U.P.B. Sci. Bull., Series A, Vol. 75, Iss. 3, 2013 ISSN 1223-7027

ETHYLENE CONCENTRATION AT FRUITS UNDER AEROBIC vs. ANAEROBIC CONDITIONS

Stefan BANITA[1] , Cristina POPA[2], Mihai PATACHIA[3] and Dan C. DUMITRAS[4]

Laser photoacoustic spectroscopy (LPAS) is a highly sensitive method for analyzing low molecular weight gases such as the natural plant hormone ethylene. Plants lack glands that produce hormones; instead, each cell is capable of producing hormones. Under normal conditions, plants grow and reproduce; yet, plants are often faced with a changing – sometime extreme – environment that can cause unfavorable conditions and in such an environment plants are considered to be stressed.

To evaluate the concentration of ethylene released by plants we have used a very performant laser photoacoustic system able to measure concentrations at sub-ppb level. We started the measurements to compare the ethylene concentrations of fruits under aerobic conditions compared to fruits under anaerobic conditions.

In this work we measured the level of ethylene at fruits both under stress conditions and in normal conditions.

Trace gas detection techniques based on photoacoustic spectroscopy make it possible to discover and control plant physiology mechanisms.

Keywords: ethylene biosynthesis, ACC synthase, photoacoustic spectroscopy

1. Introduction

Ethylene (C_2H_4) is a colorless gas that is naturally produced by plants and acts as a plant (growth) hormone. Fruits may be classified depending on their response to C_2H_4. Climacteric species produce C_2H_4 as they ripen, and the harvested produce is capable of ripening during the postharvest period. These fruits, such as bananas, apples, and peaches, tend to get sweeter and softer after harvest.

[1] Reseach assist., Department of Laser, National Institute for Laser, Plasma and Radiation Physics, Magurele, Bucharest, ROMANIA and PhD Student at University POLITEHNICA of Bucharest, Faculty of Applied Science, Romania , e-mail: stefan.banita@inflpr.ro

[2] CS3, Department of Laser, National Institute for Laser, Plasma and Radiation Physics, Magurele, Bucharest, ROMANIA

[33] Reseach assist., Department of Laser, National Institute for Laser, Plasma and Radiation Physics, Magurele, Bucharest, ROMANIA and PhD Student at Faculty of Physics, University of Bucharest, Romania

[4] CS1, Department of Laser, National Institute for Laser, Plasma and Radiation Physics, Magurele, Bucharest, ROMANIA

Non-climacteric plants, such as leafy vegetables, do not continue to ripen after harvest; they will soften and rot, but this is due to moisture loss, decay, and tissue deterioration.

So, a climacteric fruit can be picked at full size or maturity, but before it is ripen. Generally, there is an increase in flavor quality, juice, sugars and other factors. Non climacteric fruits tend to maintain what ever quality they had at harvest without many beneficial changes. More technically, in climacteric fruit, ripening is controlled by the fruits production of C_2H_4 and a significant increase in CO_2 production. Non climacteric fruits produce little or no C_2H_4 and no large increase in CO_2 production. Some fruits are picked full sized and green in color and held under refrigeration with C_2H_4 gas added to make them suitable for sale. It seems that much of what we know about the ripening of non climacteric fruit remains poorly understood [1, 2].

The definition of a climacteric fruit has three distinct component parts: (a) an autocatalytic increase in C_2H_4 production; (b) an associated increase in respiration, which is referred to as the respiratory climacteric; and (c) that (a) and (b) are accompanied by phenotypic (and genetic) changes in the fruit that lead them to be identified as ripe [3-5].

The division of fruits into climacteric and non-climacteric types has been very useful for postharvest physiologists. However, some fruits, for example kiwifruit and cucumber, appear to blur the distinction between the groups. Increase in respiration rate occurs during stress and other developmental stages, but a true climacteric only occurs coincident with fruit ripening [5-7].

In Table 1 is given a general classification of fruits according to their respiratory behavior during ripening.

