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Investigation of Local Ethylene Emission from Intact Cherry Tomatoes by Means of Photothermal Deflection and Photoacoustic Detection[1]

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The function of the coronet region of the cherry tomato (*Lycopersicon esculentum* Cherry) as the main emission channel for ethylene was investigated. Ethylene was measured employing two laser-based detection systems, the photothermal deflection instrument and the photoacoustic detection setup. It is possible to detect minimum ethylene concentrations of 1 nL L^{-1} locally and rapidly with the first instrument and concentrations of 6 pL L^{-1} in a continuous flow system with the second setup. The continuous flow system makes it possible to change the air composition and to monitor its influence on the ethylene production of the tomato. The response times of the two instruments are 30 s and 4 min, respectively. The local character of the measurements makes it possible to determine the emission sites of the gaseous plant hormone ethylene and their relative importance. Transient anoxic conditions stop production of ethylene; return to aerobic conditions shows the evolution of the accumulated ethylene precursor 1-aminocyclopropane-1-carboxylic acid and its renewed production on the measured ethylene emission, with a time resolution of several minutes. Temporarily sealing the main emission channel yields results comparable to anoxia.

Starting with Met the ethylene biosynthesis proceeds to SAM, which is converted into ACC, the immediate precursor of ethylene (Yang and Hoffman, 1984). This biosynthetic pathway is regulated mainly by two enzymes, ACC synthase and ACC oxidase (Kende, 1993; Voesenek and van der Veen, 1994). This last enzyme depends on a certain level of oxygen, one of its substrates, for full activity.

The role of ethylene in fruit ripening, especially in (cherry) tomato (*Lycopersicon esculentum* Cherry), has been well studied (Abdel-Rahman, 1977; Biale and Young, 1981; Abeles et al., 1992; Theologis, 1992). As was demonstrated by Brecht (1987), ethylene synthesis starts in the fruit's interior concomitant with gel formation in the locules and prior to its initiation in the pericarp; this is followed by an augmentation at the breaker stage, the so-called climacteric rise in ethylene production. The latter rise in ethylene production coincides with the climacteric rise in respiration (Biale and Young, 1981). As has been remarked by Saltveit (1993), classification of fruit as climacteric is based on experiments with harvested fruit, and a rise in respiration and ethylene production are considered climacteric only if both coincide with ripening. However, there are characteristic differences between attached and detached tomato fruits. Although detached and attached tomatoes both demonstrate the same ripening score and strong increase of ethylene production, the emission of CO_2 for harvested fruit shows a more pronounced increase than for attached fruit (Saltveit, 1993).

In this context the determination of the stem scar as the predominant avenue for gas exchange merits attention. The stem scar is the place where the pedicel with sepals connects the berry to the stem (Coombe, 1976). As early as 1965 Burg and Burg mentioned that more than 60% of ethylene diffuses out of the stem scar of tomatoes of normal size. This value was estimated by sealing the stem scar with lanolin paste. A drawback of this method is that the gas exchange is disturbed completely. More recently, Cameron and Yang (1982) reported a stem-scar efflux percentage of 97% by preloading the tomato with ethane and afterwards determining the ethane efflux out of the stem scar and skin by GC. However, local measurements of ethylene emissions for undisturbed, intact tomatoes are lacking, because local and nonintrusive detection instruments have not been available.

The aim of this study was to determine locally the ethylene emission of cherry tomatoes employing sensitive and fast laser-driven trace gas detection systems. The PTD technique is new within biological research concerning ethylene (de Vries et al., 1993). PTD is most suitable for fast (time response of 30 s), local (no forced gas flow, no perturbation of equilibrium), and relatively sensitive measurements (1 nL L^{-1} for ethylene). The second technique is PAD, which is even more sensitive and is coupled with a flow-through setup (Harren et al., 1990; Meyer and Sigrist, 1990). PAD has already been successfully applied in dynamic studies of ethylene emission from single orchid

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Abbreviations: PAD, photoacoustic detection; PTD, photothermal deflection; SAM, S-adenosylmethionine.

flowers during emasculation (Woltering et al., 1988), *Rumex* species after soil waterlogging and submergence (Voesenek et al., 1993), and single seeds during germination (Petruzzelli et al., 1994).

