Plant Physiol. (1997) 113: 925–932

Dynamics of Acetaldehyde Production during Anoxia and Post-Anoxia in Red Bell Pepper Studied by Photoacoustic Techniques[1]

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Acetaldehyde (AA), ethanol, and CO_2 production in red bell pepper (*Capsicum annum* L.) fruit has been measured in a continuous flow system as the fruit was switched between 20% O_2 and anaerobic conditions. Minimum gas phase concentrations of 0.5 nL L^{-1}, 10 nL L^{-1}, and 1 mL L^{-1}, respectively, can be detected employing a laser-based photoacoustic technique. This technique allows monitoring of low production rates and transient features in real time. At the start of anaerobic treatment respiration decreases by 60% within 0.5 h, whereas AA and ethanol production is delayed by 1 to 3 h. This suggests a direct slow-down of the tricarboxylic acid cycle and a delayed onset of alcoholic fermentation. Re-exposure of the fruit to oxygen results in a 2- to 10-fold upsurge in AA production. A short anoxic period leads to a sharp transient peak lasting about 40 min, whereas after numerous and longer anoxic periods, post-anoxic AA production stays high for several hours. High sensitivity of the fruit tissue to oxygen is further evidenced by a sharp decrease in post-anoxic AA production upon an early return to anaerobic conditions. Ethanol oxidation by the "peroxidatic" action of catalase is proposed to account for the immediate post-anoxic AA upsurge.

Fermentation occurs in fruits when oxygen flux to respiring cells is reduced below a critical value. Hypoxia occurs in bulky fruits under natural conditions during normal ripening due to impaired gas exchange with the atmosphere. The metabolic response and adaptation of plants to anaerobiosis has been extensively reviewed (Perata and Alpi, 1993; Pfister-Sieber and Braendle, 1994). Low oxygen concentrations are widely used in controlled atmosphere storage of harvested fruit, e.g. apples, with the goal of prolonging fruit shelf-life (Knee, 1991). Reduction of respiration rate and ethylene biosynthesis during controlled atmosphere storage does not, however, induce fermentation. Recently, storage in 1% O_2 was shown to extend the postharvest life of bell peppers (Luo and Mikitzel, 1996). Similarly, short-term anoxic treatments of harvested fruit improve fruit aroma and quality (Pesis, 1995). The physiological basis of the observed effects has yet to be clarified.

AA, the precursor of ethanol, accumulates in almost every fruit during ripening (Fidler, 1968) and is one of the natural aroma compounds. The suggested biosynthetic pathway for AA is anaerobic alcoholic fermentation. If the availability of oxygen to the tissue is locally limited by the characteristics of the tissue itself, AA may be produced even under external aerobic conditions. To determine at what level of hypoxia the fermentation pathway becomes functional, and to what degree, requires highly sensitive detection methods. Of equal importance is the study of the metabolic changes occurring during transition from anoxia back to aerobic conditions. The concern here is the potential toxicity of the accumulated ethanol and AA (Jackson et al., 1982; Perata and Alpi, 1991) and of active oxygen species formed upon re-exposure of the tissue to oxygen. AA fumigation is known to possess fungicidal properties (Avissar and Pesis, 1991), as can result from anoxic treatments of a 24-h duration (Pesis and Avissar, 1988). Is the fungicidal effect a direct product of fumigation with AA or an indirect one due to synthesis of another antifungal compound? We can also ask if these effects are a direct effect of anoxia itself or of post-anoxic metabolism. Active oxygen species play an important role in plant defense against pathogens (Mehdy, 1994) and are believed to be a primary cause of post-anoxic injury in plants (Pfister-Sieber and Braendle, 1994).

PA techniques of trace gas detection achieve higher sensitivities than GC methods for a variety of gases. A lower detection limit of 10 pL L^{-1} for the gaseous plant hormone ethylene was achieved by Harren et al. (1990). The usefulness of the PA detector can be assessed if it is used as a flow-through setup so that very low production rates and transient features can be monitored in real time. Recently, a CO-laser-based PA detector was developed and successfully applied to study, in particular, anaerobic processes in fruit (Bijnen, 1995). In this work detection limits for ethanol and AA were 10 nL L^{-1} and 0.5 nL L^{-1}, respectively.

In the present study the high sensitivity of the PA technique and its fast time response are exploited to study the dynamics of ethanol and AA when red bell peppers are switched between 20% O_2 and anaerobic conditions. To demonstrate the potentials and the advantages of the PA

[1] This work was supported by the Dutch Technology Foundation (STW, Project NNS55.3608) (H.Z.) and formed part of the EC Science program (contract no. BRFSC1*-CT91-0739).