Consequently, we used a sensitive photoacoustic technique for the detection and monitoring of C_2H_4 at low levels. The instrument, a CO_2 laser-based photoacoustic spectroscopy, ensures high output power in a wavelength region (9-11 μm) where more than 200 molecular gases of environmental concern for atmospheric, industrial, medical, military, and scientific spheres exhibit strong absorption bands. This laser can be only stepwise tuned when operated in cw, and is an ideal source to push the sensitivity of PA (photoacoustic) gas detection into the concentration range of part-per-bilion-by volume (ppbV) or even lower [8, 9].

Table 1

Examples of climacteric and non-climateric fruits

Climacteric			**Non-Climacteric**	
Apple Papaya Apricot Passion fruit Avocado Peach Banana Pear, Feijoa	Soursop Fig Tomato Guava Watermelon Jackfruit Kiwifruit Mango	Biriba Persimmon Breadfruit Plum Muskmelon Nectarine Cherimoya Sapote	Blueberry Cacao Caju Cherry Cucumber Grape Grapefruit	Lemon Lime Olive Orange Pepper Pineapple Strawberry Tamarill

LPAS was used to quantify the C_2H_4 that is produced by green apricots, cherries and zucchini fruits while they developed in planta. We investigate the physiological response of fruits under aerobic vs. anaerobic conditions.

Like the other plant hormones, C_2H_4 is considered to have pleiotropic effects. This essentially means that it is thought that at least some of the effects of the hormone are unrelated. What is actually caused by the gas may depend on the tissue affected as well as environmental conditions. In the evolution of plants, C_2H_4 would simply be a message that was coopted for unrelated uses by plants during different periods of the evolutionary development [10].

2. Ethylene biosynthesis

C_2H_4, regulates many diverse metabolic and developmental processes in plants. It has been shown that C_2H_4 is produced from essentially all parts of higher plants, including leaves, stems, roots, flowers, fruits, tubers, and seedlings [11].

The biosynthesis of the endogenous hormone C_2H_4 inside plant tissues depend on the activities of certain enzymes, the rate of outward diffusion and the rate of metabolization: it starts with the conversion of the amino acid methionine into S adenosyl-L-methionine (SAM or AdoMet) by the enzyme Met Adenosyltransferase. SAM is then converted into 1-aminocyclopropane-1-carboxylic-acid (ACC) by the enzyme ACC synthase (ACS). The activity of ACS is the rate-limiting step in C_2H_4 production, therefore regulation of this enzyme is the key for the C_2H_4 biosynthesis (figure 1). The final step requires oxygen and involves the action of the ACC-oxidase enzyme (ACO), formerly known as the C_2H_4 Forming Enzyme (EFE) [12-18].

C_2H_4 biosynthesis can be induced by endogenous or exogenous C_2H_4. The action of C_2H_4 is not only controlled by endogenous ethylene concentrations in tissues, but also by the tissue sensitivity. It is widely assumed that molecules involved in C_2H_4 perception and in the transduction of the signal probably controls how much ethylene is required to evoke a physiological response.

