LASER PHOTOACOUSTICS: A NOVEL METHOD FOR ETHYLENE DETERMINATION IN PLANT PHYSIOLOGICAL STUDIES.

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Abstract

A CO_2 laser-based photoacoustic method was used to determine the initial changes in ethylene production following emasculation of selected orchid species.

The laser photoacoustic equipment consisted of a line tunable CO_2 laser in conjunction with a single pass resonant acoustic cell. The minimum detection limit of the system for ethylene was 0.03 nl/l.

In all tested orchid species an increase in ethylene production was measured within a few hours after emasculation. The time dependent pattern of ethylene production in *Cymbidium* and *Oncidium* showed an optimum. In *Phalaenopsis* and *Epidendrum* it was characterized by a gradual increase.

The principle of photoacoustics and its application in plant physiological studies is discussed.

1. Introduction

Ethylene evolution from intact plants and ornamental products such as flowers, is generally very low. When a standard gas chromatograph (GC) equipped with a flame ionization detector (FID) is used, the products have to be placed in a closed environment for a prolonged period, allowing ethylene to accumulate. A sample of the head space may then be analysed for ethylene. This method does not allow detailed kinetic studies and the changing concentrations of carbon dioxide, oxygen and ethylene may interfere with ethylene production (Dhawan et al., 1981; Kao and Yang, 1982; Bassi and Spencer, 1983; Yang and Hoffman, 1984).

In order to avoid problems arising from the changed gaseous composition, collection methods (De Greef et al., 1976; Bassi and Spencer, 1979), and the use of a GC equipped with a photo ionization detector (PID) have been used. PID's appeared to be several-fold more sensitive than the FID (Bassi and Spencer, 1985).

Recently, laser photoacoustic (LPA) spectroscopy has been described for analysis of ethylene in the atmosphere (Perlmutter et al., 1979).

When used with a CO_2 laser as the radiation source, this technique possesses high sensitivity and would allow on-line measurement of ethylene from plants in a continuous flow system.

We report here on a laser-driven photoacoustic (PA) system capable of determining low concentrations of ethylene in a flow-through system. The system was used to measure ethylene evolution of selected orchid species after removal of the pollinia (emasculation) which includes removal of the anther cap.

2. Basic Principle of photoacoustic (PA) spectroscopy in gases

The PA effect is based on the generation of acoustic waves due to the deposition of heat in the sample following the absorption of energy. The effect was discovered by A.G. Bell (1881) on solids and was confirmed by Tyndall and Röntgen on aqueous and liquid samples.

An early photoacoustic set-up using the frame of an old sewing machine is shown in figure 1. The radiation from the sun was periodical interrupted by means of a mechanical chopper and directed into a small vessel containing a gaseous sample that absorbs in the infra-red. Bell and contemporaries listened with a stethoscope connected to the vessel and perceived the feeble sound production at a frequency that corresponded to that at which the radiation source was modulated.

Nowadays it is understood that this optically induced sound production results from the periodical absorption of the infra-red portion of the energy provided by the sun. Following the absorption of energy, the gas molecules are excited from the ground state into the rotational level of a higher vibrational state and de-excitation processes will then redistribute the energy (Fig. 2).

In the infra-red region the probability for radiative decay is small and, in general, relaxation takes place along the non-radiative channel. For the majority of molecules the relaxation time at atmospheric pressure is fast (in the order of μs) as the rotational-translational de-excitation through collisions prevails. It causes an increase in kinetic energy of gas molecules and hence also of gas temperature.

Consequently, in a vessel of constant volume the increase in temperature leads to a corresponding increase in pressure. Thus, when the radiation source is modulated at audio frequency, the generated pressure changes can be detected by a microphone. The magnitude of the generated signal is proportional to the number of absorbing molecules (concentration) and the amount of power absorbed by the sample.

In principle, regardless of the excitation beam (electro-magnetic radiation from very short to very long wavelengths, charged particles or other) the detection principle remains the same (Rosencwaig,1980).

Fifty years after Bell's discovery, the almost forgotten PA effect found its first application when Viengerov constructed an instrument to acoustically record spectra of N_2O. Later on concentration measurements were also performed (Viengerov, 1938).