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Abbreviations: AA, acetaldehyde; ADH, alcohol dehydrogenase; PA, photoacoustic.

technique, this fruit was chosen because of the absence of a watery endocarp that would otherwise introduce a high diffusion barrier to the escape of volatiles. We show that AA is a most sensitive indicator of metabolic changes occurring as the fruit switches between respiration and alcoholic fermentation.

MATERIALS AND METHODS

Israeli red bell peppers (*Capsicum annum* L. cv "Maor") were bought in a local store during February to March of 1995. Dutch red bell peppers (*Capsicum annum* L. cv "Spirit") grown in a greenhouse during June to July of 1995 were used within 1 to 24 h after harvest. The stem pedicel was cut either at the abscission zone or 1 cm above the fruit. For some experiments, a 1-mm hole was punctured through the stem of the fruit, to facilitate the gas exchange. The average fresh weight of each fruit was about 160 g.

PA Detection Technique

A review of the PA technique and its applications can be found elsewhere (Harren et al., 1990; de Vries et al., 1995; Bijnen et al., 1996); only a brief description will be given here. The PA effect is based upon conversion of radiation into acoustic energy. Via collisional relaxation, the excited molecules transfer their laser-excited vibrational energy to translational energy, which gives rise to a pressure increase. The laser radiation is modulated at a frequency of about 1 kHz by a chopper so that the gas absorption is generating a periodic pressure modulation, i.e. sound. The CO laser is line-tuneable over a large IR frequency range (350 lines between 1200 and 2100 cm^{-1}), where many gases possess a strong fingerprint absorption. The absorption cell (PA cell) is built as an acoustic resonator to optimally sustain this periodic pressure modulation. A condenser microphone mounted at the anti-node of the resonator detects the sound. The PA cell is placed inside the CO-laser cavity to profit from the one-order-of-magnitude increase in laser power as compared with an extracavity position. The PA setup used in the measurements is presented in Figure 1.

A multicomponent analysis of the gas emitted by red bell peppers was performed by tuning the grating to 15 laser lines and measuring the PA signals that were proportional to the absorption; the PA signals were normalized to the intracavity laser power. The absorption coefficients of AA, ethanol, CO_2, and H_2O at these laser lines were determined beforehand. The concentrations of the gases under investigation were calculated using the mathematical formalism of Meyer and Sigrist (1990) involving matrix manipulation. A full cycle of positioning the grating and measuring on 15 lines took about 15 min.

Fast time response measurements of AA were performed by switching solely between two neighboring laser lines, the radiation of one strongly and one weakly absorbed by AA, and by taking the difference signal. We used the $P(11)_{13}$ (α = 28.4 atm^{-1} cm^{-1}) and $P(8)_{13}$ (α = 12.7 atm^{-1} cm^{-1}) transitions of CO at 1765.46 and 1776.55 cm^{-1}, respectively. The time between two measurements of AA concentration could be reduced to 1 min. The major inter-

