. Later on, it was demonstrated that PID had a significant improvement over FID, becoming the most efficient detection system concerning ethylene measurements (Bassi and Spencer, 1985). The PID is also a popular detector in the field of environmental-pollution and industrial-process monitoring, as it is specifically sensitive to aromatic hydrocarbons and sulfur compounds. A low detection limit of sub-nL L^{-1} has been reported using such a detector (Degreef and Deproft, 1978); however, the claim was not well supported by the data (Pham-Tuan *et al.*, 2000).

Headspace collection of ethylene. The sampling procedure and subsequently injection of the sample into the GC column are two aspects that require careful consideration (Tholl *et al.*, 2006). In GCs, a small volume of the gas sample is needed for injecting into the column (typically having the inner diameter of 5 mm for packed columns and 250 μm for capillary columns, respectively). If too much of the sample is injected, the peaks of the chromatogram show a significant tailing, which causes a poorer separation (Bassi and Spencer, 1985).

In the early 1970s, ethylene was sampled from the headspace of a closed cuvette, in which the plant was enclosed for a few hours, and manually injected into the GC column with a gas-tight syringe (Abeles *et al.*, 1992) (Fig. 1A). Although this is a simple technique, it was time consuming for the analyst and not very reproducible as the sampled amount could differ slightly; not to mention the induced-physiological impact on the plant material due to the enclosure needed to obtain a high enough ethylene concentration. A solution was quickly developed, with automatic samplers connected to the GC raising the standard GC performance and reliability. In modern chromatography systems, concentric rotary valves allow a discrete sample to be automatically introduced into the column for separation (Fig. 1B). These samplers provided accuracy, superior reproducibility, optimum injection flexibility, reliability and were user-friendly. The use of valves to switch between gas streams simplified the analyst's work and made the GC more attractive.

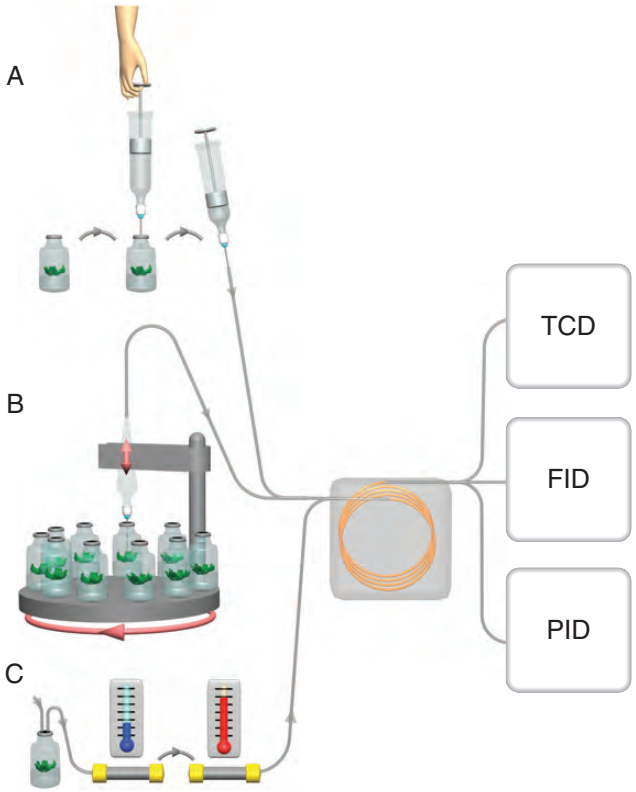


FIG. 1. Gas chromatography detection. Several configurations have been used with GCs. Both sampling and injection into the column have been improved over the years. (A) Manual GC injection was done with syringes. (B) Auto-injector-facilitated injections brought higher accuracy and could process a large number of samples. (C) Adsorption/desorption units were used to accumulate ethylene for minutes to increase the detection limit to the nL L^{-1} range. Several detection schemes were implemented using a non-destructive, although not sensitive sensor, such as the thermal conductivity detector (TCD). Later on detectors sensitive to hydrocarbons – the flame ionization detector (FID) – or to aromatic and olefin hydrocarbons – the photoionization detector (PID) – were developed and became the most commonly adopted detector technologies.

Furthermore, 'pre-concentration' of ethylene contributed to an even better sensitivity and more efficient sampling. The GC systems were coupled with adsorption–thermodesorption devices enabling them to store the emitted ethylene (Segal *et al.*, 2000) (Fig. 1C). The plants were placed in closed cuvettes and continuously flushed with air, minimizing the accumulation effects. The adsorption–thermodesorption device samples from the headspace of the cuvette typically at a 50-mL min^{-1} flow rate for several minutes up to several hours, depending on the ethylene concentration. Ethylene is thus trapped inside a tube containing an appropriate adsorption material (e.g. carbon molecular sieve) and is then released into a smaller volume by heating the adsorbent. Important features of the adsorbent are: the trapping efficiency which should be close to 100 %, its facility to release of the sample during desorption and its selectivity. Several adsorbents have been proposed for efficient ethylene trapping (Pham-Tuan *et al.*, 2000). However, thermal desorption suffers from the lack of repeated sample injections and the degradation of the trapping media (Clausen and Wolkoff, 1997).

Nowadays, such GC systems can be fully automated to continuously monitor ethylene emission at a repetition rate of <10 min. Commercial GC systems are offering, for moderate costs, a few tens of nL L^{-1} of detection limit for ethylene. However, rather than costs, accuracy, speed and repeatability should be the major considerations in the selection of a GC.

Efficient and fast measurements. For highly sensitivity systems, the sampling is done semi-continuously. The gas sample is periodically introduced into the GC system thanks to a slight overpressure of the input or by pumping the sample directly (Gaspar, 1991). As the length of the analysis is determined by the RT of different components in the gas mixture, efforts were devoted to reduce the response time of GCs to below 10 min (Cramers *et al.*, 1999; Peters *et al.*, 1991). One way is what is known as 'back flow measurement' or 'back cleaning' (Fett, 1963; Klemp *et al.*, 1993). It consists of dividing the GC column into two parts: a short 'stripper' column and a long 'analysis' column. In this way, the slowest compounds travelling into the GC are not injected into the GC analysis column. As soon as ethylene enters the analysis column, the flow inside the stripper column is reversed and all the molecules travelling at a lower speed are flushed backwards.

Several companies (e.g. Synspec) have developed compact and fast GC systems with a response time of a few minutes. GC systems have evolved from complex, bulky systems to portable, battery-operated units, capable of accurate, reliable measurements at low concentration (standard levels as low as 100 nL L^{-1}) (Inficon/Photovac).

