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Ethylene and CO₂ emission rates and pathways in harvested fruits investigated, in situ, by laser photothermal deflection and photoacoustic techniques

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Abstract

 CO_2 laser photothermal deflection (limit 1 nl l⁻¹) and photoacoustic detection (limit 6 pl l⁻¹) systems were used to measure ethylene emission rates from point sources of a wide range of mature nonclimacteric and climacteric fruits. In addition, an infrared gas analyzer was used to measure CO_2 from the same samples. As well as total rates, the percentages emanating from the skin, tissue at the distal end (floral scar) and the pedicel were established. Possible links with fruit behaviour during abscission are discussed.

Keywords: Fruit; Postharvest; Ethylene; CO₂; Laser detection

1. Introduction

In the past fleshy fruits have been classified either according to their internal structure or to their physiological behaviour. Their internal structure depends on which floral parts constitute the ripened ovary (or ovaries) of a flower. The development of fleshy fruit, originating from the diversity of floral structures, is reviewed by Coombe (1976). The main distinguishing features of physiological behaviour by fruits are their respiration and ethylene emission patterns. Based on their respiratory behaviour fruits are generally classified as climacteric or nonclimacteric. Climacteric fruits like tomato, fig, mango and banana show a respiratory rise during ripening while others like cherry, strawberry, lemon and

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mandarin, belong to nonclimacteric fruits (Biale and Young, 1981; Seymour et al., 1993). This distinction appears to be valid for harvested fruit, while for fruit attached to the plants the behaviour may be different as discussed by Saltveit (1993) for tomatoes. In a number of different climacteric as well as nonclimacteric fruit species ripening is retarded when fruits are attached to the tree for not well understood reasons comprised in the so-called 'tree factor' (Ben–Yehoshua and Eaks, 1970; Burg and Burg, 1965a; Lau et al., 1986).

Besides elevated levels of CO_2 , climacteric fruits produce increased levels of ethylene during ripening. Ethylene is a gaseous plant hormone; its biosynthesis starts from methionine and leads through two intermediates, *S*-adenosylmethionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC), to ethylene (Yang and Hoffman, 1984). It is known that ethylene regulates many processes, e.g. fruit ripening (yellowing, softening, respiration, autocatalytic ethylene production) and (in combination with decreasing auxin levels) abscission (Abeles et al., 1992; Osborne, 1989; Sexton et al., 1985; Ulrich, 1958). For ethylene the exit channels may be of importance because of their role in ripening and especially in abscission; local increase of the ethylene concentration may lead to local stimulation. The possible interaction of CO_2 cannot be left out of consideration, e.g. because it may slow down the conversion of ACC to ethylene, and thus locally inhibit ethylene effects (Abeles et al., 1992).

To measure local ethylene emission rates sensitive laser-driven detection systems are used. Photothermal deflection (PD) is used to measure ethylene concentrations at the spot of emission, i.e. very close to the fruit surface, pedicel or floral end. Photoacoustic (PA) detection is employed in combination with a continuous flow-through system to determine quantitative ethylene emission rates. An infrared gas analyzer (IRGA) sampled the concomitant CO_2 production parallel to PA apparatus.

In this paper we describe ethylene and CO_2 emission pathways in cherry tomato, round tomato, red bell pepper, fig, pear, apple, banana, cucumber, kiwi and mandarin. For ethylene the emission per surface area was calculated. Finally, we investigated whether the relative values for different emission routes changed during tomato maturation.

2. Material and methods

Plant material

The ethylene and CO_2 emission rates for mature fruits were measured directly after they were obtained from local sources. Cherry tomatoes and bananas were evaluated in the immature green and full-ripe stage. The fruits used in the experiments are listed in Table 1 which also indicates the respiration behaviour according to Biale and Young (1981). The time between harvest and the measurements was not documented. Therefore, the data are an indicative measure of the gas production and emission pathway of these particular batches of fruit. Transport and storage conditions as well as age may affect the results.