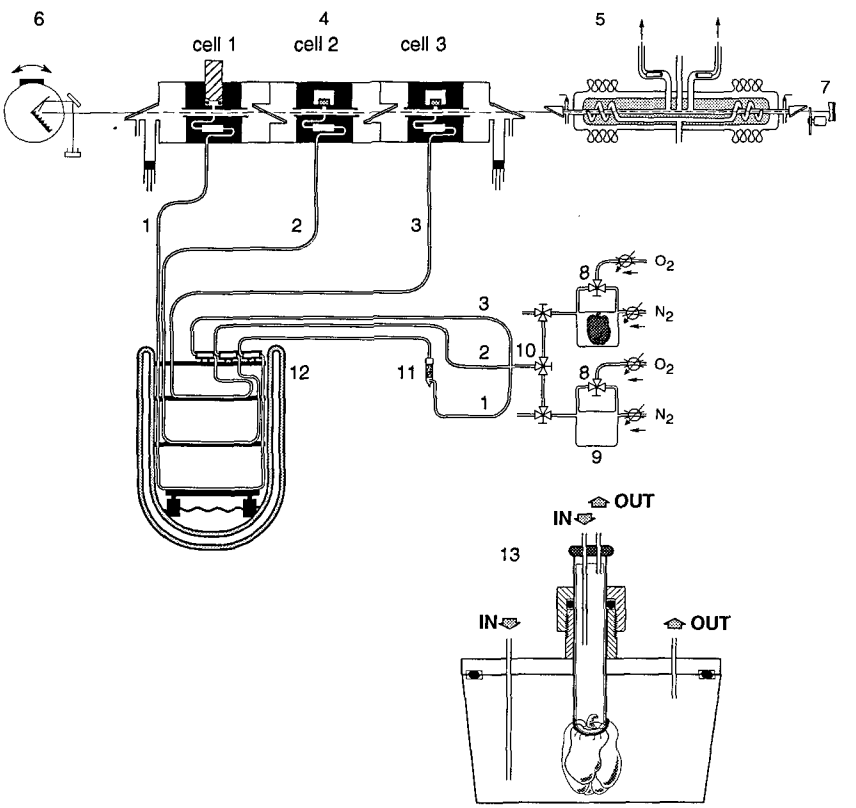

Figure 1. PA detection. 1, 2, and 3 indicate the gas flows to 4, the triple PA cell, through 12, the different cooling traps, after the gas emissions were sampled in the measuring cuvettes, 9; 5, liquid-nitrogen-cooled CO laser; 6, grating to select the appropriate transition; 7, chopper; 8, switching valve to establish (an)aerobic conditions; 9, cuvettes, one containing a red bell pepper, the other empty for reference; 10, switching valve to select a cuvette; 11, KOH scrubber to remove CO_2; 13, a special sample cuvette: there are two compartments to distinguish between stem and cuticle responses to various (an)aerobic treatments.

fering gases, CO_2, H_2O, and ethanol, have nearly equal absorption strengths on these two lines, so their contribution to the signal can be easily subtracted. The time response of the PA detector to fast changes in gas concentration inside the sample cuvette also depends on the gas flow rate. At a flow of 1 L h^{-1} the residency time of the gas in the PA resonator is only 40 s (1/e time). The results presented in this work were obtained using flow rates of 2 to 5 L h^{-1}.

Water vapor may influence the absorption of other gases and its strong adsorption to the wall material can further perturb the performance of the detector. To reduce these problems we used a cooling trap and Teflon (FEP) tubing for the gas flow from the sampling cell to the PA cell. The cooling trap consisted of a liquid nitrogen reservoir, in which the liquid level was kept constant, and three trapping stages. The gas flow from the fruit cuvette was split into three equal flows, each leading to a different trapping stage. The top one, approximately 500 mm above the liquid nitrogen level and at a temperature of approximately −20°C, removed most of the water vapor and constituted the first stage of trapping. The second trapping stage consisted of a metal plate 300 mm above liquid nitrogen and at a temperature of −70°C. This stage reduced water vapor concentration to μL L^{-1} levels while allowing ethanol to pass at about 10 times higher concentrations. In the third trapping stage, the gas flow came into contact with a metal plate 150 mm above the liquid nitrogen kept at a constant temperature of −120°C. At this temperature AA and CO_2 pass freely at μL L^{-1} and mL L^{-1} levels, respectively, whereas residual water vapor and ethanol are reduced to concentrations of about 40 pL L^{-1} and 3 nL L^{-1}, respectively.

It is of relevance to mention here that AA is about 15 times more volatile than ethanol at ambient pressure and 23°C (Kimmerer and MacDonald, 1987). This has a direct influence on the capability of the PA technique to detect small changes in ethanol production rates. An increase of 200 nL L^{-1} in AA concentration in the gas phase corresponds to a change of about 130 μg L^{-1} in the liquid phase. However, an equal change in ethanol concentration in the liquid phase corresponds to a change of only 13 nL L^{-1} in the gas phase. A practical PA detection limit for ethanol is 10 nL L^{-1} (Bijnen, 1995). Thus, a measured change of 13 nL L^{-1} in ethanol concentration was considered as noise if it appeared on a background equal to or higher than 130 nL L^{-1} of ethanol. In view of this and the relatively high background of ethanol in all red bell peppers measured, even under normoxic conditions, time-resolved ethanol measurements were not continued.

A single fruit was placed either in a simple glass cuvette or in one consisting of two compartments (Fig. 1), which allowed for separate measurements of gas exchange through the stem and the cuticle. During the anoxic period the fruit was exposed to either dry or humidified N_2; independent flow could be passed through each compartment. Ambient air conditions (20% O_2) were established by admixing 100% oxygen at the inlet instead of the outlet of the cuvette to raise the total flow rate through the fruit cuvette by 20%. The desired oxygen concentration inside the fruit cuvette was established within about 5 min after the start of the specific treatment. We did not attempt to determine the corresponding O_2 concentrations within the pepper. The change in O_2 concentration in the atmosphere around the fruit could be applied to the stem and the cuticle sites independently. All of the experiments were conducted in normally illuminated laboratory conditions at approximately 23°C.

