Use of a Laser-Driven Photoacoustic Detection System for Measurement of Ethylene Production in Cymbidium Flowers

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ABSTRACT

A laser-based photoacoustic method was used for determination of ethylene (C2H4) production of emasculated orchid (Cymbidium) flowers in a flow-through system. The laser photoacoustic equipment consisted of a line-tunable CO2 laser in conjunction with a single-pass resonant acoustic cell. The minimum detection limit of the system for C2H4 in air was 0.03 nanoliter per liter. C2H4 production of intact Cymbidium (cv Mary Pinchess 'Del Rey') flowers was very low (0.015 nanoliter per gram per hour) and showed an increase within 3 hours following emasculations (removal of pollinia plus anthercap). Production peaked (0.14 nanoliter per gram per hour) 8 hours after emasculation and decreased thereafter. Production again increased 45 hours after emasculation. Coloration of the labellum appeared shortly after the first peak; wilting of the petals and sepals appeared during the second rise in ethylene production. The use of the laser photoacoustic technique in plant physiological studies is discussed.

These problems can be overcome through the use of a GC equipped with a PID (3) and, to a lesser extent, through the use of a system capable of controlling the carbon dioxide and oxygen concentrations (29). The PIDs are several-fold more sensitive than FIDs and have even been used to measure C2H4 production of plants in a flow-through system (3).

In another approach, collection of the gas is achieved by trapping it on a solid support maintained at a low temperature, as described by De Gref et al. (12) and by Bassi and Spencer (1). Although long sampling intervals are necessary in some cases, these methods allow measurements of C2H4 production of intact plants in a flow-through system.

In the last decades, LPA spectroscopy has been used for detection of atmospheric pollutants. High power, step-tuneable CO2 and CO lasers have been frequently used and, in principle, more than 250 gases of environmental interest (including C2H4) could be investigated with such a laser (17, 22).

The photoacoustic effect was discovered in 1881 by A. G. Bell (4) and has gained renewed attention following the advent of powerful infrared lasers. In photoacoustics, for instance, the radiation source (e.g. a CO2 laser beam) is periodically interrupted by means of a mechanical chopper. The chopped beam is directed into a small vessel (PA cell) containing for example a gaseous sample assumed to absorb at the emission frequencies of the laser.

Following the absorption of energy the gas molecules are excited from the ground state into a rotational level in a higher vibrational state and de-excitation processes will then redistribute the energy.

In the infrared region the probability for radiative decay is small and, at atmospheric pressure, relaxation generally takes place along the nonradiative channel. This causes an increase in kinetic energy of gas molecules and, hence, also of gas temperature.

Consequently, in a closed or semiclosed vessel of constant volume, the increase in temperature leads to a corresponding increase in pressure. When the radiation source providing the energy is modulated at audio frequency, the generated pressure changes (acoustic waves) can be detected by a microphone.

We report here on an LPA system for measurement of C2H4 production of ornamental products in a flow-through system. Orchid (Cymbidium) flowers were chosen because intact flowers show a low C2H4 production that can be manipulated by removal of the pollinia (emasculations), which includes removal of the anthercap (15).

MATERIALS AND METHODS

LPA Detection System. The LPA detection system used in this study consists of a modified version of a line-tuneable

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1 Abbreviations: C2H4, ethylene; LPA, (laser) photoacoustic; FID, flame ionization detector; PID, photo ionization detector; GC, gas chromatography.
infrared CO₂ laser (27) and a stainless steel “organ pipe” single-pass resonant PA cell (24). Most of the equipment was constructed in the laboratories of the University of Nijmegen.

The used CO₂ waveguide-laser operates in a direct current longitudinal discharge regime on a flowing mixture of He, N₂, and CO₂ (ratio 6:1:1) as active medium. In the laser tube the typical operating pressure is 80 mbar. The maximum output power of the laser amounts to 6 W at the used frequencies, and more than 80 discrete laser wavelengths between 9 and 11 μm can be selected (16). Selection of the desired wavelength was achieved by adjusting the position of the diffraction grating by means of a micrometer driven by a stepmotor. The laser-beam was periodically interrupted (mechanically) by a phase-stabilized chopper.

In order to get a maximum signal, the chopper was tuned to the resonance frequency of the PA cell filled with a mixture of ethylene in air (572 Hz). At this frequency a “standing-wave” is generated in the inner, open resonator of the PA cell (Fig. 1; length = 300 mm, diameter = 9 mm). The resonator in turn is mounted with airtight Teflon spacers in a larger tube. In this way the laser-induced heating of the cell windows does not couple efficiently into the main resonator, thereby minimizing its effect on the PA signal. The acoustic signal is detected by four miniature microphones (Knowless, type BT 1754) mounted in 1 mm diameter holes in the middle of the resonator. The microphone signal is further processed by synchronous detection at modulation frequency using a lock-in amplifier (EG & G, Princeton, NJ).