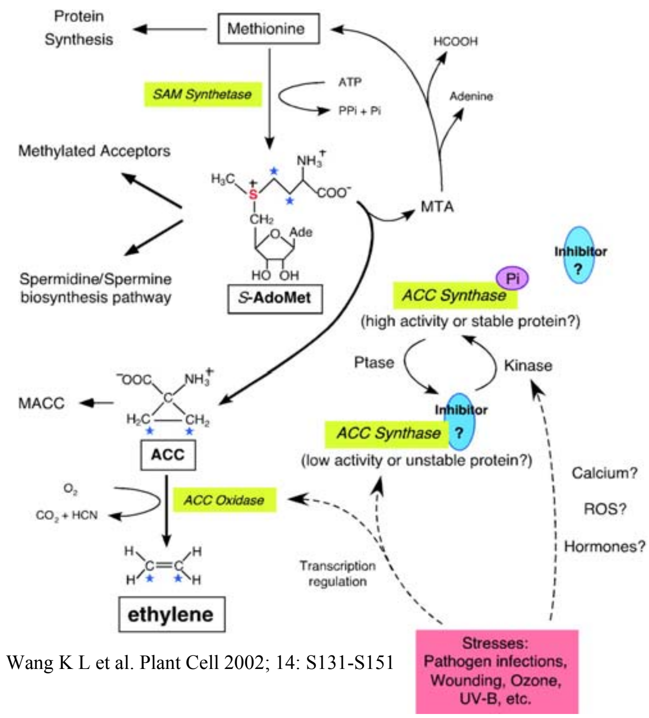

Fig. 1. The ethylene biosynthetic pathway and the methionine cycle

Environmental clues can induce the biosynthesis of the plant hormone. Flooding, drought, chilling, wounding, and pathogen attack can induce C_2H_4 formation in the plant.

In flooding, root suffers from lack of oxygen, or anoxia, which leads to the synthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC). ACC is transported upwards in the plant and then oxidized in leaves. The product, the C_2H_4, causes the epinasty of the leaves.

One speculation recently put forth for epinasty is that downward pointing leaves may act as pump handles in the wind. The C_2H_4 may or not additionally induce the growth of a valve in the xylem, but the idea would be that the plant would harness the power of the wind to pump out more water from the roots of the plants than would normally happen with transpiration [19].

Plant Responses to C_2H_4: stimulates leaf and flower senescence; stimulates senescence of mature xylem cells in preparation for plant use; inhibits shoot growth except in some habitually flooded plants like rice; induces leaf abscission; induces seed germination; induces root hair growth – increasing the efficiency of water and mineral absorption; induces the growth of random roots during flooding; stimulates epinasty – leaf petiole grows out; leaf hangs down and curls into itself; stimulates fruit ripening; induces a climacteric rise in respiration in some fruit which causes a release of additional C_2H_4; affects neighboring individuals; disease/wounding resistance; triple response when applied to seedlings – stem elongation slows, the stem thickens, and curvature causes the stem to start growing horizontally; inhibits stem growth outside of seedling stage; stimulates stem and cell broadening and lateral branch growth also outside of seedling stage; synthesis is stimulated by auxin and maybe cytokinin as well; C_2H_4 levels are decreased by light; the flooding of roots stimulates the production of ACC which travels through the xylem to the stem and leaves where it is converted to the gas; interference with auxin transport (with high auxin concentrations); inhibits stomatal closing except in some water plants or habitually flooded ones such as some rice varieties, where the opposite occurs (conserving CO_2 and O_2); where C_2H_4 induces stomatal closing, it also induces stem elongation; induces flowering in pineapples [19-21].

3. Plant material and method

Fruits were obtained from local (based on the inside of Magurele-Romania) and international producers (supermarkets). The fruits were kept in a refrigerator for several hours and then introduced into a small glass cuvette (Fig. 2), connected to the PA (photoacoustic) cell, with volume of 150 cm^3 at room temperature.

Fig. 2. The device used to contain fruits for the prelevation of the gas samples

The emitted C_2H_4 concentration measurement is analyzed by LPAS technique because it offers a high sensitivity that makes possible to evaluate absorptions coefficients on the order of 10^{-8} cm^{-1}. In a LPAS analysis the target molecules are excited through the absorption of laser radiation tuned at resonance with an active vibrational transition. The subsequent release of molecular internal energy, mainly due to *V–T* (vibration-translation) relaxation (*R*) processes, generates a pressure modulation in the gaseous medium. The signal is usually recorded with a microphone.

In order to increase the sensitivity and selectivity of the technique, the non-radiative relaxation pathways cross section is usually enhanced by adding a fixed amount of a buffer gas to the sample. Also, we took several supplementary measures, such as small glass cuvette for preserving the gas sample, or traps filled with potassium hydroxide (KOH) for retention of the CO_2 and water vapors.

A schematic diagram of the experimental apparatus for LPAS detection of C_2H_4 at trace level is given in Figure 3.