More than 10 different red bell pepper fruits were investigated. In all of them the post-anoxic upsurge was observed. In 5 fruit, repeated anoxia was applied and each fruit responded with the sharp drop in AA emission (described below).

RESULTS AND DISCUSSION

AA, Ethanol, and CO_2 Production during the First Anaerobic Treatment

Typical patterns of AA, ethanol, and CO_2 emission from red bell pepper resulted when the fruit was exposed to 100% N_2 (Fig. 2). A readily detectable AA emission was present in red bell pepper cv "Maor" (Fig. 2A) and was observed for 5 h, even under 20% O_2 conditions. Just before the start of anaerobic treatment it yielded 0.2 nL h^{-1} g fresh weight^{-1}. The onset of enhanced AA production was delayed by about 2 h from the beginning of anaerobic treatment. Thereafter, AA emission steeply increased up to 2.2 nL h^{-1} g fresh weight^{-1}, reaching the constant level about 2.5 h later.

CO_2 emission (Fig. 2B) decreased from 19.4 μL h^{-1} g fresh weight^{-1} to 8.1 μL h^{-1} g fresh weight^{-1}, within 20 min after the start of anaerobic treatment. After 8 h of anoxia (data not shown), CO_2 emission yielded 5 μL h^{-1} g fresh weight^{-1}.

Ethanol production in bell peppers was readily observable both under aerobic and anaerobic conditions. An example is shown in Figure 2C for freshly harvested red bell pepper cv "Spirit." Ethanol emission under 20% O_2 conditions from the cuticle as well as from the stem yielded 6.2 nL h^{-1} g fresh weight^{-1}, which corresponds to a steady-state ethanol production rate of 12 ng h^{-1} fruit^{-1}. A distinct rise in ethanol production could be observed with a delay of 2 h after the start of anaerobic treatment (Fig. 2C, 1.1 h). Five hours of anoxia resulted in ethanol emission of 9.4 nL h^{-1} g fresh weight^{-1}.

Separate treatments of the stem and the cuticle, and especially the measurement of their AA responses, often yielded distinct and surprising, although not always consistent, results regarding the permeability of different tissues (we will discuss this in future studies).

A change in the metabolism of the fruit is indicated by the rapid 2.5-fold drop in CO_2 emission rate at the beginning of anaerobic treatment (Fig. 2B). We have no estimate of the rate by which anoxia was established within the pepper tissue; seemingly, the TCA cycle is rapidly slowed down by decreased oxygen availability. However, the CO_2 measurement cannot serve as a reliable indicator of fermentation onset. The consistently slow CO_2 production rates, at least during the first 10 h of anoxia, demonstrate a