The experiments were performed at 21°C and 50% room humidity. Care was

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Species	Family	Origin	Climacteric	Weight (g)
Cherry tomato (Lycopersicon esculentum)	Solanaceae	Holland	yes	12
Round tomato (Lycopersicon esculentum)	Solanaceae	Holland	yes	60
Bell pepper (red) (Capsicum annuum)	Solanaceae	Holland	yes ^a	150
Fig (Ficus carica)	Moraceae	Kenya	yes	30
Pear (Pyrus communis)	Rosaceae	Argentina	yes	90
Apple (Malus sylvestris)	Rosaceae	Holland	yes	170
Banana (Musa spec. 'Grand Nain')	Musaceae	Colombia	yes	130
Cucumber (Cucumis sativus)	Cucurbitaceae	Holland	no/yes ^b	500
Kiwi (Actinidia chinensis)	Saxifragaceae	New Zealand	yes ^c	70
Mandarin (Citrus reticulata)	Rutaceae	Spain	no	50

^a Red bell pepper is climacteric according to Batal (1982), while some peppers are nonclimacteric (Saltveit, 1977).

^b According to Biale (1981) cucumbers are nonclimacteric, but are classified as climacteric by Kanellis (1986).

^c According to Hyodo (1987).

taken to prevent mechanical and thermal stress, to avoid unwanted ethylene production and differences in ripening pattern.

Methods

Two closely related laser techniques, photothermal deflection and photoacoustics, were used to determine ethylene concentrations. For detection of CO_2 an IRGA instrument was used. The three detection systems are shortly described below.

Photothermal deflection. The photothermal deflection method is based on absorption of (CO_2) laser radiation by molecules which have a characteristic absorption spectrum. The absorbed energy causes a local rise in temperature which changes locally the refractive index of air. Due to this change in refractive index a second laser beam (He–Ne laser) is deflected. The angle of deflection is proportional to the concentration of the absorbing gas (De Vries et al., 1995a, b, c).

Deflection caused by ethylene absorption is detected as a difference signal for two neighbouring CO_2 laser lines, one where ethylene shows strong absorption and a reference (weak absorption) laser line. The switching time between these laser lines was about 30 seconds, yielding the time resolution of the measurement.

In this set up, the sample is held under normal atmospheric conditions in the open air and measurements are done locally at a few mm above the sample. At ambient conditions switching is necessary to eliminate interference of other absorbing gases, of which CO₂, H₂O and ethanol (the latter may be anaerobically produced within the tissue) are the most important. For this system the detection limit for ethylene is 1 nl l^{-1} (versus 10–20 nl l^{-1} with conventional gas chromatography).

Due to the limited space for the sample in the experimental photothermal deflection set-up, the measurements were limited to fruit of small dimensions like fig, pear and cherry tomato. As a consequence of huge amounts of volatile nonethylene hydrocarbons (e.g. ethanol) produced by banana fruit, these experiments were only possible in combination with trapping of these interfering gases. We took therefore recourse to the PA technique because with this method removal of the interfering gases can be done prior to the concentration measurement.

Photoacoustics. Photoacoustics (PA) and PD are closely related. Instead of making use of the change in refractive index, the change in pressure (that accompanies also the change in temperature) is detected by a microphone (Harren et al., 1990; Voesenek et al., 1990; Woltering et al., 1988). In this set up (detection limit 6 pl 1^{-1}) measurements are performed in a continuous flow-through system. Switching between different sampling cuvettes at a flow of 2 l h⁻¹ give rise to a response time of 2 min. For the different kinds of fruit different cuvettes of various sizes and shapes were designed. To investigate ethylene emission from three different parts of the fruit simultaneously, cuvettes with separate compartments were used (e.g. for a pear see Fig. 1). To measure the emission rate from the pedicel, the tissue was sealed from the measuring line using vacuum clay around the pedicel ensuring an airtight connection and avoiding interference from surrounding tissue. An empty cuvette was used as 'blank'.