A revival of interest in photoacoustics was brought about by the advent of the laser. Soon after Kerr and Atwood (1968) demonstrated the usability of the laser to acoustically record the weak spectra of water vapour, Kreuzer (1971) used the He-Ne laser to investigate methane-nitrogen mixtures. When a high-power light source is used, the PA method becomes comparable or even superior to other spectroscopic techniques.

A true upsurge of interest has been noticed in the last decade, in particular for quantifying low concentrations of the atmospheric pollu-

tants. In principle, more than 250 gases of environmental interest could be investigated with a CO_2 laser (Hubert, 1983).

Besides having a large number of other applications of this and related techniques in agricultural, medical and other life sciences, in process engineering and areas of natural science, photoacoustic detection of photosynthetic activities has become a valuable tool in plant-physiological studies (Malkin and Canaani, 1988). The experimental results presented in this report show that the LPA technique provides a very sensitive method for determining minute changes in ethylene evolution from ornamental products.

3. Experiments with orchid flowers

The PA equipment used in our experiments consisted of a line-tunable CO_2 laser and a single pass resonant PA cell (Harren et al., 1987). The acoustic signal was detected by four miniature microphones located in small holes in the middle of the inner resonator (Fig. 3).

The microphone signal was consistently measured at two laser wavelengths (10.51 and 10.53 μm) with known absorption coefficients for ethylene (Brewer et al., 1982). From these data, the ethylene concentration could be calculated without problems arising from interpretation errors due to the presence of interfering volatiles (such as odors).

Ethylene production of individual orchid flowers was measured in a continuous flow system (0.9 l/h) consisting of small glass cuvettes fitted with in- and outlet ports (Fig. 4). The cuvettes were also provided with a septum-stoppered port to facilitate the emasculation treatment (i.e. removal of the pollinia which includes removal of the anther cap). Emasculation was carried out with a paper clip through the septum, thus not changing the gaseous composition in the cuvette.

A detailed description of the experimental set-up will be published elsewhere (Woltering et al., in press).

The changes in ethylene production following emasculation were calculated from the ethylene concentration measured in the outlet air (Fig. 5). Ethylene production of emasculated flowers was expressed as the difference between emasculated and intact flowers, the latter produced ethylene at a steady, very low rate (~ 0.01-0.02 nl/g.h) that did not show significant fluctuations during the course of the experiment.

In *Cymbidium* and *Oncidium* flowers, a relatively small ethylene peak appeared shortly after emasculation (Fig. 5), followed some days later by a more pronounced increase accompanying senescence (data not shown). In *Phalaenopsis* and *Epidendrum* there was a gradual increase in ethylene production starting almost immediately after emasculation which developed into a much more pronounced increase starting at about 12 h after emasculation (fig. 5). This upsurge in ethylene production lasted several days and coincided with wilting of the flowers (data not shown).

4. Discussion

The laser-driven PA system used in our study was very sensitive for determination of ethylene. The minimum detection limit in the present experiments was 0.03 nl/l, which is much lower than in any other method described so far. On-line detection of ethylene directly at the outlet

of a continuous flow system with a single flower appeared possible without accompanying problems arising from an inadequate control of the gaseous composition in the cuvette. The measurements therefore represent a true estimate of the ethylene production following emasculation in a natural environment.

The relatively large volume of the PA cell (~ 250 ml; Fig. 3) was a disadvantage in our experiments since, due to the flow characteristic of the system, interchanging between the various cuvettes was possible only once per 25 min. Therefore, the construction of a smaller PA cell will now have our attention.

In a number of flowers senescence is thought to be mediated by endogenously produced ethylene. These flowers show a large increase in ethylene production prior to or during the wilting process (Nichols, 1966; Goh et al., 1985). It is not clear, however, which events trigger this autocatalytic process.

In orchid flowers the autocatalytic ethylene production can be hastened by emasculation (Burg and Dijkman, 1967; Goh et al., 1985); in carnation flowers it can be advanced by treatment of the flowers with exogenous ethylene (Mayak et al., 1977).

The experiments with emasculated orchid flowers show that there is already a small change in ethylene production within a few h after emasculation. This minor change is later followed by a much more pronounced change that coincides with wilting of the flowers. We therefore suggest that these early changes in ethylene production play a role in the onset of the autocatalytic process and the senescence symptoms in these flowers.