In this paper our objectives were (a) to determine exactly the ethylene emission rates through the stem scar and epidermis, (b) to determine the physiological effect of sealing the main emission channel (the stem scar), and (c) to compare the physiological consequences of blocking the stem scar and withholding oxygen. The strategy is schematically presented in Figure 1.

MATERIALS AND METHODS

Fruit Materials

Experiments were carried out with intact detached cherry tomatoes (*Lycopersicon esculentum* Cherry), weight about 14 g and diameter approximately 30 mm. Fruits were obtained from commercial growers and transported to the laboratory within 12 h after harvesting. As indicated, tomatoes were investigated in green, orange, and red stages and observed during the entire ripening process. Tomatoes with coronet (pedicel length 10 mm; already dried out) or without coronet were used. In Figure 1, a schematic view of a cherry tomato is presented, including terms that will be used throughout this paper.

Moreover, data were obtained for tomatoes in which the stem-scar area or the top of the pedicel were sealed with high-vacuum grease (Dow Corning) or with parafilm M (American National Can, Greenwich, CT). Both sealing methods were highly effective in locally blocking the gas exchange. In the case of readmitting air to the stem scar, care was taken not to misinterpret the ethylene signal due to the production of wounding ethylene. Production of wounding ethylene is caused by removal of the grease and subsequent reopening of the channels, leading to the fruit interior, by a needle. Removal of the coronet itself also leads to production of wounding ethylene; this will be detailed in a forthcoming publication.

PTD Method

PTD (indicated in the figures) was used for directly estimating local ethylene concentrations around the intact tomato (a) with open and closed stem-scar area and (b) above an area where the skin was partly peeled off. The PTD technique is based on a change of refractive index gradient caused by a CO_2 laser beam, which is probed by a HeNe laser beam. In our application these two laser beams were crossed just above the epidermis of the tomato under investigation (left part of Fig. 2).

In an intracavity setup the CO_2 laser, tunable over about 80 different lines in the infrared (9–11 μm), was sharply focused at 1.5 mm above the peel. It was determined that no temperature change or other influence of the tissue took place. This laser is made to excite ethylene into the ν_7-vibrational state. By nonradiative collisional decay the absorption region and its surroundings experience a temperature increase of typically a few millidegrees. The refractive index of the air thus changes. The second laser beam, the HeNe probe laser, passes over this region 31 times as a result of a multipass mirror system, resulting in an overlap region of the two laser beams of about 25 mm $\times$ $\pi(0.28\ \text{mm})^2$ (de Vries et al., 1992); it is deflected by the change of the refractive index gradient, which is also known as the mirage effect (Jackson et al., 1981). The deflection is proportional to the concentration of the absorbing gas and to the available power of the CO_2 laser. The CO_2 laser is periodically blocked by a 20-Hz chopper, giving rise to a modulated deflected probe signal. This signal is monitored with a position-sensing detector (Fig. 2, PSD), from which the signal is fed into a lock-in amplifier.

Ethylene is detected as difference signal for two neighboring laser lines, the strongly absorbing 10P14 (wave-

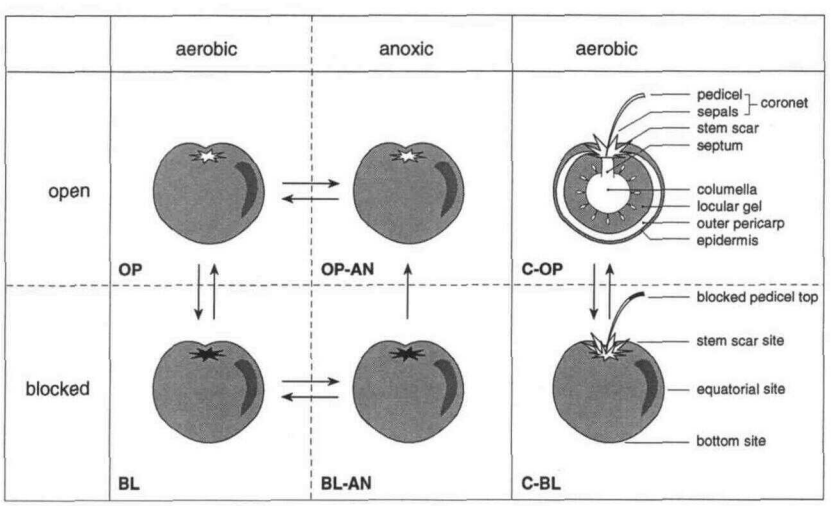

Figure 1. Schematic presentation of the different experimental conditions. Changes in experimental conditions are indicated by arrows. At the right, an intact tomato with coronet and pedicel is presented with descriptions of several parts of the tissue and ethylene monitoring areas. Blocking occurred at the upper part of the pedicel. OP, Open; BL, blocked; AN, anoxic; C, (tomato with) coronet. Unless indicated, tomatoes without coronet were observed under aerobic conditions.