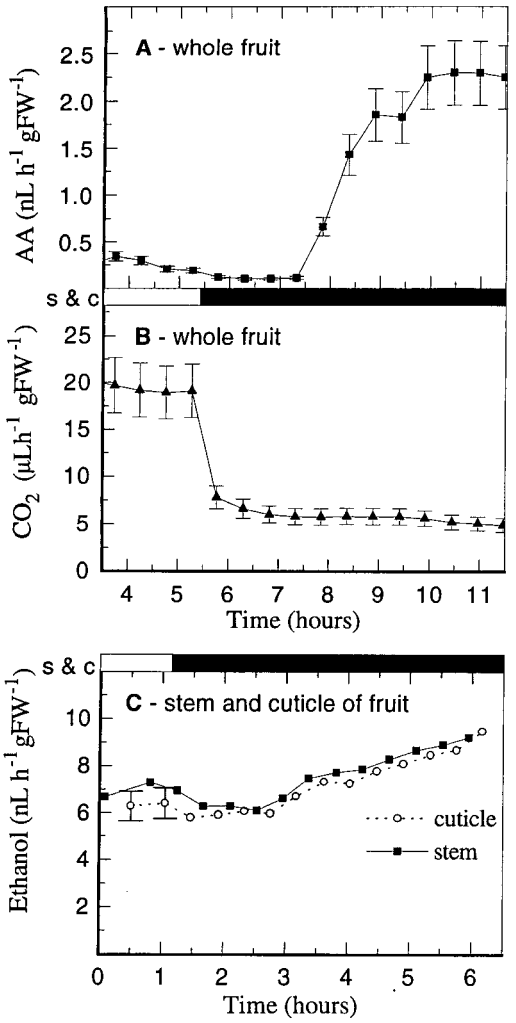

Figure 2. Total AA (A) and CO_2 (B) emission rates from stem and cuticle of red bell pepper cv "Maor" (160 g fresh weight), first under aerobic conditions, followed by anaerobic conditions imposed 5.4 h after fruit was placed in its cuvette. To facilitate gas exchange, the stem site was punctured. A dry air flow was used. Each experimental point was obtained by measuring the absorption on 15 laser lines, for the cuvette containing the fruit and for an empty cuvette. The signal difference yields the concentrations, taking into account the absorption coefficients for the contributing gases. The time resolution was 40 min. Note the delay of 2 h in the AA response to denied O_2. Zero on the time axis corresponds to the moment the fruit was enclosed in the cuvette. C, Ethanol emission rate from freshly harvested red bell pepper cv "Spirit" (160 g fresh weight; humid air flow). Stem (s) and cuticle (c) emissions were measured simultaneously and do not show significant differences. Anaerobic treatment started at 1.15 h (s and c). FW, Fresh weight; □, aerobic conditions; ■, anaerobic conditions.

lack of any marked acceleration of glycolysis (the "Pasteur effect").

In contrast to the fast decrease of respiration at the beginning of anaerobic treatment, the increase in AA and ethanol production shows a significant delay of 1 to 3 h. We propose that this increase marks the onset and further development of ethanolic fermentation through the fruit tissues. Previous measurements of AA during anoxia in leaves (Kimmerer and MacDonald, 1987) used head space sampling techniques or enzymic assays; long accumulation times and low sensitivity mostly precluded real-time evaluation of the metabolic changes during the transition to anoxia. In maize root tips, ethanol production appears within 10 min from the start of hypoxia (Roberts et al., 1984; Fox et al., 1995) and is triggered by the decrease in cytoplasmic pH at the onset of anoxia. Measurements of respiratory activity of bell peppers following storage under 1.5% O_2 (Rahman et al., 1993) showed that about 2 h is necessary to re-equilibrate the atmosphere inside the locules of fruit with the outer atmosphere of 20% O_2. These results strongly suggest that in the present study, as the bell pepper fruit was deprived of O_2, anoxia within the locular cavity and the ensuing fermentation were delayed by about 1 to 3 h with respect to the establishment of anoxia in the cuvette atmosphere. However, once fermentation started, a stationary AA emission was reached within 2.5 h, compatible with a sharp threshold for fermentation.

Post-Anoxic AA Emission

Readmission of O_2 after an anoxic period had a dramatic effect on AA emission. Both cultivars responded to reaeration with an upsurge in AA from both stem and cuticle sites. "Maor" peppers showed AA emission from stem and cuticle, whereas "Spirit" peppers were active mostly through the stem.

Results in Figure 3 show five post-anoxic AA upsurges from the cuticle of a bell pepper cv "Maor" following anoxic periods varying from 40 min to 20 h. First, a 3-fold upsurge from 3.2 nL h^{-1} g fresh $weight^{-1}$ to 12 nL h^{-1} g fresh $weight^{-1}$ was seen from the cuticle when O_2 was supplied to the stem at 6.62 h, ending a 4.3-h anaerobic treatment. AA evolution reached a maximum 25 min after transfer to 20% O_2; the asymmetric peak had a full width at half maximum of 36 min. Second, a 3-fold upsurge from the cuticle occurred at the end of a second period of stem anoxia (20.73 h). A maximum AA emission rate of 15.3 nL h^{-1} g fresh $weight^{-1}$ was measured 25 min after O_2 readmission to the stem. Third, a 2-fold upsurge from the cuticle with a maximum output 34 min after the end of third anoxia (44.03 h) was seen when O_2 was re-admitted to the cuticle site only. Fourth, an additional 2-fold upsurge in AA evolution was measured at the cuticle when O_2 was also re-admitted to the stem site (44.82 h). This last post-anoxic upsurge did not exhibit a peak but, rather, a plateau. AA decreased by only 5% after 2 h, compared with the 40% decrease seen 1.4 h after the end of the second period of anoxia. Fifth, AA emission surged up promptly (49.02 h) after a 40-min interval of (fourth) anoxia.