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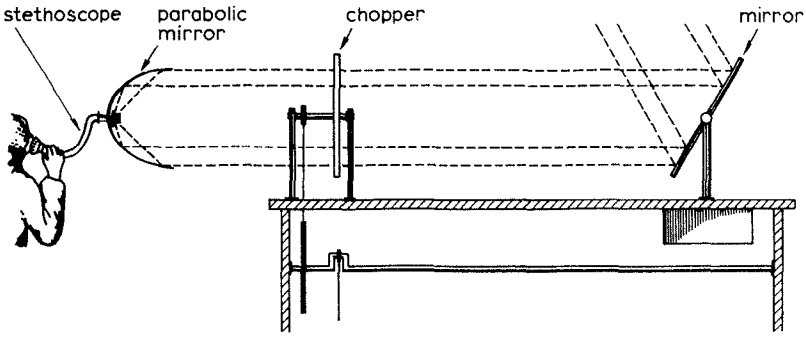

Figure 1: Early photoacoustic detector (Bell, 1881).

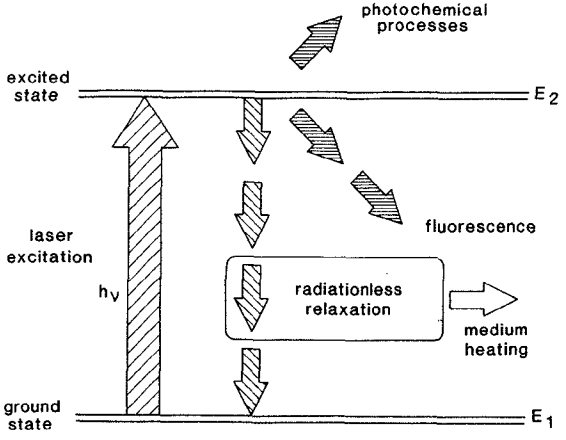

Figure 2: Redistribution of energy on absorption of radiation from the excitation beam. Radiationless relaxation gives rise to a photoacoustic signal (Zharov and Letokhov, 1986).

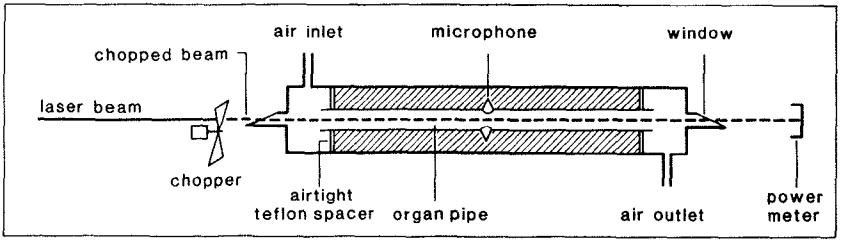

Figure 3: Schematic presentation of the laser photoacoustic detection system (Woltering et al., in press)