Infrared gas analyzer. To determine CO₂ concentrations simultaneously with ethylene, an infrared gas analyzer (IRGA Hartmann & Braun, URAS 2T 42CG48-2) was installed in parallel with PA. Its response time is about 5 min when a flow of 1 l h⁻¹ is used. The minimum observable CO₂ concentration is 50 μ l l⁻¹. In order to measure the CO₂ concentrations a flow of 2 l h⁻¹ was split directly after the cuvette containing the fruit (Vries de et al., 1995b).



Fig. 1. A triple chamber cuvette is depicted containing a pear. Ethylene and CO_2 emission rates from pedicel, floral end and skin surface are separately monitored with help of the PA and IRGA setup, respectively. In the case of measuring emission rates through the pedicel, a silicon tube connection was made.

3. Results

The measurements of ethylene emission were performed on intact fruit, for which a distinction was made between emissions through the external skin, skin at the floral end, and the pedicel. In the case of tomato and pepper the pedicel is regarded as part of the floral end.

As an example, the local ethylene emission rates in pear determined with the PD setup are shown in Fig. 2. The measurements were performed just above (1.5 mm distance) the calyx at the floral end, above the skin and above the pedicel and were compared with ambient ethylene levels. For the floral end of the pear, ethylene levels were monitored during 2.5 hours. As shown in Fig. 2, the concentration fluctuations at these higher levels (12–18 nl l^{-1}) are much bigger than observed for ambient concentrations (air). The larger fluctuations are likely due to air turbulence disturbing the ethylene concentration gradient around the floral end; ambient concentration levels are steady; fluctuations due to interfering gases (like CO₂ and H₂O) are negligible. Measurements of local ethylene emission rates using the PA method and the three compartment cuvette yielded essentially similar results.

The data for ethylene emission detected by PA, and the data for CO_2 emission detected by IRGA are presented in Table 2. Besides total emission rates for ethylene, relative emission values are indicated; for CO_2 only relative values are presented. In tomatoes and bell pepper most of the produced ethylene was emitted through the pedicel. In other fruit species emission through the pedicel did not contribute significantly to the total ethylene emission; in these species most of the ethylene was emitted through the skin. In figs and pear the floral end contributed significantly to the total ethylene emission (18.5% and 17%, respectively). For pear



Fig. 2. A PD measurement of ethylene concentrations 1.5 mm above the equatorial (skin) side, floral end and pedicel of a pear. The ambient ethylene level is also shown.

Table 2

Total ethylene and relative ethylene and CO_2 emission rates for several kinds of fruits; values are obtained for single pieces of fruit.

Туре	Ethylene emission				CO ₂ emission			
	Total rate (nl h^{-1} fruit ⁻¹)	Skin (%)	Floral end (%)	Pedicel (%)	Integral skin (%)	Floral end (%)	Pedicel (%)	
Cherry tomato (green)	5	17.5	_	82.5	37.5	-	62.5	
Cherry tomato (red)	50	5	-	95	22	-	78	
Tomato (round, red)	70	14.5	-	85.5	45.5	-	54.5	
Bell pepper (red)	4	25	-	75	nd	nd	nd	
Fig	14	75	16	9	90	8	2	
Pear	300	76	16	8	89.5	8	2.5	
Apple (Elstar)	2500	98	1	1	>99	<1	<1	
Apple (Granny Smith)	1000	99.5	0.5	0	nd	nd	nd	
Banana (green)	1.2	>99	<1	<1	>99	<1	<1	
Banana (yellow)	50	≫99	≪1	≪1	≫99	≪1	≪1	
Cucumber	7	>99	_	<1	>99	-	<1	
Kiwi	1	>99	<1	-	>99	<1	_	
Mandarin	0.3	>99	<1	-	>99	<1		

nd = not determined. The absolute error in relative values amounts to 1%. Emission values for floral end are corrected for emission from the skin surrounding the floral end covered by the measuring line.

the difference with apple (Elstar 2%, Granny Smith 1.5%) is remarkable since both species are related and both floral ends are open for gas diffusion. The lower emission rate through the skin for pear as compared to apple can probably be related to the difference in internal air space, 25% for apple and 5% for pear (Knee, 1991; Seymour et al., 1993).