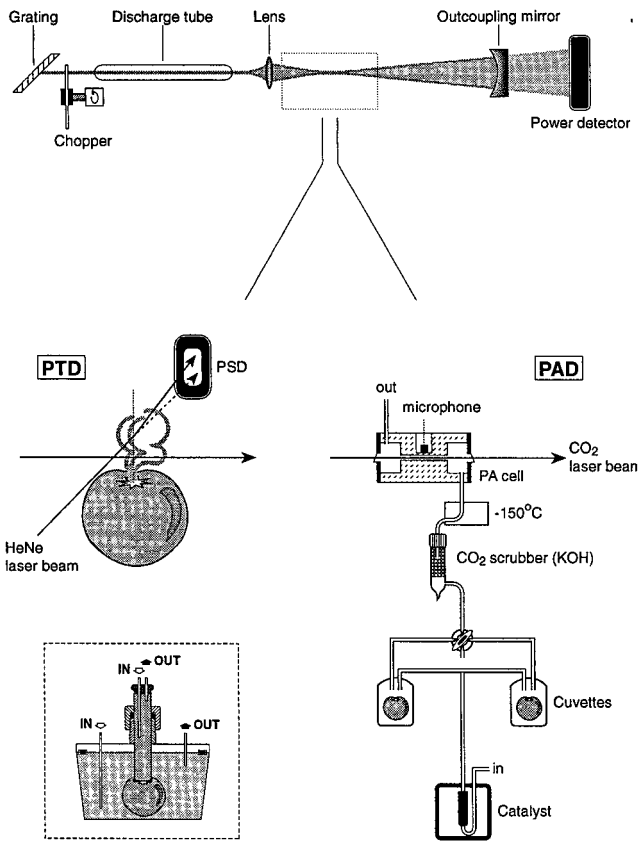

Figure 2. Two different laser-based detection systems. The excitation laser is an IR CO_2 waveguide laser, which is employed in an intracavity setup. PTD and PAD experiments were performed on tomatoes inside the cavity, as is presented in the lower part of the figure. In the PTD configuration a cherry tomato is placed 1.5 mm under the excitation laser. The deflection of a HeNe laser beam is detected. In the PAD setup a continuous flow system (consisting of a catalyst to remove ethylene and cuvettes with sample), a scrubber (KOH) to remove CO_2, a cooling trap to remove ethanol, and a photoacoustic cell with three microphones are used. A double cuvette is inserted to sample gas from different parts of the fruit.

length = 10.532 μm) and weakly absorbing 10P12 (wavelength = 10.513 μm) laser lines (Harren et al., 1990). Including piezo optimization, the switching time between the two laser lines is about 30 s, which is the time response of the total detection system.

Under normal air conditions, switching between two laser lines is often necessary due to interfering absorbing gases of which CO_2, H_2O, and ethanol are most important. These gases absorb almost equally on the 10P14 and 10P12 laser lines, and thus the difference signal yields the ethylene concentration. The practical detection limit is 1 nL L^{-1} for ethylene in this specific, open-air application.

For calibration purposes a reference cell was filled with nitrogen containing ethylene at different concentrations starting from a certified gas bottle with 1.2 μL L^{-1} of ethylene. The cell was installed at the interaction point of the probe laser beam and the CO_2 laser beam.

PTD experiments were performed in a laboratory with an air ventilation system, resulting in a constant temperature of 21°C and an RH of about 50%. Care was taken to prevent mechanical and thermal stress of the tomato. The measurements were performed in the dark. The fruit was placed such that air currents could not perturb the measurements. One location at a time was investigated for local ethylene emission. All measurements were repeated at least 20 times for the same tomato. The full procedure was applied to at least three different tomatoes.