The responsivity of the PA cell was determined using a 95 nL/L certified mixture of C₂H₄ in N₂ (Matheson). In other experiments (data not shown), it was shown that the PA signal was strictly linear with the concentration of C₂H₄ (0.05 up to 1000 nL/L).

In an actual experiment, the relevant signals were fed to an Apple IIe computer for calculation of the C₂H₄ concentration.

Measurement of Ethylene Production. Ethylene production of individual Cymbidium flowers was measured in a flow-through system (flow rate 0.9 L/h) consisting of small glass cuvettes (volume 80 mL) fitted with in and outlet ports (Fig. 2). The cuvettes were also provided with a septum stoppered port to facilitate the emasculation treatment. A copper-constantan thermocouple connected to a data-logger was inserted in each cuvette to monitor temperature. Relative humidity inside the cuvettes was regularly measured using a “Murata” dew sensor coupled to an ohm-meter. The experiments were carried out under continuous light of about 15 μmol/m²·s.

Compressed air (0.035% CO₂) (v/v) was first passed through a copper tube (6 m) filled with a platinized aluminum oxide catalyst (at 350°C) to remove hydrocarbons. Through a flow-controller (Brooks) the purified air was admitted into the cuvettes.

Air from the cuvettes passed through a mass-flow meter (Brooks) and infrared carbon dioxide monitor (ADC) both connected to a data-logger, in order to monitor flow and CO₂ concentration, respectively. The air was then passed through a glass column (10 × 1 cm) filled with KOH grains to remove water and CO₂. Thereafter, the air was passed through the PA cell. When not in line with the PA cell, the effluent was vented to atmosphere.

The photoacoustic signal was measured at two laser wavelengths (10.51 and 10.53 μm) at which the absorption coefficients of C₂H₄ are known (5).

The experiments were repeated several times with essentially identical results.

Plant Material. Cymbidium flowers (cv Mary Pinchess ‘Del Rey’), obtained from commercial growers, were placed in the cuvettes well before start of the measurements in order to acclimatize. Each cuvette contained one flower placed in 10 to 20 ml water to avoid desiccation. By means of a lever (paperclip), fitted in a silicon rubber septum, emasculation (removal of pollinia plus anthercap) was carried out from the outside without changing the gaseous composition inside the cuvette.

RESULTS

The typical CO₂ laser photoacoustic spectrum of C₂H₄ is shown in Figure 3. Very high absorption (high microphone signal) was found at 10.53 μm while absorption at the adjacent laser transition (10.51 μm) was much lower. The C₂H₄ concentration was calculated from the microphone signal recorded at both discrete laser transitions, thereby eliminating possible interpretation errors due to the effect of interfering volatiles (such as odors) that generally have comparable absorption over a broad range of laser transitions.

The flow characteristic of the system was determined by injecting a known amount of C₂H₄ into an empty cuvette that was flushed with purified air at a flow rate of 0.9 L/h. The PA signal was monitored during a period of 1 h after injection (Fig. 4). The half response time was found to be about 10 min; the signal reached its initial level after approximately 1 h. The total volume of the system (including the PA cell) was calculated to be 225 mL. At this flow rate, switching between the various cuvettes will only yield valuable information if a sampling interval of at least 45 min is sustained.

When a Cymbidium flower was placed in the cuvette and an amount of water was added, the outlet air contained about 0.045% CO₂ (v/v) and was nearly saturated with water vapor (about 90% RH). The glass column packed with KOH grains reduced the CO₂ concentration and water vapor pressure to about 1 μL/L and 1 Torr, respectively. At these low levels, the contribution of these constituents to the PA signal is negligible. It was checked that the KOH grains did not retain any C₂H₄.

During the experiments no significant fluctuations in the air flow were found (less than 5%); the temperature fluctuations were within 1.0°C.

Figure 5 represents data from two cuvettes containing Cymbidium flowers. The rotary valve (see Fig. 2) was operated alternatively between a cuvette with an intact flower and a cuvette with an emasculated flower. Each cuvette was connected for a period of 45 min to the PA cell.

Changing from one cuvette to the other produces a rapid change in C₂H₄ concentration until, after about 40 to 45 min, the actual concentration in the cuvette is reached. This experi-

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FIG. 2. Schematic presentation of the experimental setup for measurement of C2H4 production.

FIG. 3. Photoacoustic signal at 84 discrete laser transitions between 9 and 11 μm of a gas mixture containing 1000 nL/L C2H4 in N2.

FIG. 4. Characteristic of the flow-through system (flow rate = 0.9 L/h). At t = 0, C2H4 was injected into the cuvette.

FIG. 5. Characteristic of the flow-through system (flow rate = 0.9 L/h). C2H4 concentrations (expressed as microphone signal) from a cuvette with an intact Cymbidium flower (---) and from a cuvette with an emasculated Cymbidium flower (-----) were measured for periods of 45 min. Emasculation was carried out 2 h before the start of the measurements.

Emasculation shows that sampling intervals of about 45 min are indeed necessary to obtain reliable measurements of the C2H4 evolution. The C2H4 concentration (expressed as the microphone signal) in the outlet of the emasculated flower showed an increase and a decrease during this experiment; the C2H4 concentration from the intact flower showed no significant fluctuations.