Compact GC for field measurements. If GCs are considered an efficient tool for biological and chemical analysis, the range of applications in remote, on-site monitoring or monitoring hostile environments is limited due to the nature of the technology. Miniaturization of GCs into a compact, robust and low-power consumption device would enable their range of applications to be extended. The first attempt to develop micro-GC was reported in 1979 (Terry *et al.*, 1979). The 1·5-m-long GC column was fabricated in silicon using photolithography. Coupled with a TCD, the resolution and sensitivity was poor in comparison to standard GCs. The driving idea behind miniaturization was to decrease the analysis time as well as the amount of reagents required for analysis. In the 1990s, portable gas chromatographs were manufactured by MTI (Microsensor Technology Inc.) based on their solution of 1979. Since then, several fabrication processes of an integrated GC column have been reported in the literature. Attempts were made with silicon (Reston and Kolesar, 1994; Lambertus *et al.*, 2005; Radadia *et al.*, 2009) and other materials (Noh *et al.*, 2002; Bhushan *et al.*, 2007; Lewis *et al.*, 2010) to achieve sufficient resolution. The technology is quite promising and commercially available, although the detection limit is still in the low μL L^{-1} or hundreds of nL L^{-1} range. To our knowledge, micro-GC has neither been designed nor applied to ethylene detection.

As more compounds could be measured with a single device, the GC is a widely used instrumentation within the plant research community, although other powerful techniques specialized in ethylene monitoring are becoming commercially available.

Advantages of GC detection
Small sample requirements
High selectivity, good at separating complex mixtures and compounds
Fast analysis (minute time scale)
Easy to operate (fully automated)
Portable GCs available for field measurements

Disadvantages of the GC
Limited sensitivity
Requires a pre-concentration step for better sensitivity and optimized plant conditions
High costs for the highest performing systems

Electrochemical sensors

Various electrochemical devices, e.g. for measuring pH or oxygen, have been routinely used in the past. Recent advances in electrochemical sensor technology have expanded the application of these devices towards a wide range of compounds, including ethylene.

An electrochemical sensor transforms the concentration of a gas into a detectable physical signal such as: electrical current, resistance, etc. In detail, the target gas undergoes a chemical reaction with the active sensing material, which in the presence of an electrical circuit will generate a change in an electrical parameter (Bard and Faulkner, 1980). Electrochemical sensors can be classified accordingly to the physical change measured. If the measurand is current (A) we refer to 'amperometric sensors', where we measure resistance (Ω) we refer to 'chemoresistive sensors' and if we measure a change in capacitance the sensors are referred to as 'capacitive' (Janata, 2009). Below, these three groups of electrochemical sensors will be discussed as they are the most popularly employed for ethylene monitoring.

Amperometric sensors. In its simplest form, an electrochemical sensor consists of a diffusion barrier, a sensing-electrode (or anode), a counter-electrode (or cathode) and often a third electrode (or reference electrode), separated by a thin layer of electrolyte (usually sulfuric or phosphoric acid) (Fig. 2). The electrodes are commonly fabricated by fixing a precious metal with a high surface area onto a porous hydrophobic membrane. For example, currently available amperometric electrochemical sensors use gold anodes. When a voltage is applied between a gold electrode and a reference electrode, ethylene that diffuses into the sensor through the barrier is catalytically oxidized at the surface of the gold electrode (Schmidt and Pastor, 1994). This results in a current change that is proportional to the concentration of the ethylene gas. A few years later, a sensor was reported using a layer of gold, plated onto a Nafion membrane as anode, and sulfuric acid (0·5 M H_2SO_4) as catalyst (Jordan *et al.*, 1997*a*). The sensor had a detection limit of 40 nL L^{-1} of ethylene based on a signal-to-noise ratio of 3 and showed linear response up to 500 μL L^{-1}. Moreover this sensor was found to be dependent on the ethylene flow rate and suffered from baseline drift (one reason could be electrolyte evaporation).

Despite the significant effort dedicated to the design and fabrication of electrodes and electrolytes, the electrochemical sensors have demonstrated that they are not completely gas specific; they are also sensitive to other compounds. Oxidation of ethylene on a gold–Nafion electrode also generates acetaldehyde, nitrogen oxides and sulfur compounds which are considered interfering gases (Pastor and Schmidt, 1995). A trap–release method used for pre-concentrating ethylene was proposed as a filter and was predicted to improve the sensor sensitivity to ethylene down to the nL L^{-1} level (Jordan *et al.*, 1997*b*). The use of a filter may cause delay in the sensor response which was not the apparent case in this report. It is acknowledged, however, that retention of the interference gases in the trap could shorten its lifetime.

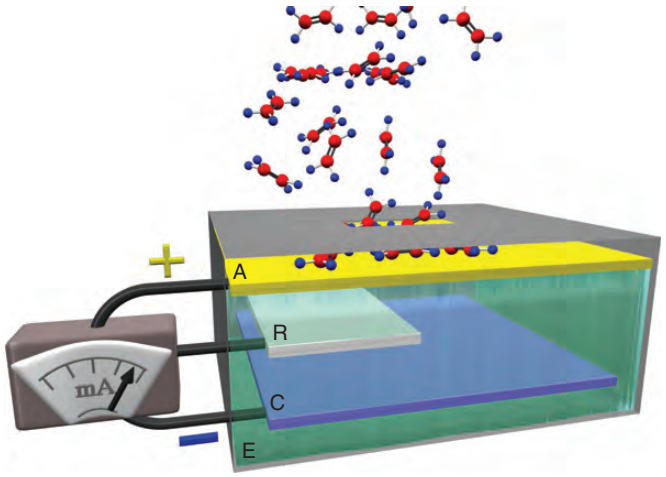


FIG. 2. Electrochemical sensor. Ethylene diffuses through a barrier into the sensor, which consists of a sensing electrode (anode, A), a counter electrode (cathode, C) and a reference electrode (R) covered by a thin layer of an electrolytic solution (E). If an electrical potential is applied to the anode (most recently made of gold particles) ethylene is catalytically oxidized, resulting in a current change proportional to the ethylene concentration.

As the performances of the amperometric sensors strongly depend of the anode material, much effort over the last few years was devoted to electrode fabrication. Nanoparticle technology has provided innovative solutions to improve sensor performances (Thompson, 1999). Although gold was known as a noble and non-reactive metal, it appears that 2- to 20-nm gold clusters have catalytic properties, beginning a 'new gold rush' research area. The nanoporous gold sensor technology has been recently implemented by Fluid Analytics, Inc. (which further licensed the technology to Absoger, CID and EMC), resulting in a portable sensor for detecting ethylene down to 10 nL L^{-1} in air (Shekarriz and Allen, 2008).

An acid electrolyte is necessary to prevent gold oxide formation before the oxidation of ethylene. Research is ongoing to elucidate the role of the composition and strength of the electrolyte in the mechanism of ethylene oxidation. Instead of the acid electrolyte, a non-acidic thin ionic-liquid layer has been proposed recently (Zevenbergen *et al.*, 2011). This type of electrolyte is nontoxic and has a low drift since it virtually evaporates. Such a sensor has a detection limit of 760 nL L^{-1} (at a signal-to-noise ratio of 3) and a linear response up to 10 μL L^{-1}. In 2012, Holst Centre and Imec launched the news through their website that they had demonstrated for the first

time the use of this electrolyte in a single-chip electrochemical sensor, monitoring ethylene with 100-nL L^{-1} step changes in a concentration range below 1 μL L^{-1}; the detection limit of the sensor was 200–300 nL L^{-1}.