PAD Method

In addition to the PTD technique, a PAD setup was used to determine ethylene concentrations in out-flowing gas streams from cuvettes, more specifically (a) the climacteric ethylene pattern under normal aerobic conditions, (b) the effects of interchanging aerobic and anoxic conditions, (c) the exact emission rates from stem scar and epidermis, (d) the influence of sealing and anaerobically deblocking the stem scar, and (e) the effect of blocking and deblocking the top of the pedicel.

PAD is closely related to PTD; instead of making use of a gradient in refractive index, a change in pressure, caused by a CO_2 laser, is detected with three microphones placed on a resonator pipe. An acoustical signal in the pipe is produced at the resonance frequency of 1600 Hz by appropriately adjusting the modulation frequency of the CO_2 laser beam. This resonance frequency depends only slightly on the air composition in the resonator pipe (Harren et al., 1990).

In this setup, cuvette measurements were performed in a continuous flow system as can be seen in Figure 2 (right part). The concentration was related to the emission rate by multiplying the measured concentration with the flow rate. The flow rate was monitored by a mass-flow sensor (Brooks 3850E; maximum flow rate 5 L/h, accuracy of 1%). A column filled with KOH removed CO_2 and water. In addition, a liquid nitrogen cooling trap (−150°C) served to remove other interfering gases like ethanol. For ethylene a minimum concentration of 6 pL L^{-1} can be detected. Switching between cuvettes with a flow of 2 L/h causes a delay of 4 min before the photoacoustic cell is completely refilled. Figure 2 also shows a double cuvette (on the left), which allows determination of emission rates for different pathways simultaneously.

In all our experiments ethylene levels from an empty cuvette were subtracted to obtain the emission rates by the incubated fruit.

RESULTS

Climacteric Ethylene Emission Pattern

Using the flow-through system coupled with PAD, the total ethylene emission rate was observed during the ripening process of a cherry tomato, without coronet (OP) from the immature green to the orange stage (Fig. 3). The ethylene emission started at a low level and showed a huge upsurge within a few days as the fruit started to turn orange. Cherry tomatoes with coronet (COP) demonstrated the same pattern (data not shown).

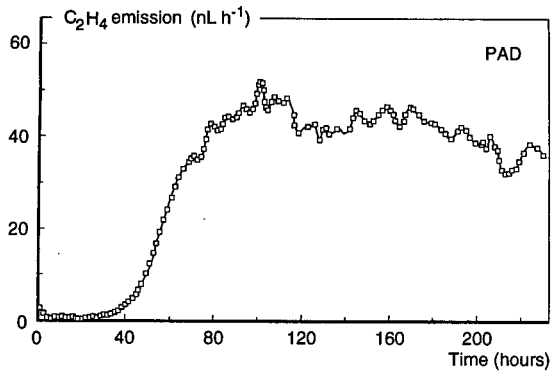

Figure 3. Ethylene emission rates measured with PAD of a single cherry tomato, with coronet, under aerobic conditions. At the start the tomato was still green, changing its color to orange from 40 h onwards. After 200 h the tomato was fully orange.

Aerobic/Anoxic Conditions

The continuous flow system of the photoacoustic setup allows switching from air to nitrogen flow. Interchanging aerobic and anoxic conditions (OP→OP-AN) greatly influenced the ethylene emission pattern (Fig. 4, solid line). First, the tomato was kept under aerobic conditions, followed by an anoxic period from 2.2 to 5.2 h, and finally the aerobic conditions were restored. Note that the ethylene pattern in time differed when air was changed to nitrogen and vice versa. Following application of anoxic conditions, it took about 2 h before ethylene levels dropped to zero. Switching from anoxic to aerobic conditions resulted in an increase of ethylene production, leading to a maximum after 1 h and a decline afterwards. Later, a second, much slower increase was observed that reached an equilibrium state after 10 h.