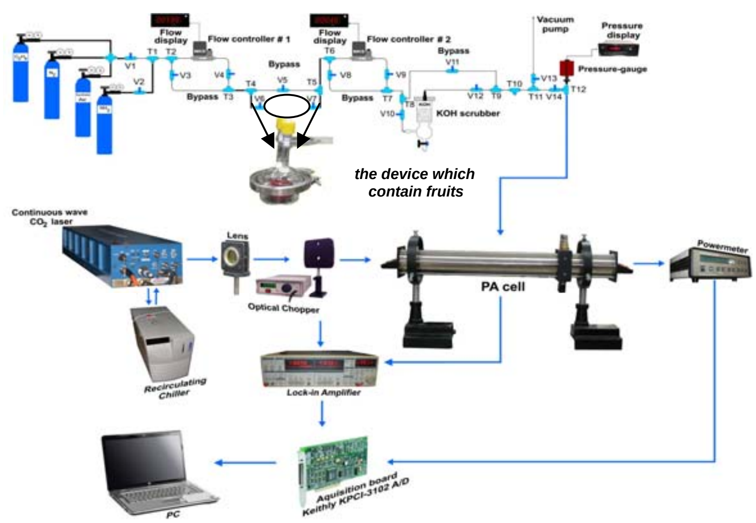

Fig. 3. Scheme of the LPAS system for ethylene detection

The radiation from a cw frequency stabilized and line-tunable CO_2 laser (tuned on the 10P (14) emission line at 10.53 μm) was modulated by a chopper (model DigiRad C-980: operated at the appropriate resonant frequency of the PA (photoacoustic) cell - 564 Hz). The generated laser pulses were then slightly focused by a lens into a PA (photoacoustic) stainless steel cell sealed with coated ZnSe optical windows. Four sensitive miniature microphones (Knowles electret EK-3033 or EK- 23024 connected in series) are placed at the center of the PA (photoacoustic) cell and are extremely sensitive to pressure changes inside the PA (photoacoustic) cell. The electric signal from the microphones, (after being visualized on an oscilloscope), was coupled to a lock-in amplifier (Stanford Research Systems model SR 830) synchronized with the frequency of the chopper. The processed signal was then transmitted by a data acquisition interface to a computer for data processing and storage. More details about the PA (photoacoustic) cell and the experimental protocol are found in other publications [22-26].

4. Results

The measurements were made for organic and non-organic fruits, but a clear delimitation is possible only with supplementary analysis. For this reason, all the fruits were considered non-organic.

Cherries (about 20 g), zucchinis (about 150 g) and green apricots (about 25 g) were kept at 4^0C in a refrigerator for several hours and then introduced into a small glass cuvette, at room temperature (25^0 C). Before starting C_2H_4 emission monitoring, the fruits were allowed to acclimatize for 2 hours. They were continuously flushed with an air flow of 1 L/h. After about 30 minutes from the measurement start, when the C_2H_4 production became stable, the air flow (aerobic conditions) was gradually reduced and replaced by a nitrogen flow (anaerobic conditions) of 1 L/h.

To analyze the glass cuvette contents, firstly we evacuated thoroughly the previous gas mixture from all the handling system, including the PA (photoacoustic) cell, traps, pipes etc., and then we flushed the system with pure nitrogen at atmospheric pressure for 10-15 minutes. After a second vacuum cleaning, the gas from the sample was transferred in the PA (photoacoustic) cell and analyzed.

Fig. 4 presents the levels of C_2H_4 experimentally measured for cherries and zucchinis under aerobic vs. anaerobic conditions.

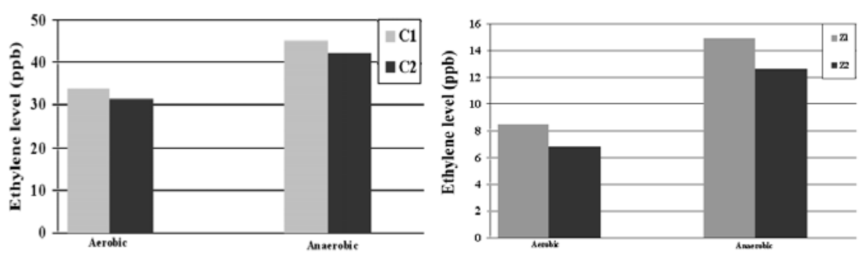

Fig. 4. Ethylene concentrations at cherries and zucchinis under aerobic/anaerobic conditions

The concentration level of C_2H_4 for the cherries (C1, C2) before the interruption of air flow was ~ 35 ppb and 30 ppb and after the air flow replacement with nitrogen flow increases to ~ 38 ppb and ~ 36 ppb respectively. For the zucchinis (Z1, Z2) the level of C_2H_4 was ~ 8 ppb and ~ 5ppb before the air flow interruption and ~ 12 ppb and ~ 9 ppb for nitrogen flow.