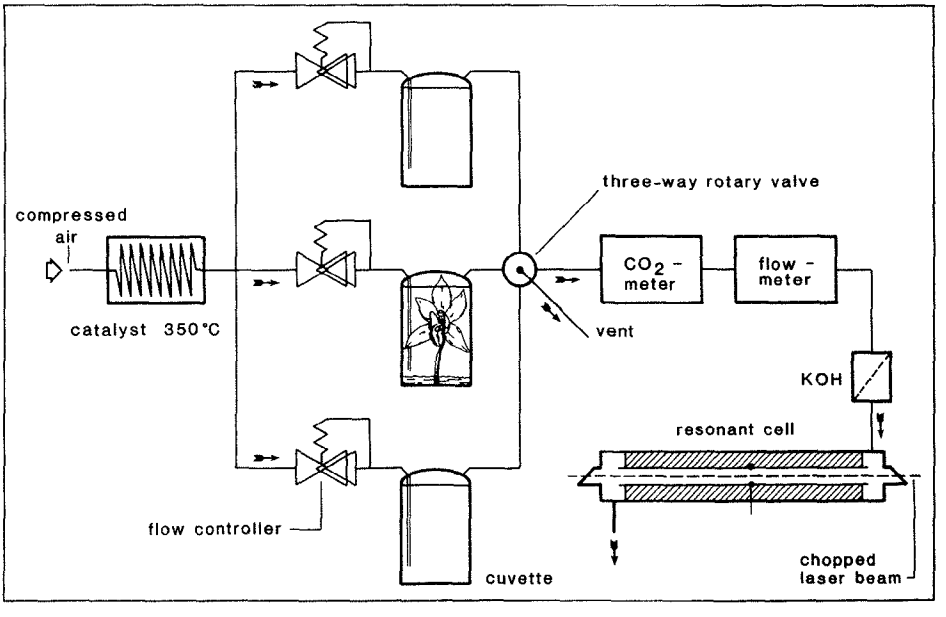

Figure 4: Schematic presentation of the experimental set-up for measurement of ethylene production (Woltering et al., in press)

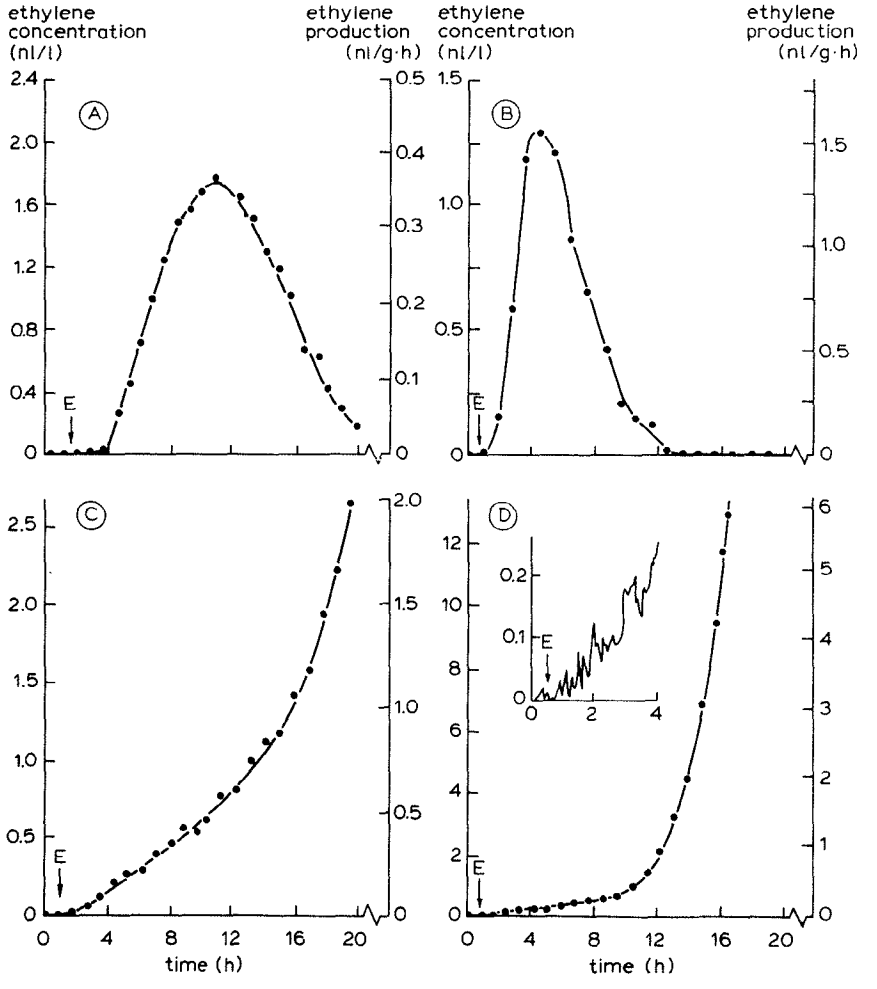

Figure 5: Calculated ethylene production (right ordinate) and difference between ethylene concentration in the outlet air from a cuvette with an intact flower and a cuvette with an emasculated flower (left ordinate) as a function of time. Arrow indicates time of emasculation.
Flow rate = 0.9 l/h; Temperature = 21.5 ± 0,5°C; Relative humidity = 50 ± 10%; Carbon dioxide concentration = ~ 0.04%
A = *Cymbidium* Mary Pinchess 'Del Rey';
B = *Oncidium splendidum*
C = *Epidendrum* (hybrid);
D = *Phalaenopsis* 'Red Lips';