Oxygen is an essential ingredient in the reaction with the gas and in maintaining the generated current when ethylene is present. If the oxygen supply is inadequate, the sensor will not operate properly, thus the sensor cannot be used for certain applications. Measurements in low-oxygen conditions can be performed if a separate access of air to the counter-electrode is available.

Interestingly, electrochemical sensors have been indicated as suitable for post-harvest storage under low-oxygen conditions. Obviously, the main considerations for this choice were their low cost, small size (some sensors are portable) and low power consumption. However, in the agricultural sector, to monitor ethylene down to tens of nL L^{-1} becomes more demanding. Softening of kiwifruit in the presence of ethylene at levels as low as 10 nL L^{-1} in ambient storage air represents a critical issue (Pranamornkith *et al.*, 2012).

Chemoresistive sensors. The use of a liquid electrolyte makes this sensor sensitive to environmental conditions such as temperature and humidity. Novel types of electrochemical sensors are currently being developed using carbon nanotubes (i.e. sheets of carbon atoms rolled into cylinders that act as 'superhighways' for an electron flow) or SnO_2 (tin dioxide) nanoparticles (Fig. 3). The first type of sensor is a chemoresistance sensor: the active sensing material is a mixture of carbon nanotubes and a copper complex placed between gold electrodes. Ethylene binds to the copper complex (known as the electrical conductor), changing the resistance of the nanotubes (Esser *et al.*, 2012). According to the authors the choice for copper was inspired by the role of copper as a cofactor for ethylene-binding activity in the ethylene receptors. This sensor allows detection of ethylene concentrations as low as 0·5 μL L^{-1}.

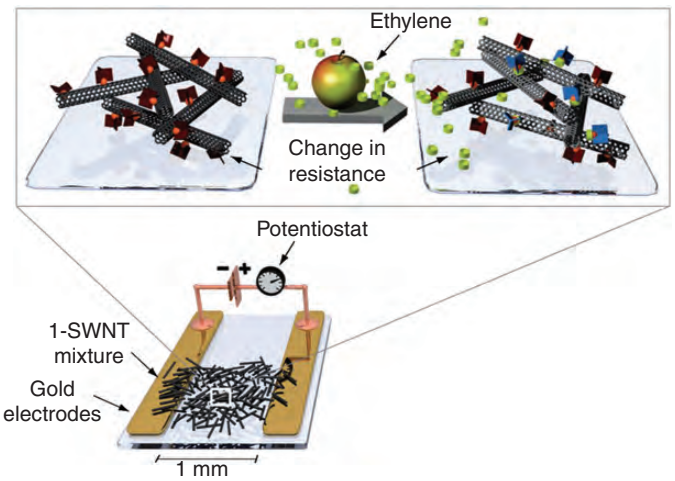


FIG. 3. Chemoresistance sensor. The sensing material is a mixture of single-walled carbon nanotubes (SWNTs) and copper complex placed between gold electrodes. Ethylene binds to the copper complex, changing the resistance of the nanotubes according to the gas concentration (adapted from Esser *et al.*, 2012).

Capacitive sensors. The second type of sensor is a capacitor-based sensor at room temperature. The active sensing material is SnO_2 nanoparticles (10–15 nm in diameter) used as a dielectric material between two copper electrodes (Balachandran *et al.*, 2008). In the presence of ethylene the capacitance of the sensor is changed; ethylene concentrations in the range from 1 μL L^{-1} to thousands of μL L^{-1} can be measured. The addition of a palladium/platinum layer between the nanoparticles and the metals resulted in increased sensitivity, but the sensor showed a faster degradation (Straumal *et al.*, 2010). The use of this kind of sensor would be desirable due to its low power consumption and short response times of a few minutes. Moreover the sensor can be integrated with a microstrip patch antenna for passive wireless detection of ethylene gas. The sensor also responds strongly to other, interfering compounds such as ethanol, acetic acid, ammonia, acetone and ethyl acetate and to humidity changes.

In general, the operating lifetime of electrochemical sensors depends on the sensor type, which can vary from 6 months to over 2 years. The lifetime is shortened by a variety of environmental factors, such as low humidity, high temperatures (as the electrolyte may dry out) and exposure to the target gas and gas interferences (consumption of electrolyte). Electrochemical cells are active even when they are stored; therefore, they have a limited lifetime even when not in use. It is recommended to keep them in a refrigerator when not in use.

Electrochemical sensor technology is a proven technology and, like any other technology, it has its advantages and disadvantages.

Advantages of electrochemical sensor technology

Ethylene gas specific in a μL L^{-1} down to tens of nL L^{-1} range
Good repeatability and accuracy
Relatively fast response time to ethylene (below 1 min) and recovery time of minutes
Low power consumption
Lightweight (2·5–10 kg)
Low cost
Portable and easy to use in laboratory or field conditions (some are battery operated up to max. 8 h)

Disadvantages of electrochemical sensor technology.

Sensitive to interfering gases
Sensitivity to temperature and humidity changes
Limited temperature range
Requires oxygen to operate correctly
Limited shelf-life
Reduced lifetime when continuously exposed to higher ethylene concentrations

A challenge remains where, on one hand, the electrochemical sensor works better at higher ethylene concentrations (by overruling the influence of the interfering gases) and, on the other hand, continuous operation over several days exhausts the anode and affects the sensitivity.

Optical gas sensors. When light interacts with ethylene molecules it can be absorbed, emitted or scattered. Ethylene, like many other molecular gases, has its specific absorption characteristics, which are the strongest in the mid-infrared (IR)

region; the so-called fingerprint region (2–12 μm). By knowing the absorption strength of ethylene at a specific IR light frequency, the molecular ethylene concentration can be quantified.

There are several schemes of such sensors and generally they can be classified as dispersive or non-dispersive sensors (Fig. 4).

Typically, an optical sensor consists of an appropriate light source (IR lamp or laser) that passes through an absorption cell containing the ethylene sample and reaches an optical detector that measures the light intensity. Obviously, the light source has to be chosen in the wavelength region where ethylene has absorption features. The measured intensity of the transmitted light is altered due to the light absorption of the ethylene molecules and from this change the ethylene concentration can be determined.