Each re-exposure of red bell pepper fruit to 20% O_2 after a period of anoxia was accompanied by an upsurge of AA production. Short periods of anoxia resulted in a transient post-anoxic AA peak (full width at half maximum of about 40 min) whereas after numerous and longer anoxic treatments large rates of AA production were sustained for several hours. The post-anoxic upsurges occurred with a

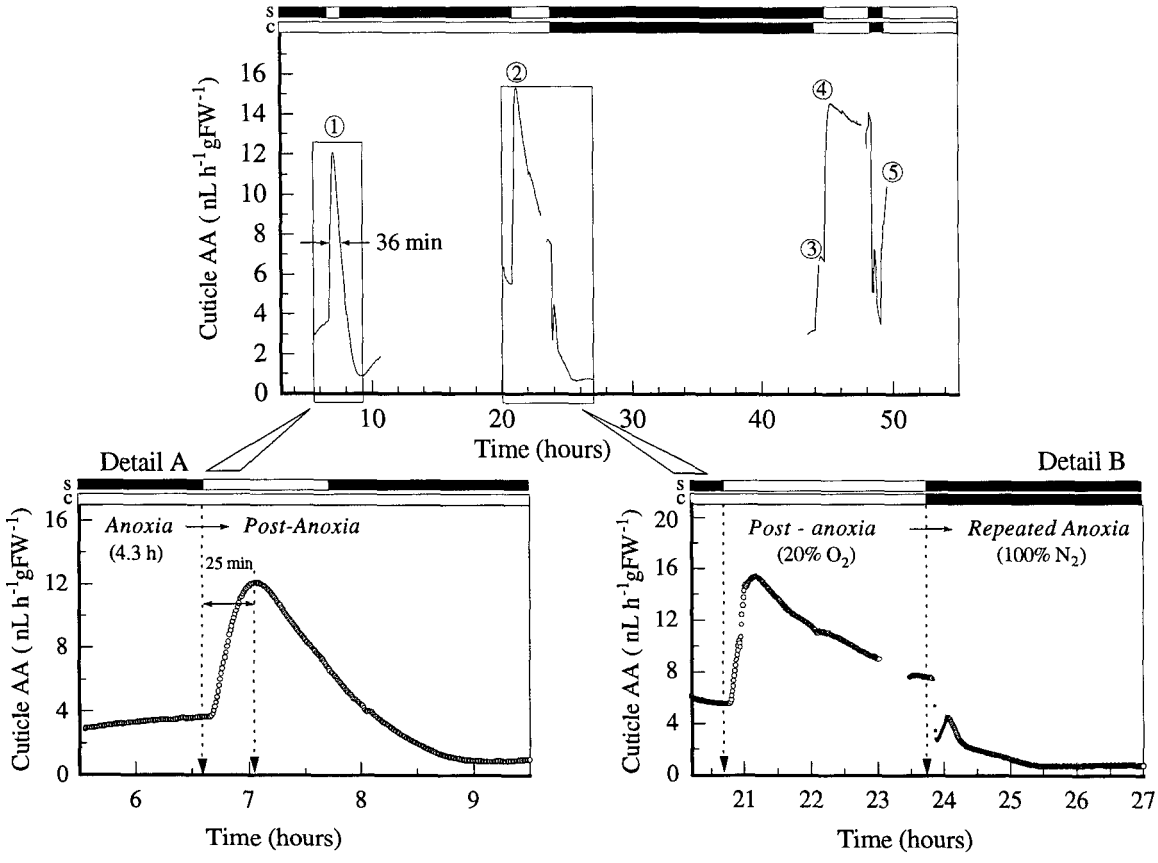

Figure 3. AA emission rate from the cuticle of red bell pepper cv "Maor" (124 g fresh weight; 1-mm hole punctured through the stem site; dry air flow). Aerobic or anaerobic treatments were given to cuticle (c) and stem (s) sites separately. Anaerobic treatments started at 2.33 h (s), 7.62 h (s), 23.83 h (s and c), and 48.33 h (s and c). Oxygen was re-admitted at 6.62 h (s), 20.73 h (s), 44.03 h (c), 44.82 h (s), and 49.02 h (s and c). A, Detail with the time scale expanded. B, Detail with the time scale expanded. The existence of a peak in AA production at about 24 h is due to memory effects of the tubing. □, Aerobic conditions; ■, anaerobic conditions.

delay of only 5 min, entirely attributed to instrumental effects. The upsurge itself took place on a time scale considerably shorter than what was found for the enhanced AA emission during the first anaerobic treatment (Fig. 2A). Our attempt to account for the burst-like post-anoxic AA emission inevitably raises many questions regarding the detailed dynamics of post-anoxic metabolism, e.g. how fast does the fermentative pathway switch off? How fast do the electron transport and the TCA cycle switch on? What are the dynamic changes of the NAD^+ to NADH ratio both in the cytosol and inside the mitochondria during the first minutes of post-anoxia?