In Fig. 5 it was illustrated the C_2H_4 concentrations for green apricots (A1, A2) for aerobic and anaerobic process. We can observe that before the air flow interruption, C_2H_4 was about 2.5 ppb and 3 ppb respectively for two samples, whereas after air flow replacement with nitrogen flow the C_2H_4 concentration increases at about 3 ppb and 4 ppb, respectively.

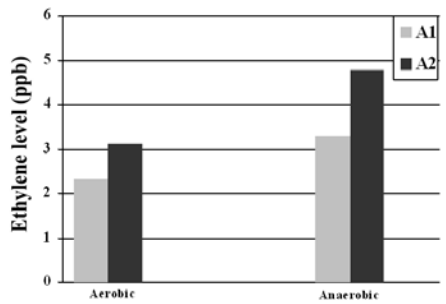

Fig. 5. Ethylene concentration at green apricots under aerobic/anaerobic conditions

So, all the fruits (cherries, zucchinis and green apricots) that were monitored by us produced C_2H_4 (Figs. 4 and 5). Since C_2H_4 biosynthesis requires the presence of O_2, the replacement of aerobic by anaerobic conditions determined an increase of C_2H_4 concentration. Anaerobic stress accelerates synthesis and accumulation of C_2H_4.

All the study and the results exposed here are only preliminary measurements. In order to verify this hypothesis further determinations are required. We have exemplified the potential of laser photoacoustic spectroscopy to detect and measure quantitatively very low traces of C_2H_4 in plant physiology.

5. Conclusions

In this work we investigate the level of ethylene at cherries, zucchinis and green apricots under stress conditions and under normal conditions with help of LPAS. We obtained a higher level of ethylene at fruits under stress conditions, whereas for fruits under aerobic conditions we obtained a lower level of ethylene.

The possibilities of LPAS and PA (photoacoustic) cell design are not yet fully utilized and hence the aim of its numerous applications in different fields of science can be widened. The future of low cost applications of LPAS may be the realization of fully integrated micromachined photoacoustic instruments. The application of LPAS to complicated materials and systems are possible only with the parallel improvement in the technique in terms of methodology and instrumentation.

Acknowledgments

Authors thank to Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-RU-TE-2011-3-0269 for financial assistance in the form of research grant.