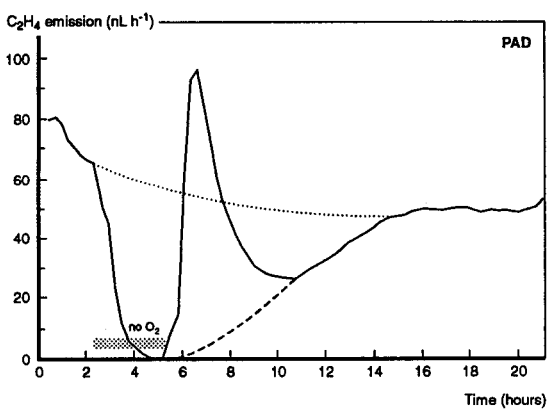

Figure 4. PAD measurements of ethylene emission rates of a single orange cherry tomato under initially aerobic conditions. After 2.2 h an anoxic period was introduced and after 5.2 h the aerobic conditions were restored. The dotted line indicates undisturbed aerobic ethylene emission; the dashed line represents the predicted ethylene production if the presumed effect of the conversion of accumulated ACC to ethylene is neglected.

Local Ethylene Emission

The main ethylene exit channel of an intact cherry tomato without coronet (OP) was determined by scanning over its surface in the PTD setup. Measurements performed above the stem scar and the bottom site opposite the stem scar demonstrated that about 90% of the internally produced ethylene was released at the stem-scar site (Fig. 5, left part). The amount of ethylene emitted at the bottom site and at the equatorial site yielded equal concentrations (data not shown), both slightly above ambient concentrations of 4 nL L^{-1}.

These data were compared with data from measurements carried out with the PAD setup, using the double cuvette (Fig. 2, inset). The total emission rates for ethylene through the skin and through the stem-scar area (OP) are presented (Fig. 6A). At the start ethylene production by the tomato was half of that seen at the climacteric rise in ethylene production (green/orange stage) (see also Fig. 3). The emission rates were observed until the orange stage was reached. Data for green tomatoes just before the climacteric rise demonstrate that 80% of the total ethylene diffused out of the stem scar (data not shown). The greater part of the ethylene was evolved at the stem scar independent of the maturity stage. However, the contribution of the stem scar to the total emitted ethylene increased over time. Emission rates per surface area were not calculated first because the contribution of a small part of the skin around the stem scar to the total emission through the stem scar is entirely negligible, and second because the determination of the calyx-shaped area of the stem scar is difficult and uncertain.

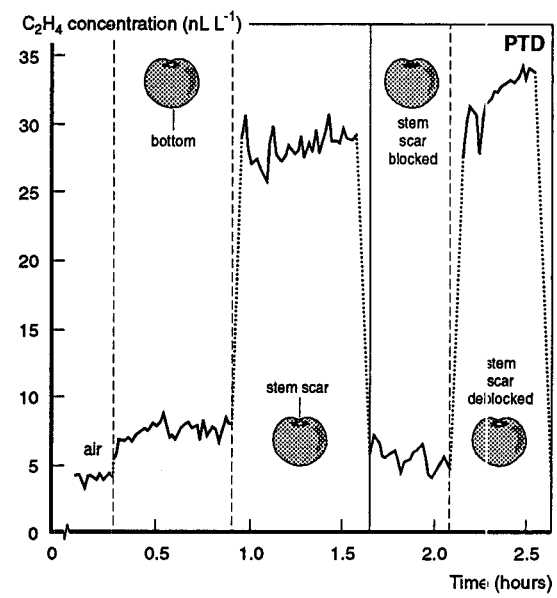

Figure 5. Left, Concentrations of ethylene were measured at 1.5 mm distance from a single orange tomato, using the PTD setup. For comparison the background ethylene concentration in air was determined and is represented at the left. Right, At 1.6 h the stem scar was blocked, and 30 min afterwards the stem scar was reopened.