High post-anoxic levels of AA have been observed in potato tubers 5 h after re-aeration, following anoxic treatment for 24 to 72 h (Pfister-Sieber and Braendle, 1994). A 5-fold increase in AA has been observed 30 min after transfer to air for anoxic *Glyceria maxima* rhizomes (Monk et al., 1987a). The conversion of anaerobically accumulated ethanol to AA may account for these observations and may be one of the causes of post-anoxic injury (Studer and Braendle, 1988). Two main pathways of ethanol oxidation, i.e. NAD^+-dependent conversion of ethanol to AA catalyzed by the action of ADH, have been proposed (Monk et al., 1987a):

$$AA + NADH \leftrightarrow \text{ethanol} + NAD_+ \quad (1)$$

and H_2O_2-dependent catalase-controlled peroxidation

$$\text{ethanol} + H_2O_2 \rightarrow AA + 2H_2O. \quad (2)$$

The flow-through technique and the fast time response of the present study led us to conclude that the AA upsurge in peppers is a direct response to oxygen re-admission, presumably as a consequence of ethanol oxidation by the "peroxidatic" action of catalase. We will emphasize here the importance of the second mechanism, although it is highly probable that both mechanisms play a role, especially in long-term, post-anoxic AA production. In a study of air adaptation of submerged rice seedlings, Shibasaka and Tsuji (1991) addressed in detail the changes in the respiratory system upon re-exposure of the seedlings to air. It was found that O_2 uptake increased rapidly, reaching a plateau 16 h after exposure to air, and that restoration of mitochondrial activity might have been NAD^+-limited.

Guided by these results and by intuition we find it unlikely that a significant surplus of NAD^+ would be available to ADH during the first minutes after re-exposure of pepper fruit to O_2. In other words, the NAD^+-dependent ADH-reaction would be too slow on re-exposure to O_2 to account for the 3- to 10-fold AA upsurge observed within a few minutes. Thus, the "peroxidatic" action of catalase is held responsible for the AA post-anoxic upsurge rather than ADH-mediated ethanol oxidation.

This reaction requires H_2O_2, and the most probable source is dismutation of the superoxide radical (O_2^-) within mitochondria by superoxide dismutase (Monk et al., 1987b; Scandalios, 1994). The high level of reducing equivalents (NADH) within mitochondria prevailing in the anoxic tissue just before re-exposure to O_2 (Reggiani at al., 1985; Van Toai and Bolles, 1991) may well favor a rapid generation of active O_2 species, e.g. O_2^-, H_2O_2, and OH• during the first minutes of post-anoxia. The post-anoxic ethanol oxidation rate is then dependent upon the rate of H_2O_2 production. It reaches a peak value and the subsequent decrease in ethanol oxidation is the (indirect) result of diminishing reducing power when the mitochondria resume normal functioning and the steady-state normoxic metabolism is eventually re-established. The use of the catalase-inhibitor 3-amino-1,2,4-triazole to substantiate our hypothesis that post-anoxic AA originates from the "peroxidatic" action of catalase might provide a clear answer; however, based on previous indications (Oshino at al., 1973), we concluded that unequivocal conclusions from such experiments are not warranted.

Repeated Anoxia

The start of the second anaerobic treatment (Fig. 3, 7.62 h) of red bell pepper cv "Maor" barely affected the already existing trend of a fast AA decrease. However, the third (23.83 h) and fourth (48.33 h) anaerobic treatments of both the stem and the cuticle showed a dramatic change of the cuticle AA emission. The time scale of Figure 3 is expanded in detail in Figure 3B to show the start of the third anaerobic treatment. Remarkably, switching to anaerobic conditions (Fig. 3B, 23.83 h) resulted in a 50% decrease within about 15 min, down to the (extrapolated) anoxic level. Only 1.6 h later, constant, very low concentrations were found. The sharp decrease at the start of repeated anoxia, together with the burst-like upsurge caused by post-anoxic admission of O_2, led us to the conviction that the responsible reaction site of the bell pepper must be sought at tissue that is directly exposed to the changing O_2 conditions in the cuvette. In contrast, the locular cavity tissue is expected to react with a time delay of about 2 h (Rahman et al., 1993).