 CO_2 emission showed the same pattern for most fruits (Table 2). The data are in agreement with published data on local ethylene emission from fruit (Abeles et al., 1992; Seymour et al., 1993). However, in tomato the relative emission via the skin of CO_2 is higher than for ethylene.

As shown in Table 2 for the Granny Smith and Elstar apples a high percentage of ethylene is emitted through the skin. This is in contrast with the results reported for Golden Delicious apples, where 42% of the ethylene is emitted through the calyx and, as a consequence, no more than 58% through the skin (Ben–Yehoshua and Cameron, 1989). This indicates that substantial differences may exist in ethylene production and emission rates in various cultivars while postharvest treatments (storage period, waxing) may additionally affect ethylene emission.

In Table 3 emission rates have been normalized for the surface area over which the emission rates were determined in order to obtain resistance values for skin, floral end and pedicel. Some caution should be taken during interpretation of the data. Pedicel emissions were calculated on basis of actual surface areas on top of the pedicel. However, the measured ethylene and CO_2 may partly have been from gases that laterally diffuse out of the pedicel. Similarly, measurements done at the floral end inevitable included some surrounding skin; the data in Table 3 are corrected for that.

Table 3

Ethylene emission of fruits for skin, floral end and pedicel; emission rates are normalized on the total skin surface, the floral end surface and the surface on top of the pedicel, respectively. Emission values for floral end are corrected for emission from the skin surrounding the floral end covered by the measuring line. For fig the obtained pedicel emission include part of the emission through the upper 8 mm of the skin surrounding the pedicel. In the case of emission rates for tissue below the detection limit, the indication '0' is given.

Туре	Skin emission (nl m ⁻² h ⁻¹)	Floral end emission (nl $m^{-2} h^{-1}$)	Pedicel emission (nl m ⁻² h ⁻¹)
Cherry tomato (green)	310	_	1.5×10^{5}
Cherry tomato (red)	890	_	1.7×10^{6}
Tomato (round, red)	1.3×10^{3}	_	1.2×10^{6}
Bell pepper (red)	64	-	2.4×10^{3}
Fig	2.0×10^{3}	8.0×10^{4}	1.0×10^{4}
Pear	2.0×10^4	1.7×10^{6}	3.4×10^{6}
Apple (Elstar)	1.6×10^{5}	8.0×10^{5}	$3.5 \times 10^{6} a$
Apple (Granny Smith)	6.3×10^{4}	1.5×10^{5}	0
Banana (green)	30	0	0
Banana (yellow)	1.4×10^{3}	0	0
Cucumber	150	_	0
Kiwi	1.1×10^{3}	0	-
Mandarin	60	0	-

^a This value is rather uncertain because the small pedicel emission is of the order of our experimental error (Table 2).

The Table shows that on a surface basis, especially in tomato and pear, the pedicel is a low resistance pathway for ethylene. As, in pear, the surface area of the pedicel is rather small, it does not serve as an important route for ethylene diffusion. In apple, pear and fig diffusion resistance of the floral end was clearly less than resistance of the skin.

The total skin emission data for pear were cross-checked with a measurement of emission only from an area of 50 mm^2 (data not shown). The skin and floral end emission obtained with PD are well in agreement with the PA data. Relative to the ethylene emission from the floral end, the pedicel emission is low (PD, Fig. 2), which is in contrast to PA measurements. This can be attributed to the small size of the pedicel in comparison to the PD detection zone.