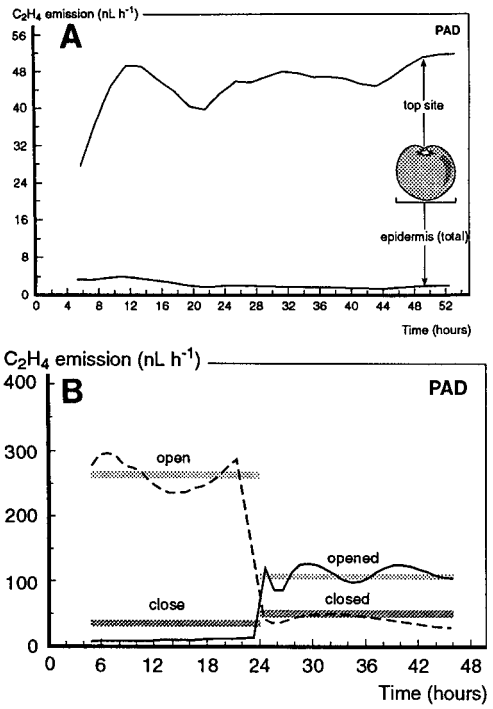

Figure 6. A, PAD measurements of ethylene emission rates for the stem scar and for the total skin surface of a single cherry tomato. Data were obtained for mature green tomatoes; part of the climacteric rise of ethylene production is visible at the left (compare Fig. 3); the orange stage was reached at the right. B, Two PAD measurements are presented for mature green/orange tomatoes, one with the stem scar blocked after 24 h (solid curve) and the other with the stem scar reopened after 24 h (dashed curve).

Blocking the Gas Outlet/Inlet

Aerobic Conditions

The local ethylene emission measured with PTD vanished instantaneously if the stem-scar emission route of green/orange tomatoes was effectively blocked (OP→BL), e.g. with high-vacuum grease (Fig. 5, right part). PAD measurements also showed that the total emission of the tomato dropped by about 90% when the stem scar was smeared with vacuum grease (Fig. 6B, dashed curve at 22 h) for the green/orange stage. Tomatoes in the orange stage and deep-red stage demonstrated the same behavior (data not shown).

Different ripening parameters, like softening and red coloration and the ethylene emission, were examined after blocking periods of variable duration. Deblocking after short blocking periods of about 30 min (BL→OP) re-established the normal emission of ethylene (Fig. 5, right part) and normal ripening took place. After a blocking period of 24 h a rise in ethylene emission following deblocking was observed (Fig. 6B, solid curve), but normal ripening did not occur. The tomato kept its originally green/orange color but softened drastically within a few hours. Besides, brown spots were observed on the epidermis. After deblocking the stem scar a higher background signal was found. Experiments in which the stem scar was blocked with Parafilm showed very similar results. In addition, sealing with Parafilm led to a gas bubble with slight overpressure between the stem scar and the Parafilm, and water accumulated under the Parafilm.

We have to remark that during storage of 1.5 weeks at 5°C, blocking of the stem scar did not cause any difference in ripening pattern compared to that seen in tomatoes with open stem scars.

Interchanged Aerobic/Anoxic Conditions

Ethylene production of intact tomatoes was measured with PAD after removal of the Parafilm that obstructed the stem scar. A special cuvette was employed in which the Parafilm could be removed without disturbing the flow rate and flow conditions. In the first instance the stem scar of the tomato was sealed and measurements were performed under aerobic conditions (BL, Fig. 7A). Small ethylene emission rates of 1 nL/h were observed. At 4.1 h switching to nitrogen flow (BL→BL-AN) caused a slow decrease in this small ethylene emission. Removing the Parafilm under anoxic conditions (BL-AN→OP-AN) at 4.8 h yielded an immediate increase in ethylene emission. Within 1.5 h the ethylene was back at a low level. The dotted curve (Fig. 7A) was obtained after injection of air with appropriate concentrations of ethylene into an empty cuvette.

In another experiment the Parafilm was removed after 4.6 h under anoxic conditions (BL-AN→OP-AN, Fig. 7B). The first peak was comparable with the peak in Figure 7A. Switching after 6.2 h from nitrogen to a normal air flow (OP-AN→OP) again yielded a direct response, within 1.5 min, resulting in a local maximum, finally ending in a steady emission.

Obstructing the Pedicel Top

Instead of sealing the stem scar, the top of the pedicel of a green and orange tomato was blocked (COP→CBL). This did not produce any difference compared to an open top (data not shown). Reopening of the stem top (CBL→COP) was realized by cutting 2 mm off the stem. Normal ripening occurred in about the same number of days as for the untreated control tomatoes. Tomatoes with or without coronet did not show a difference in ethylene levels.

Alternative Emission Channel

To create an alternative gas emission channel at the bottom site of the tomatoes, a piece of the epidermis with a diameter of 4 mm (comparable to the dimensions of the stem-scar area) was peeled off (Fig. 8). Measurements above the stem scar (B) and above the skinned part at the bottom site demonstrated that still most of the ethylene (>85%) was exhausted at the stem-scar site. The amount of ethylene emitted at the skinned opening (D) was equal to an undamaged epidermis (A and C). In the case of blocking the stem-scar outlet at 2.4 h, a slightly increased signal was observed at the skinned region (E) for several hours, but still this increase amounted to far less than the normal emission at the stem-scar site.