In another experiment, the C2H4 concentration at the outlet of a cuvette with an emasculated flower and of an empty cuvette was measured. The C2H4 concentration at the outlet of the empty cuvette was approximately 0.4 nL/L and showed no significant changes during the course of the experiment (data not shown). The difference in measured C2H4 concentration between the two
FIG. 6. Calculated ethylene production (right ordinate) and difference between ethylene concentration in the outlet air from an empty cuvette and from a cuvette with a single Cymbidium flower (left ordinate) as a function of time. Arrow indicates time of emasculation. After 24 h the experiment was interrupted briefly to change the laser supply gases. Flow rate = 0.9 L/h; temperature = 22°C.

cuvettes is the amount of C2H4 produced by the flower (Fig. 6; left ordinate). From these data, C2H4 production (nL/g fresh weight·h) was calculated (Fig. 6; right ordinate).

The calculated C2H4 production was found to be very low before emasculation (0.015 nL/g·h) with a relatively minor, but readily detectable change within 3 h after emasculation. The production peaked (0.14 nL/g·h) at about 8 h after emasculation and showed a gradual decline thereafter. The production rose again about 45 h after emasculation.

**DISCUSSION**

During the last decade, PA detection of photosynthetic activities has become a valuable tool in plant science. Applied to a leaf, the method yields information about the conversion of light into heat and, hence, indirectly about the conversion of light into chemical energy. Furthermore, it has been found that PA signals also reflect oxygen evolution in the leaves. The results of numerous experiments are summarized in review articles (e.g. 8, 19).

To our knowledge, no reports are available about the use of PA systems for detection of C2H4 emanation (from plants) in plant physiological studies.

The laser-driven PA system used in our study was extremely sensitive for determination of C2H4. The minimum detection limit was 0.03 nL/L in the present experiments, which is much more sensitive than any other method described. While concentrations were well below the nL/L level, on-line detection of C2H4 directly in the outlet of a flow-through system (flow rate = 0.9 L/h) with a single Cymbidium flower appeared possible, without accompanying problems arising from an inadequate control of the gaseous composition in the cuvette.

The relatively large volume of the PA cell, however, was a disadvantage in our experiments since, due to the flow characteristic of the system, interchanging between various cuvettes was possible only once per 45 min.

Constructing a smaller sized PA cell is theoretically possible and will eliminate this problem. In addition, it is also still possible to considerably improve the sensitivity of the LPA system either by using more sensitive microphones and a more powerful laser or by placing the PA cell inside the laser cavity (future work). With such a system, a sensitive detection at sampling intervals of several minutes becomes accessible.

An important advantage of the LPA method in comparison to the use of a GC either with PID or FID and collection unit is the direct character of detection, which makes it possible to accurately measure small changes in concentration upon a background of several nL/L C2H4. The use of extremely pure air (<0.1 nL/L C2H4), which is a prerequisite for proper functioning of the trapping methods, also introduces a steep concentration gradient between the site of C2H4 synthesis and the gaseous phase that might alter the pattern of C2H4 synthesis within the tissue (26).

In a number of flowers, senescence is thought to be mediated by endogenous C2H4 production. Flowers like carnations (21) and various orchid species (6, 15) show a large increase in C2H4 production prior to or during the wilting process. It may be suggested, however, that this increase in C2H4 production—although necessary for integration of the wilting process—is not the initial event in senescence because changes in membrane permeability and microviscosity have been observed well before the onset of the C2H4 peak (10, 13).

Since treatment with exogenous C2H4 hastens both the onset of the increase in C2H4 production and the changes in membrane properties, we suggest that fluctuation in the basal C2H4 production might play a role in triggering the senescence processes. The validity of this hypothesis can only be tested with very sensitive methods.

The role of endogenous C2H4 production in abscission is also a matter of debate (23). Changes in C2H4 production during this process might be restricted to specific tissues, and sensitive equipment is necessary to establish with certainty whether abscission is accompanied by increased C2H4 production.

The presented results on C2H4 production in emasculated Cymbidium flowers demonstrate two distinct, readily detectable peaks following emasculation. The first peak is relatively small and has apparently not been detected by other authors who studied the C2H4 production in emasculated Cymbidium flowers (9, 15).

The first peak coincides with coloration of the labellum, the second increase in C2H4 production coincides with wilting of the sepals and petals. In intact flowers these symptoms (coloration and wilting) manifest themselves after a much longer time span.

It is therefore suggested that the first (relatively small) peak in C2H4 production following emasculation initiates both the synthesis of anthocyanins in the labellum and the autocatalytic process responsible for wilting of the sepal and petals in these flowers. This view is supported by the observation that treatment of these flowers immediately after emasculation with the gaseous inhibitor of C2H4 action, 2,5-norbornadiene (25), at 1000 μL/L considerably delays the development of emasculation-induced coloration and wilting (data not shown).

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