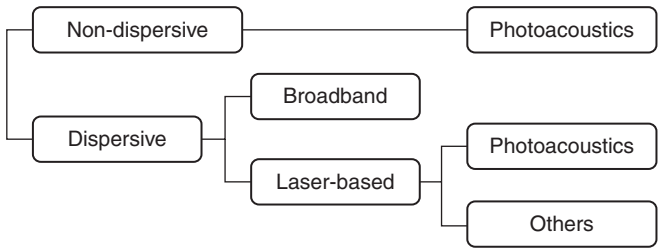


FIG. 4. Classification of the optical gas sensors. In the non-dispersive sensors the light source used for gas absorption is broadband and multiple narrow-band optical filters are required for detecting ethylene by subtracting the interference gases. The dispersive broadband sensors have dispersive elements, such as prisms to separate the wavelengths. The laser-based optical sensors are using lasers to selectively excite the ethylene molecules and sensitive detection methods (i.e. photoacoustics) for fast detection at low concentrations.

Non-dispersive sensors. In the non-dispersive infrared (NDIR) instruments, as all the 'wavelengths' from a broadband source are considered at the same time without separation, the absorption spectrum cannot be resolved. An important element for the NDIR sensors is their band-pass filter. This is necessary to increase the sensor selectivity for ethylene and to attenuate the undesired absorbents. Like the electrochemical sensors, NDIR devices also suffer from lack of selectivity. Although they are simple and robust, NDIR instruments have limited sensitivity because the measured transmitted light can also be affected by the absorptions of other gases A possible solution to this problem is to use a single source and two identical detectors with two filters: one for measuring ethylene and the other for all (also interfering) gases.

Presently, there are NDIR sensors based on photoacoustic spectroscopy manufactured by LumaSense Technologies and Gasera Ltd (Fig. 5A). This is a simple approach in which ethylene released by a biological sample is transported to the absorption cell (in this case called the photoacoustic cell). Once inside it absorbs the light of a pre-selected wavelength and converts it into heat. By modulating the light (switching the light on and off with a certain frequency) with a mechanical chopper, pressure waves (i.e. sound) are generated and detected with sensitive, miniature microphone(s). The amplitude of the waves is proportional to the concentration of ethylene in the photoacoustic cell (Kreuzer, 1977).

These NDIR sensors need multiple narrow-band optical filters that can be mounted in a filter wheel (Fig. 5A). The filters are chosen for ethylene as well as for the interfering gases; the data analysis is performed using a mathematical model based on non-linear compensation. If you desire to measure solely ethylene, you will need to also consider purchasing gas filters to remove interference gases (price increases proportionally). The detection limits are in μL L^{-1} and few hundreds of nL L^{-1} level (100 nL L^{-1} in nitrogen

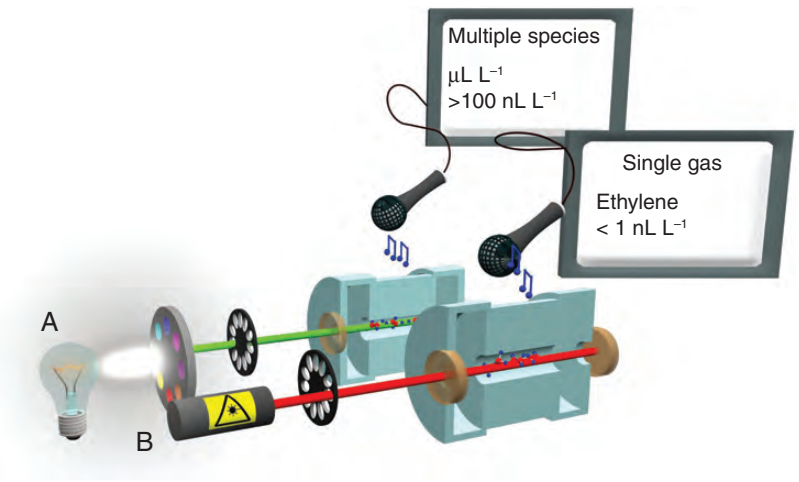


FIG. 5. Non-dispersive versus laser-based sensor using photoacoustic spectroscopy. Light is generated by a broadband source passed through a filter wheel (A) or by a laser (B) and directed into an absorption cell where it is absorbed by the ethylene molecules and converted into heat. By switching the light on and off with a mechanical chopper the temperature changes periodically, giving rise to a periodic pressure change, resulting in acoustic energy detected by a miniature microphone. The intensity of the sound is proportional to the concentration of absorbing gas molecules present in the cell. The non-dispersive sensors detect multiple species at concentrations of μL L^{-1} of hundreds of nL L^{-1} levels (e.g. ethylene and the interfering gases) using optical filters. The laser-based sensors, using a single-frequency light source, are more selective and provide the lowest detection limits so far.

with a noise level of 0·05 μL L^{-1} for LumaSense Techn. and sub-μL L^{-1} for Gasera Ltd ethylene sensor).

In a different configuration from Gasera Ltd the mechanical chopper is replaced by a low-power electrically pulsed IR-source such as a diode laser, and the microphone used is a patented cantilever pressure sensor.

The sensors have the advantages of a low sample volume of 10–30 mL, are transportable and provide simultaneous quantitative analysis of more gas compounds over a large dynamic range.

Broadband dispersive sensors. Some dispersive sensors make use of a prism or diffraction grating to disperse a spectrally broadband source such as an IR lamp (Kneubuhi *et al.*, 1966). In this way a wide range of wavelengths may be provided. Each wavelength is changed by rotating the dispersive element and monitoring their intensities; by this method an absorption spectrum (light intensity vs. frequencies) of certain molecules can be obtained. These are broadband analytical instruments, usually bulky and complex and used for molecular spectroscopy purposes, which make them too expensive for single-component detection.

Laser-based sensors. Other dispersive sensors are founded on laser-based sensing techniques. They take the advantage of using tuneable lasers to selectively emit in the absorption range of ethylene in combination with detection techniques, such as photoacoustic spectroscopy (Fig. 5B) or cavity ring-down spectroscopy. This results in unprecedented detection limits and a short response time, which no other devices can achieve, namely nL L^{-1} and sub-nL L^{-1} levels in seconds/minutes time scale.