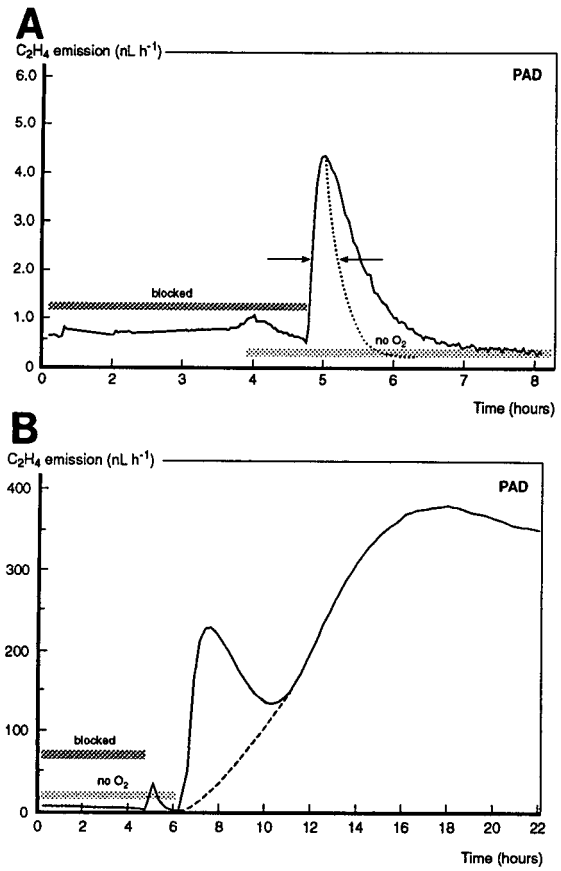

Figure 7. The seal of the stem-scar area of a single, mature orange cherry tomato was removed under anoxic conditions. A, At time = 0 h the stem scar was sealed with Parafilm for 4.8 h. After 4 h, air was replaced by a nitrogen flow (lower horizontal bar), and at time = 4.8 h the Parafilm was removed. The observed peak is due to accumulated ethylene. B, An orange tomato, the stem-scar outlet of which was reopened after being sealed for 5 h, showed, in addition to the first peak of accumulated ethylene, the conversion of entrapped ACC to ethylene (peak at time = 8 h) and of newly produced ACC to ethylene (rise at time = 12 h) when aerobic conditions were restored.

DISCUSSION

In this paper we have focused our attention on ethylene emission of ripening cherry tomatoes to determine the major gas diffusion routes. In addition, ethylene evolution—as affected by interchanging aerobic and anoxic conditions in comparison to opened and blocked stem scars—was investigated (Fig. 1). The measurements were performed on intact tomatoes in the open air and in a flow-through system using two CO_2 laser-driven detection instruments (PAD and PTD). Both instruments permit local and fast ethylene concentration measurements at subnanoliter per liter levels. The determination of emission rates through tissues with high gas resistance barriers is now possible.

Under ambient circumstances the pattern of ethylene emission demonstrates a climacteric behavior (Fig. 3), starting with low pre-climacteric rates, followed by a 60-fold rise in production from the green/orange stage onwards.

Changing from aerobic to anoxic conditions (OP→OP-AN; Fig. 4, solid line), the ethylene emission of an orange tomato drops to zero within 2.5 h. This drop is a consequence of the interrupted ethylene biosynthesis needing oxygen (Yang and Hoffman, 1984). The 2.5 h reflect both the gradual depletion of the already produced ethylene and the consumption of the still-available oxygen.

Switching back from nitrogen to air (OP-AN→OP) gives rise to an increase of ethylene emission within 4 min (Figs. 4 and 7B). This implies that sufficient oxygen is directly available and that ACC is immediately converted to ethylene. The first asymmetric high maximum—observed after 1 h with a width of 2 h at half the peak value—is attributed to direct conversion of ACC entrapped under anoxic conditions (Zhen-guo et al., 1983; Abeles et al., 1992). The second rise of ethylene production (partially indicated by the dashed curve) leads to a constant value after 10 h (Fig. 4); the dotted line reveals that normal ACC and ethylene production are resumed.