R E F E R E N C E S

[1]. *C. B. Watkins, J. F. Nock*, “Production Guide for Storage of Organic Fruits and Vegetables” NYS IPM Publication No. 10, Department of Horticulture, Cornell University (2012).
[2]. http://www.quisqualis.com
[3]. *P. P. M. Iannetta, L.-J. Laarhoven, N. Medina-Escobar, E. K. James, M.T. McManus, H. V. Davies, F. J. M. Harren* “Ethylene and carbon dioxide production by developing strawberries show a correlative pattern that is indicative of ripening climacteric fruit”, Physiologia Plantarum, Vol. 127, Issue 2, pp: 247–259, (2006).
[4]. *L. Trainotti, A. Pavanello, G. Casadoro* , “Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits?” J. of Exp. Botany, Vol. 56, Issue 418, pp. 2037-2046 (2005).
[5]. *Z. Lin, S. Zhong, D. Grierson*, “Recent advances in ethylene research”, J.of Exp.Botany, Vol. 60, Issue 12, pp. 3311-3336, (2009).
[6]. *A. B. Bleecker, H. Kende* “Ethylene: a gaseous signal molecule in plants”, Annu. Rev. Cell Dev. Biol. Vol. 16, pp 1-18 (2000).
[7]. www.postharvest.com.au
[8]. *D. C. Dumitras, D. C. Dutu, C. Matei, A. M. Magureanu, M. Petrus, C. Popa* “Laser photoacoustic spectroscopy: principles, instrumentation, and characterization”, Journal of Optoelectronics and Advanced Materials, Vol. 9, No. 12, pp. 3655-3701 (2007).
[9]. *D. C. Dumitras, D. C. Dutu, C. Matei, A. M. Magureanu, M. Petrus, and C. Popa*, ’’Improvement of a laser photoacoustic instrument for trace gas detection”, U. P. B. Sci. Bull., Series A, Vol. 69, No. 3, pp. 45-56 (2007).
[10]. http://en.wikipedia.org
[11]. *K. L.-C. Wang, H. Li, J. R. Ecker*, „Ethylene Biosynthesis and Signaling Networks”, The Plant Cell, Vol. 14, No. suppl 1, pp: S131-S151 (2002)
[12]. http://www.ru.nl/tracegasfacility/life_science_trace/plant_physiology/plant_hormone
[13]. *Abeles F.B., Morgan P.W., Saltveit M.E. Jr.* **“**Ethylene in Plant Biology**”**,Academic Press, London (1992).
[14]. *Osborne D.*, “Abscission” Crit. Rev. Plant Sci. 8, 103-129 (1989).
[15]. *Yang S.F., Hoffman N.E.*,**”**Ethylene biosynthesis and its regulation in higher plants” Ann. Rev. Plant Physiol. 35, 155-189 (1984)
[16]. *Kende H,.* **“**Ethylene biosynthesis”**,** Ann. Rev. Plant Physiol. Plant Mol. Biol. 44 283-307, (1993).
[17]. *Fluhr R., Mattoo A.K.* “Ethylene: Biosynthesis and perception” Crit Rev. Plant Sci. 12 479-523, (1996).
[18]. *Martin M.N., Cohen J.D., Safter R.A.* “A new aminocyclopropane-1-carboxylic acid conjugating activity in tomato fruit” Plant Physiology 109 917-926 (1995).
[19]. http://en.wikipedia.org/wiki/Ethylene
[20]. *M. A. Hall, A. R. Smith,* “Ethylene and the responses of plants to stress” Bulg. J. Plant Physiol., 21(2–3), 71–79 (1995).
[21]. *P. W. Morgan, M. C. Drew,* “Ethylene and plant responses to stress”, Physiologia Plantarum, Vol. 100, Issue 3, pp 620–630, (1997).
[22]. *D.C. Dumitras, S. Banita, A.M. Bratu, R. Cernat, D.C.A. Dutu, C. Matei, M. Patachia, M. Petrus, C. Popa* “Ultrasensitive CO2 laser photoacoustic system”, Infrared Physics & Technology Journal, Vol. 53, pp. 308-314 (2010).

[23]. *C. Popa, R. Cernat, D. C. A. Dutu, D. C. Dumitras,* “Spectroscopic studies of ethylene and ammonia as biomarkers at patients with different medical disorders”, U. P. B. Sci. Bull., Series A, Vol. 73, No.2, pp. 167-174 (2011).
[24]. *C. Popa, A. M. Bratu, C. Matei, R. Cernat, A. Popescu, D. C. Dumitras*, “Qualitative and quantitative determination of human biomarkers by laser photoacoustic spectroscopy methods”, *Laser Physics,* Vol. 21, No. 7, pp. 1336–1342 (2011).
[25]. *D. C. Dumitras, A. M. Bratu, C. Popa* „CO_2 Laser Photoacoustic Spectroscopy”, Chapter I „Principles”, pp: 1-42, Intech, Croatia (2012) ISBN 979-953-307-712-2, Ed. D. C. Dumitras.
[26]. *D. C. Dumitras, A. M. Bratu, C. Popa* „CO_2 Laser Photoacoustic Spectroscopy”, Chapter II „Instrumentation and Applications”, pp: 43-102, Intech, Croatia (2012) ISBN 979-953-307-712-2, Ed. D. C. Dumitras.