The main advantages of the photoacoustic detection over other laser-based techniques are simple design, high

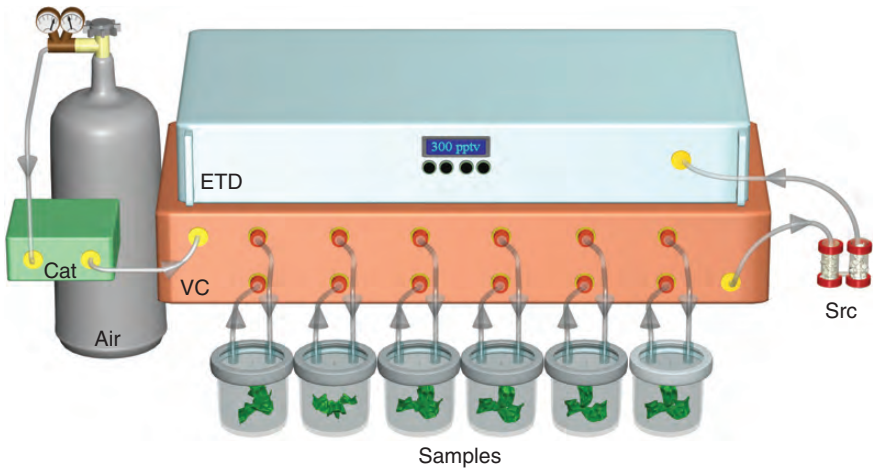


FIG. 6. Experimental set-up for on-line ethylene monitoring with a laser-based photoacoustic detector. Compressed air from a cylinder is passed through a catalyser (Cat) to remove the hydrocarbons and then distributed at a controlled flow rate to six cuvettes enclosing the biological samples by a valve controller (VC). Ethylene released in the headspace of the cuvettes is alternately monitored by the laser-based detector ETD-300 (sensor sense) for a certain period. Prior to entering the ethylene detector, CO_2 and H_2O vapour are eliminated using a scrubber (Scr) with KOH and $CaCl_2$, respectively.

TABLE 1. *Applications of the laser-based ethylene detector ETD-300 in plant biology*

Research theme	Key words	References
Signalling	Receptors, signal transduction, silver addition, arabidopsis seedlings	McDaniel and Binder, 2012
	Blue light, circadian clock, hypocotyls, arabidopsis seedlings	Ellison *et al.*, 2011
Post-harvest	Firmness, soluble solids, rot, kiwifruits	Pranamornkith *et al.*, 2012
	AVG, 1-MCP, phenylalanine ammonia-lyase, endive	Salman *et al.*, 2009
Development	ABA-deficiency, cell enlargement, fruit development, tomato	Nitsch *et al.*, 2012
	Apical hook, auxin, gibberellins, arabidopsis seeds/seedlings	Gallego-Bartolome *et al.*, 2011
	SAM cycle, methionine synthase, carboxyl methyltransferase, tobacco flowers	Piechulla *et al.*, 2009
	Auxin, cryptochrome, hyponasty, petiole, phytochrome, arabidopsis plants	Millenaar *et al.*, 2009
Abiotic stress	Aquatic macrophytes	Forni *et al.*, 2012
	Jasmonic acid, salicylic acid, thermotolerance, arabidopsis plants	Clarke *et al.*, 2009
Plant–pathogen interaction	Metabolomics, *B. cinerea*, defence, arabidopsis mature plants	Lloyd *et al.*, 2011
	B. cinerea, *in vitro*, tomato	Cristescu *et al.*, 2006
	Nitric oxide, haemoglobin, *P. syringae*, *B. cinerea*, salicylic acid, jasmonic acid	Mur *et al.*, 2012
Plant–insect interaction	Insect (*Diprion pini*) egg deposition, jasmonic acid, herbivore, Scots pine trees	Schroder *et al.*, 2007
Mineral nutrition	Magnesium depletion, circadian rhythm, arabidopsis mature plants (hydroponics)	Hermans *et al.*, 2010*b*
	Nitrogen nutrition, root architecture, arabidopsis seedlings (*in vitro*)	Hermans *et al.*, 2011
	Potassium starvation, stomatal conductance, sunflower plants	Benlloch-Gonzalez *et al.*, 2010
Unicellular organisms	Cell death, *Chlamydomonas reinhardtii*, nitric oxide, unicellular algae	Yordanova *et al.*, 2010
Nitrogen fixation	Nitrogen fixation, cyanobacteria	Staal *et al.*, 2007

sensitivity, wide linear dynamic range, fast response and low sample volume. The technique has been described in detail elsewhere including a comprehensive list of applications (Harren *et al.*, 2012).

At the Life Science Trace Gas Facility (Nijmegen, The Netherlands), the potential of this technique has been fully exploited since the 1990s. Since the sensitivity of this method strongly depends on laser power, a high power source has to be employed, such as the CO_2 laser (Watts's laser power), to reach very low detection limits. Moreover, it is a fortunate coincidence that ethylene displays the highest absorption strength in the emission range of the CO_2 laser at 10 μm. Using these features, the laboratory-developed set-up was capable of measuring ethylene in a continuous flow system down to 10 pL L^{-1} over 90 s (Cristescu *et al.*, 2006).

In close collaboration with biologists, the system was used over the years to monitor various dynamic processes in plants and micro-organisms. Thanks to this excellent sensitivity and rapid time response, fast processes in plants were revealed and real-time measurements from a single plant or plant organ were possible without accumulation periods (Cristescu *et al.*, 2006; Harren *et al.*, 2012).

Only recently has the development of these detectors moved towards commercial instruments (ETD-300, Sensor Sense BV) (Fig. 6). This detector has a detection limit of 300 pL L^{-1} within a 5-s measuring time, at the moment the best-achieved sensitivity worldwide. From this limit its dynamic range goes up to 5 μL L^{-1} (or optionally to 300 μL L^{-1}). In addition, data handling software makes external data processing user-friendly for non-laser specialists. In combination with a gas handling system that includes mass flow controllers and electronic valves to perform switching between various cuvettes (up to six) containing biological samples, this detector is a powerful tool for continuous on-line measurements for long periods of time (weeks, months). An overview list with most recent applications of this detector is given in Table 1.

The main advantage of laser-based detectors is their high sensitivity and selectivity as compared with the other detection techniques mentioned previously. This relies on the choice of the laser and its combination with the detection technique. When no high power source is available, other lasers can be used (e.g. diode lasers) in combination with other optical spectroscopic techniques, such as cavity ring-down spectroscopy or cavity-enhanced absorption spectroscopy. Both methods use optical cavities consisting of two mirrors with high reflectivity that allow light to travel an extended optical path (up to kilometres) inside a small volume, increasing the interactions with the ethylene molecules. Such an instrument was developed by Picarro and tested in trace gas ethylene monitoring in ambient air in a cold storage room of a fruit-packing facility over a several-month period (Wahl *et al.*, 2006). The detection limit was 2 nL L^{-1} in 5 s.

Recent advances in the fabrication of mid-IR sources such as quantum cascade lasers (Faist *et al.*, 1994; Curl *et al.*, 2010) operating at room temperature opened new opportunities for development of quantum cascade laser-based ethylene detectors (Weidmann *et al.*, 2004). Different absorption techniques can be combined with such lasers, as previously demonstrated by J. Tulip's group who obtained a detection limit for ethylene of 20 nL L^{-1} in an average time of 5 s (Manne *et al.*, 2010) and 5 nL L^{-1} in <10 s (Manne *et al.*, 2008). Although such sensors are not yet in use for monitoring ethylene from plants, this type of laser is very promising in terms of system integration (Harren *et al.*, 2012).