Banana, cucumber, kiwi and mandarin yield relatively small normalized skin emission rates (nl m⁻² h⁻¹), though this is the only significant outlet found. Among these kiwi and mature yellow banana show the highest values, probably due to their climacteric behaviour (Table 1).

In Fig. 3 the influence of maturity is considered for cherry tomatoes, starting with immature green (IG, open bar), green orange (GO, shaded bar) ending up in the orange stage (O, black bar), i.e. just before IG, halfway (GO) and just after the climacteric rise (O) in ethylene production. The ratio of stem-scar/skin surface emissions of ethylene and CO_2 is higher for mature cherry tomatoes compared to immature ones. For bananas the opposite is observed; mature bananas have a higher relative skin/floral end pedicel ratio as compared to the immature fruit (see Table 2).



Fig. 3. PA and IRGA data for cherry tomatoes during three different stages of ripening: immaturegreen (open bar), green-orange (shaded bar) to mature-orange (black bar). Ethylene and CO_2 emission through stem-scar and skin are determined.

4. Discussion

The importance of ethylene and CO_2 concentrations and emission pathways of fruit have been discussed by Blanke (1991), Burg and Burg (1962), Kondo and Takahashi (1989) and Solomos (1989). In this paper we have focused our attention on local ethylene and CO_2 emission avenues in several kinds of fruit. As proposed by Burg and Burg (1965b) three emission avenues can be distinguished: pedicel, floral end and cuticle. In particular for the cuticle, emission takes place through stomata or lenticels and the skin; in this study we did not attempt to distinguish between these two as we measured the total emission through the skin.

Based on the measured emission rates through different parts of the tissue (Table 2), the fruits can be divided into three groups. In the first group, members of the same family (*Solanaceae*), tomato (cherry and round) and bell pepper are characterized by their ethylene and CO_2 cmission predominantly through the pedicel. The second group (fig, pear and apple) emit a substantial amount of ethylene and CO_2 through the skin but also through the floral end and/or pedicel. The third group (banana, cucumber,kiwi and mandarin, is characterized by negligible emission through both floral end and pedicel.

The first group of fruits reveals even more clearly the importance of the pedicel as gas diffusion avenue if the normalized surface area is taken into account (Table 3). One must realize the difference in internal structure between bell pepper and tomato. As earlier discussed (De Vries et al., 1995b), the locular gel layer probably forms the main resistance for gas exchange in a cherry tomato. However, a locular gel layer does not exist in bell peppers. In spite of this there is no large difference in the relative contribution of pedicel and skin to the total gas emission.

The CO_2 emission follows the pattern of ethylene emission fairly closely, but the high water content of the tissue and higher solubility of CO_2 in water (Landolt

and Börnstein, 1969) explains the shift toward skin emission in comparison to ethylene.

The importance of different emission routes for ripening and abscission is not well understood. It is likely that in fruits where emission mainly takes place through the pedicel, the ethylene pathway is involved in the control of abscission (Osborne,1989). As shown in Fig. 3 during the ripening process of cherry tomatoes the stem-scar becomes more important for ethylene emission.

In case of pear and fig the pedicel represents a significant emission avenue and fruit produced ethylene could therefore regulate the abscission process in these fruit. For apples, bananas, cucumber, kiwi and mandarin pedicel emission is negligible. Ethylene produced in these fruits presumably does not influence the abscission process.

As is demonstrated in this paper, the determination of emission rates and pathways by both laser detection techniques is helpful. It permits determination of changes of ethylene emission rates from different parts of the tissue. Changes in concentrations are detectable below nl l^{-1} level instantaneously and continuously, providing the opportunity to measure very small amounts of ethylene emitted even through tissues with a high gas resistance barrier. PD has the added advantage of quick time-resolution that provides evidence of fast fluctuations in the emission process.

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