Most of the ethylene is emitted from the stem-scar area, varying from 80 to 95% of the total emission during maturing from green to orange stage (Figs. 5 and 6), and the remainder is released through the skin of the fruit. Burg and Burg (1965) and Cameron and Yang (1982) have previously investigated ethylene emission pathways for normal tomatoes. Their findings bear on their relative importance and are in qualitative agreement with our results. However, Burg and Burg blocked the stem scar and measured the ethylene production in the sealed tomato. This has drastic consequences, as demonstrated in this paper, which were entirely neglected by Burg and Burg. The local character of ethylene production—and its possible consequences on the emission—was not taken into account by Cameron and Yang, since they simply pumped ethane into the tomato and measured the diffusion resistance of the epidermis and the stem-scar region.

If most of the ethylene is produced near the columella (center) of a mature tomato, it can diffuse directly into the

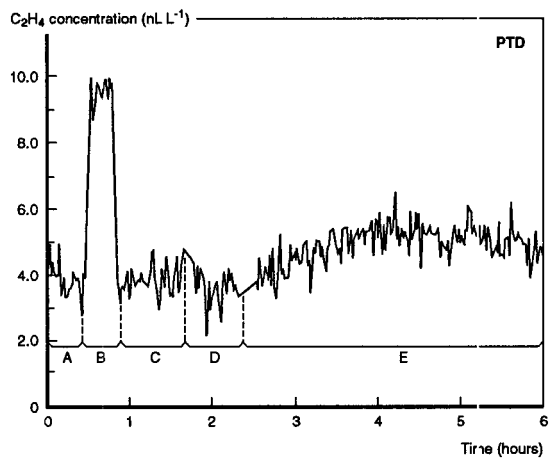

Figure 8. PTD measurements at the bottom (A), stem-scar (B), and equatorial (C) sites of a single, mature orange cherry tomato. After 1.6 h the skin was partly peeled off at the bottom site, and shortly afterwards the stem-scar outlet was obstructed while ethylene was still detected at the bottom site (D and E, respectively).

septum (air channel) leading to the stem-scar area (see Fig. 1). In the mature stage the layer of locular gel around the center acts as a high-resistance barrier for diffusion of ethylene. The diffusion rates for ethylene and oxygen are about 10^4 times slower in water than in air (Landolt-Börnstein, 1969).

Sealing the stem-scar area (OP→BL) with high-vacuum grease or Parafilm results in an immediate decrease of ethylene emission to about 10% of the total unimpeded value (Figs. 5 and 6B [dashed curve]). This percentage equals the normal value for skin emission. The skin does not take over the function of the coronet; the low-level emission through the skin, however, continues to decrease measurably, by about 2% in 24 h. We thus conclude that some diffusion of oxygen through the skin takes place, because ACC is still converted into ethylene. However, after removal of the Parafilm some ethanol evolution is observed (data not shown), i.e. fermentation also takes place; locally, anoxic conditions have thus been produced in the tomato.

Readmitting oxygen by removal of the Parafilm (BL→OP; Figs. 5 [right part] and 6B) resulted in an immediate rise in the ethylene level, similar to the pattern obtained by changing from anoxic to aerobic conditions (OP-AN→OP; Figs. 4 and 7B).

If the Parafilm is removed in a nitrogen environment (BL-AN→OP-AN), only a small broad peak of ethylene emission is observed (Fig. 7A). This peak is due to small amounts of accumulated ethylene in or near the columella during blocking; closure for 6 h (Fig. 7B) yields the same pattern as closure for 3 h.

Sealing the stem top of harvested tomatoes (COP→CBL; Fig. 8) does not restrict gas diffusion. No effects of accumulated ethylene/ACC and of delayed resumption of ACC production are observed.

Finally, PTD measurements above a small opening in the peel (Fig. 8) while the stem scar is obstructed show that this artificial channel does not form an alternative ethylene exit. The skin itself may thus not be considered the diffusion-limiting barrier.

The features of PAD are compared to those of a gas chromatograph described by Brailsford et al. (1993). With the measurements described in this paper the power of the PTD method in performing local, fast, and sensitive trace gas level determinations is demonstrated, as is that of the slightly modified PAD technique.

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