Advantages of laser-based sensors
Highest sensitivity (below nL L^{-1})
Fastest response time (seconds)
Good selectivity
Real-time monitoring

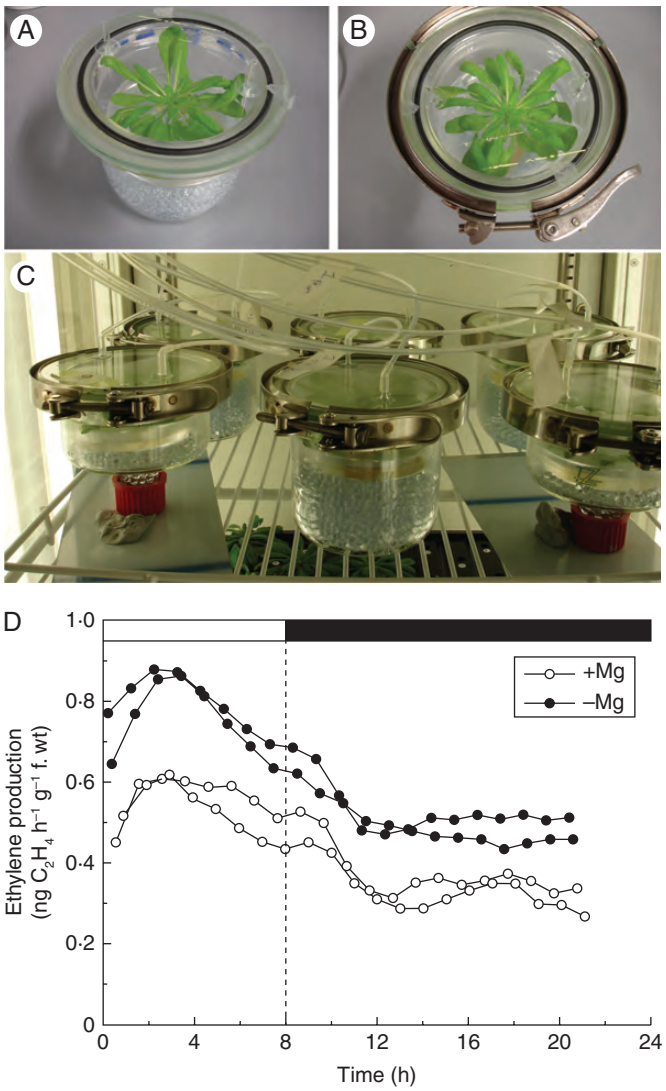


FIG. 7. Ethylene production upon Mg starvation in mature plants of *Arabidopsis thaliana* grown hydroponically. Plants (Col-0 accession) were grown hydroponically for 5 weeks in a short-day regime (8 h light/16 h darkness) with an Mg-fully replete (2 mM Mg^{2+}) solution and transferred for 1 week to the same solution or to an Mg-deplete solution. Ethylene measurements were done 7 d after transfer, while rosettes were still not showing signs of Mg-deficiency symptoms. (A, B) Cuvette containing one plant fed with nutrient solution. The cuvette was filled with glass beads to minimize the headspace volume. (C) Six cuvettes were continuously flushed with air at 1 L h^{-1} and ethylene monitored in succession of 10 min for each cuvette. (D) Evolution of ethylene production during a day/night cycle. The trends of gas emission for two individual plants are shown per treatment: +Mg = Mg-fully replete; –Mg = Mg-deplete.

Compact, transportable and user-friendly

Disadvantages of laser-based sensors.
Expensive
Single gas detection

Application: sensitive and fast ethylene monitoring. Due to their high sensitivity and fast response time the laser-based detectors can detect traces of ethylene emitted in various plant processes, thus making possible the transition from post-harvest research, requiring hundreds and tens of nL L^{-1} detection levels to fundamental research in plant physiology that requires monitoring of nL L^{-1} and sub-nL L^{-1} concentrations. As an example, we will take the recording of ethylene production from *Arabidopsis thaliana* under the depletion of two major essential elements (nitrogen and magnesium), to illustrate the suitability of the laser-based detectors for plant-physiology research. The detector (ETD-300; Sensor Sense) in combination with the gas handling system allows ethylene monitoring in three operation modes, namely continuous flow, stop-and-flow and sampling, respectively (Fig. 6).

Research into the roles of ethylene in signalling nutrient imbalances and in morphological and physiological responses of plants to mineral resource fluctuations is gaining interest. Interrelation between ethylene and mineral nutrition is frequently reported (Rubio *et al.*, 2009; Verbruggen and Hermans, 2012). In particular, modulation of the expression levels of several genes encoding enzymes in the ethylene biosynthesis, namely 1-aminocyclopropane-1-carboxylic acid