From AA measurements at the start of (repeated) anoxia it is evident that anoxia has two opposing effects on AA formation. The obvious one is promotional when the switching to fermentation leads to AA production as an intermediate. The more striking effect is inhibitory when anoxia is imposed during post-anoxic enhancement of AA formation. Here the sudden reduction of O_2 concentration in the atmosphere surrounding the fruit immediately arrests further formation of active oxygen species, which would deny H_2O_2 for conversion of ethanol to AA (Equation 2). These two opposing effects become discernible in our time-resolved measurements, mainly due to the fact that each fermentative AA production is delayed by 1 to 3 h from the start of anaerobic treatment; this delayed reaction is evident in the time course of AA response to repeated anaerobic treatment at 7.62 h in Figure 3. After the start of second anaerobic treatment at 7.62 h, a delay of 1.7 h was observed to change the AA trend from a decrease to an increase.

Post-Anoxic Response after Repeated Anoxia

Post-anoxic responses in AA production after second and third anaerobic treatment of the same pepper (Fig. 3, at 20.7 h and 44.0 h) showed that, following the initial upsurge, post-anoxic AA emission decreased more slowly than after the upsurge generated by the first (4.3-h-long) anaerobic treatment. Prolonged periods of anoxia are expected to cause damage to mitochondria and, consequently, longer times of post-anoxic aeration will be required to obtain full or partial oxidative capacity. In support of this, a diminished recovery of marker enzymes and proteins in mitochondrial fractions was reported after 6 to 15 h of anoxia in excised castor bean endosperms (Donaldson, 1985).

Postharvest Treatment to Inhibit Ripening of Fruit

Several different postharvest treatments of fruit (and flowers) are known to delay ripening (senescence) of fruit. These include exogenously applied ethanol vapor (Kelly and Saltveit, 1988; Heins, 1980), exogenously applied AA vapor (Pesis, 1995), and anoxic treatment ($O_2 < 2\%$) for 24 h (Pesis, 1995). Our results and tentative interpretation throw a new light on these effects.

If ripening is also viewed as an oxidative phenomenon (Brennan and Frenkel, 1977; Knee, 1991), any treatment that reduces the level of H_2O_2 will potentially delay ripening. In plant tissue the presence of "peroxidatic" substrates, e.g. ethanol, can help degrade H_2O_2 more rapidly (Chance and Oshino, 1971; Oshino at al., 1973) by decreasing the concentration of the catalase-H_2O_2 complex and thus providing more free catalase for rapid H_2O_2 removal. Exogenously applied AA during postharvest treatments inhibits ripening by being partially converted to ethanol, which will then promote H_2O_2 degradation. This in turn implies the presence of ADH in the fruit. A constitutive ADH enzyme system has been suggested to explain the immediate ethanol synthesis in leaves (Kimmerer and MacDonald, 1987). Special care must be exercised during application of postharvest anoxic treatments so that post-anoxic generation of H_2O_2 and free radicals will not outweigh the advantageous effects of ethanol accumulated during the anoxic period. Pre-treatment of mango fruit with low O_2 or ethanol vapor for 48 h caused an increase in the activity of catalase (E. Pesis, personal communication)

and was associated with a decrease in chilling injury symptoms. H_2O_2 production at low concentrations was suggested to induce synthesis of more catalase (Prasad et al., 1994) in maize seedlings subjected to low temperature stress. The existence of a similar mechanism in fruit may provide an alternative (or additional) physiological reason to account for the beneficial effects of an anoxic postharvest treatment. The dual role of H_2O_2 as a destructive agent and as a gene regulator is intriguing. Such a dual nature of active oxygen species has been postulated for plant-pathogen interactions (Mehdy, 1994).

CONCLUSIONS

In this work the usefulness of the PA technique for time-resolved in vivo measurement of AA and ethanol in intact fruit under anoxic and post-anoxic conditions was established. For the first time to our knowledge, prompt changes in gas emission rates of bell pepper have been observed in reaction to changing the composition of the surrounding atmosphere. The observed post-anoxic AA upsurge may serve as a sensitive indicator of post-anoxic injury and may be useful in fine-tuning various postharvest treatments.

ACKNOWLEDGMENTS

The authors would like to thank F.G.C. Bijnen and H. de Vries (Department of Molecular and Lasers Physics, University of Nijmegen) for stimulating discussions. The authors are grateful to E. Pesis (Volcani Institute, Israel) for helpful suggestions during the course of the measurements and for providing data prior to publication. We thank B. Visser (Huissen, The Netherlands) for providing the fruit material. The critical reading of the manuscript by A. Alpi, C.W.P.M. Blom, R. Braendle, M. Jackson, T.K. Prasad, and L.A.C.J. Voesenek is greatly appreciated. We thank C. Sikkens, C. Timmers, and H. Schoutissen for technical support.

Received September 23, 1996; accepted November 25, 1996.

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