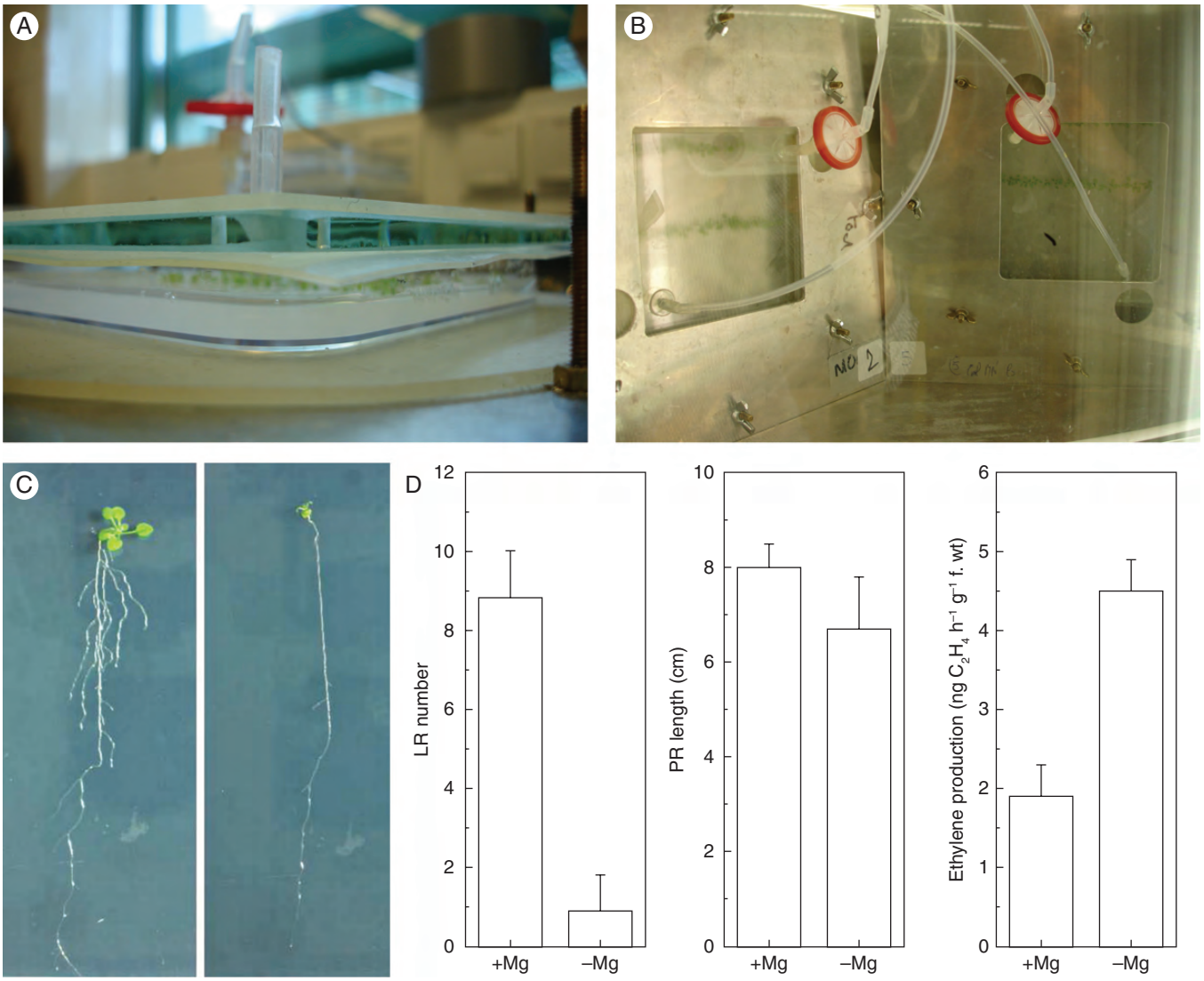
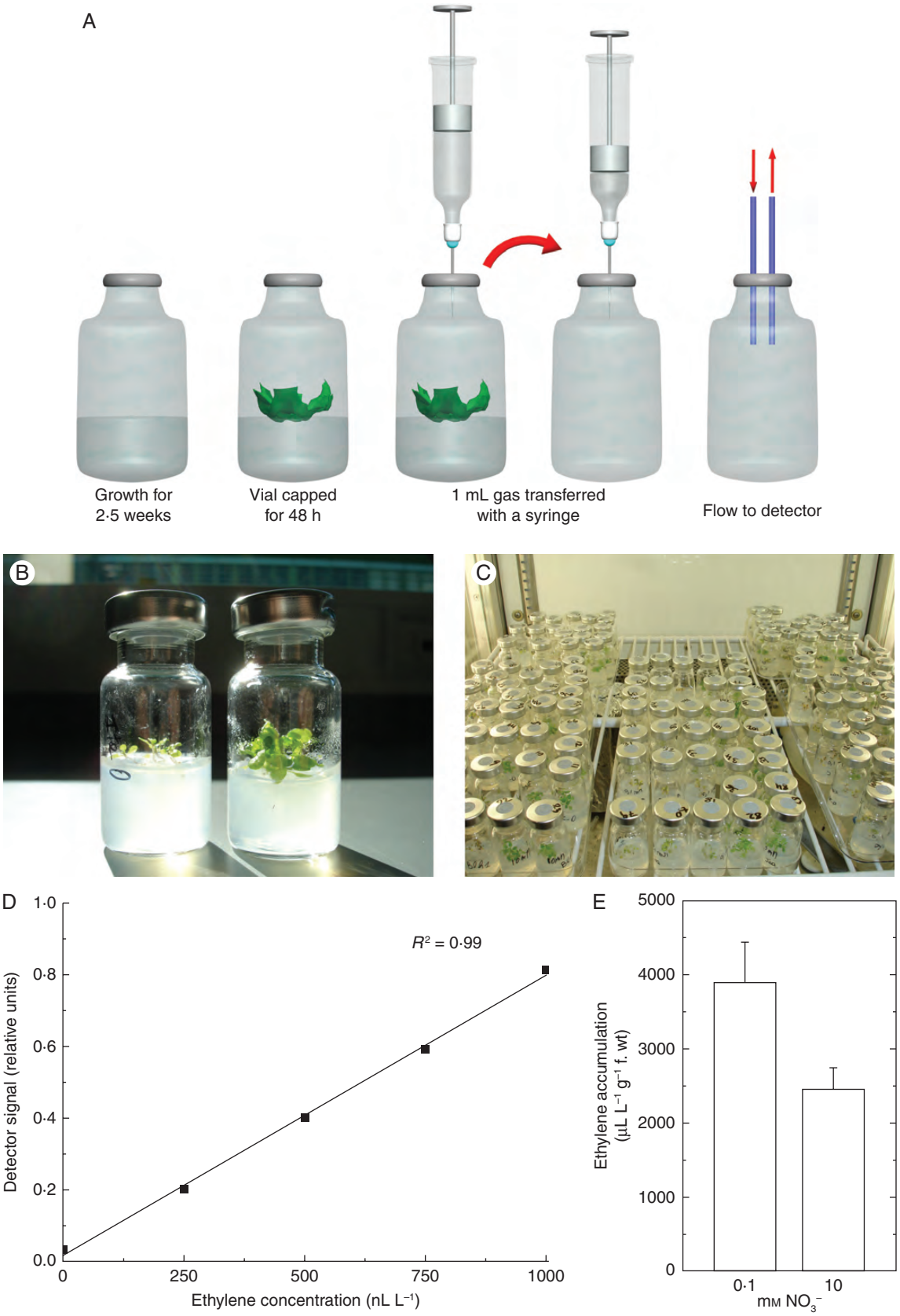


FIG. 8. Ethylene production as a function of the Mg supply in *Arabidopsis thaliana* seedlings grown *in vitro* on vertical plates. In (A) and (B) the custom -made cuvettes using Petri plates are shown. The plate was covered with a glass top having an inlet and outlet for gas flow, and assembled between two metal pieces. (C) Representative seedlings grown on agar media with 1·5 mM (left) or without (right) Mg^{2+} added. The pictures were taken 15 d after germination. (D) Means (± s.e.) for number of lateral roots (LR, visibly emerged, >1 mm) on primary root ($n = 15$ seedlings), primary root (PR) length ($n = 15$ seedlings), and ethylene production ($n = 3$ measured Petri plates containing 15 seedlings). +Mg, with 1·5 mM Mg^{2+}; –Mg, without Mg.



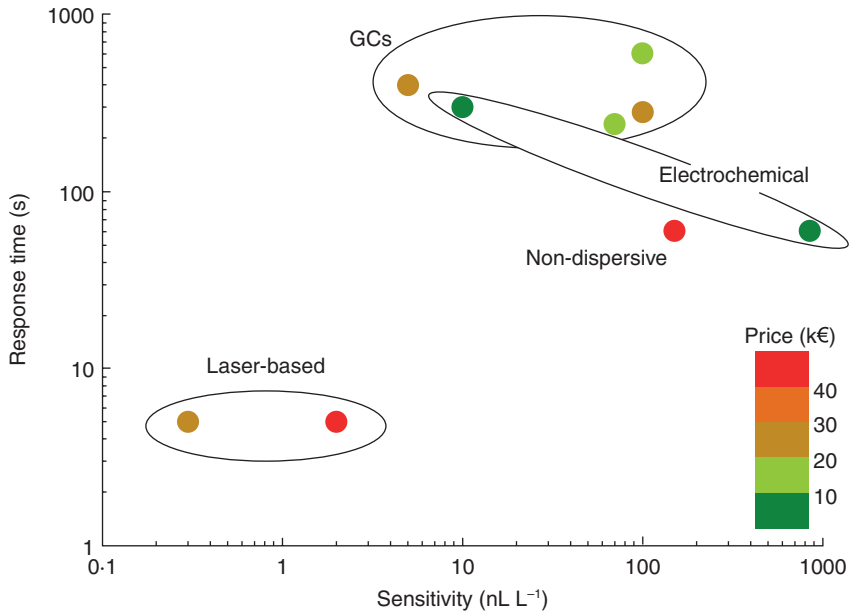


FIG. 10. Comparison of different sensors described in this study. Gas chromatographs, electrochemical sensors and non-dispersive and laser-based sensors are compared in terms of sensitivity (*x*-axis) and response time (*y*-axis). The colours are indicative of prices for the existing commercial devices. Note: the prices for GCs do not include an automatic sampler unit.

synthases and ethylene production were observed upon fluctuation of nitrogen (Khan *et al.*, 2008; Tian *et al.*, 2009; Iqbal *et al.*, 2011) and magnesium (Hermans *et al.*, 2010*a*, *b*) supplied to the plant.

Continuous flow. Up to six cuvettes with biological samples are continuously flushed with air at a constant flow rate of 1 l h^{-1} (Fig. 6). Ethylene released in the headspace is transported to the ETD-300 alternately, allowing a succession of typically 10 min for each cuvette. In this way, the dynamics of the ethylene emission can be studied in sufficient detail over several hours or for an even longer period. Here, we measured ethylene evolution of arabidopsis rosettes subjected to Mg depletion (Fig. 7); their growth conditions are described in detail elsewhere (Hermans *et al.*, 2010*b*). Briefly, 5-week-old plants *Arabidopis thaliana* (Col-0), grown hydroponically, were transferred to Mg-depleted nutrient solution for 7 d. At the end of the treatment, ethylene production was doubled in Mg-starved plants compared with control ones (Fig. 7D). A possible implication of ethylene as a response to Mg shortage could be via adaptation of the photosynthetic apparatus (Hermans *et al.*, 2010*b*).

Stop-and-flow. In some applications, the plant biomass is very small and the ethylene concentration is below the nL L^{-1} level. Therefore, in a stop-and-flow mode, ethylene is allowed to accumulate for a period of time (e.g. 1 h) before it is transported to the ethylene detector. While ethylene from a cuvette is measured (typically 15–30 min) no air flow is applied to the other cuvettes, hence optimizing the measurement efficiency. As such an ethylene production as low as 40 pL h^{-1} can be recorded. To illustrate that procedure, the ethylene production of very young seedlings, grown *in vitro* vertically on agar media was measured (Fig. 8A–C). Agar media (0·8 % w/v) contained 1× MS strength with 10 mM NO_3^- as the sole source of nitrogen. Fifteen days after germination, ethylene production was doubled in Mg-starved seedlings, which had smaller shoots and fewer lateral roots than control seedlings (Fig. 8D). Ethylene is identified as a central actor in shaping root architecture upon mineral element fluctuation (Tian *et al.*, 2009; Hermans *et al.*, 2011; Verbruggen and Hermans, 2012). Further characterization of the involvement of ethylene in the response to Mg limitation is essential.

Sample mode approach. Other applications require multiple samples to be screened. Ethylene production needs to be quantified once per sample, rather than monitored over time. Using the sample mode approach, ethylene production of arabidopsis seedlings, grown at two different nitrogen supplies, was measured. Six seedlings (Col-0) were grown *in vitro* in small vials (10 mL) for 2·5 weeks at a continuous light regime. Agar media (0·8 % w/v) contained 1× MS strength with 0·1 or 10 mM NO_3^- as the sole source of nitrogen (J. De Pessemier

FIG. 9. Ethylene production as a function of the nitrate supply in *Arabidopsis thaliana* seedlings grown *in vitro* in small vials. (A) Summary of the experimental procedure. Six seedlings (Col-0 accession) were gown *in vitro* in small vials (10 mL) for 2·5 weeks with a continuous light regime. Vials were capped for 48 h and then 1 mL was extracted with a syringe and injected into an empty vial. Vials were stored prior to their content flowing to the detector. (B) Picture of capped vials with plants grown at 0·1 mM (left) and 10 mM (right) NO_3^-. (C) Analysing the gas content of a vial with flow inlet and outlet. (D) Calibration curve with ethylene standards for these conditions. (E) Ethylene accumulation of seedlings grown at 0·1 and 10 mM NO_3^- ($n = 3$ measured vials, ± s.e.). Fresh biomass was 13 ± 2 mg or 41 ± 11 mg for six pooled seedlings grown at 0·1 or 10 mM NO_3^-, respectively.

et al., unpubl. res.). Vials were capped for 48 h and then 1 mL was extracted with a syringe and injected to an empty vial (Fig. 9A). The advantage of this procedure is that large amount of samples can be collected within a short time period and that vials can be stored prior to flowing their content into the detector (Fig. 9B, C). The detector was calibrated with known ethylene concentrations under similar gas transfer procedure (Fig. 9D). The biomass of seedlings grown with a low nitrate supply was almost three times lower than those grown with a high nitrate supply, while ethylene production was only one-third higher under the same conditions (Fig. 9E). Ethylene involvement has been linked with features critical for the improvement of the efficiency of nitrogen use by crops, namely the control of biomass production and the timing for senescence induction (J. De Pessemier *et al.*, unpubl. res.). The biodiversity in ethylene emission offered by a large set of natural arabidopsis populations can be exploited for a better understanding of the controlling function of ethylene over biomass production at low nitrogen input.

CONCLUSIONS AND OUTLOOK

Several types of sensors for monitoring ethylene production in plants were presented. Ideally, a sensor should monitor ethylene quickly, sensitive, with high selectivity and should be available at low costs. Unfortunately, such a sensor does not exist at the moment (Fig. 10). Therefore it is important to review your application information carefully, before deciding on the best-suited technology. Different applications require different solutions. If sensitivity and real-time analysis are required laser-based sensors should be considered. If less sensitivity and selectivity is needed, but portability is most important, electrochemical sensors are recommended. When more gases need to be measured, then a GC system is better suited.

Each type of sensor presented in this review has its own field of applications. The technical challenges for the future may include the improvement of sensitivity, selectivity and measuring time. One might imagine a faster GC system, a more selective electrochemical sensor or even a more sensitive laser-based detector. To fulfil such goals, new materials have to be designed and new laser sources must be developed.

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