Plant Physiol. (1984) 76, 88–91
0032-0889/84/76/0088/04/$01.00/0

Effect of a Brief CO_2 Exposure on Ethylene Production

Received for publication November 23, 1983 and in revised form April 5, 1984

ALICIA R. CHAVES*[1] AND JORGE O. TOMÁS
Centro de Investigación y Desarrollo en Criotecnología de Alimentos, Universidad Nacional de La Plata, CONICET—CIC, Calles 47 y 116, (1900) La Plata, Argentina

ABSTRACT

Ethylene production and respiration by Granny Smith apples were inhibited by treatment with 20% CO_2 for 2 hours. A similar effect was observed in tissue slices when treated at either 0 or 25°C.

The inhibition continued even after an extended aeration period. There is also an inhibition of ethylene emission in tissue slices incubated with exogenous 1-aminocyclopropane-1-carboxylic acid (ACC).

In general, CO_2 treatment increased the ACC content of the tissue. These observations are consistent with the idea the action of CO_2 is directed toward the enzyme system responsible for the conversion of ACC into ethylene.

The storage life of apples can be increased by treating the fruit with a high concentration of CO_2 during a short period.

Burg and Burg (9, 10) found that CO_2 is a competitive inhibitor of ethylene action, and Beyer (5, 6) reported that such an action is related with ethylene metabolism. CO_2 can affect this metabolism by inhibiting ethylene oxidation to CO_2.

The action of CO_2 in delaying ripening rate has been related to a decrease in respiratory activity and an inhibition of the succinic oxidase complex and especially of the succinic dehydrogenase (18–20).

In addition to its respiration blocking action, CO_2 at high concentrations also decreases ethylene production in many fruits including apples, pears, tomatoes, etc. (7, 8, 14–16, 22). The pathway of ethylene production has been shown as methionine → SAM[2] → ACC → ethylene (2, 12).

This paper presents results on the effects of CO_2 at high concentrations on ethylene production and ACC levels in apple fruit tissue.

MATERIALS AND METHODS

Plant Material. Granny Smith apples were harvested during the growing season of 1982 (April 1982). The fruits were stored in air at 0°C during a short period until used.

Experiments with Whole Fruit. A current of moist air or a mixture containing 10, 20, or 30% CO_2 was flushed (flow rate: 4 L h^{-1}, for different periods of time, through jars containing one fruit.

Experiments with Tissue Slices. Discs (2 × 10 mm) of cortical tissue were incubated (about 1 g) in 25 ml containers with 3 ml of the following incubation medium: 0.6 M sorbitol, 10 mM K-phosphate buffer (pH 6.8), and 50 μg/ml chloramphenicol (4).

In some experiments a HCO_3^--CO_2 buffer was used to control the CO_2 concentration of the gas phase (21). The experiments were carried out with 10, 20, and 30% of CO_2 at 0 and 25°C.

Experiments with Exogenous ACC. In some cases, ACC at 4 and 100 μM was added to the incubation media. The experiments were carried out at 0 and 25°C.

Ethylene Determination. Ethylene production from whole fruit was measured by GC using a flame ionization detector. Samples were taken at the exit of the flushing gas stream as well as in the inner part of the fruit. A glass tube closed at one end with a septum was inserted into the apple core through its open end for internal ethylene sampling.

CO_2 Determination. CO_2 was measured with a thermal conductivity detector GC fitted with a silica gel and molecular sieve columns.

ACC Determination. Once the experiment was over, the tissue slices were removed from the incubation solution, washed and ACC extracted with 80% ethanol. After evaporation, the residue was taken up into water and ACC determined by the method of Lizada and Yang (13).

Statistical Analysis. All experiments were repeated at least three times. The effects of CO_2 treatment were evaluated by analysis of variance and the least significant differences between means (LSD) at the 0.05 level were calculated.

RESULTS

When whole fruits were treated with 20% CO_2 for either 2 h or 5 d, ethylene production was inhibited. An initial 2-h exposure to 20% CO_2 inhibited internal ethylene production by 28.4% after 5 d, whereas a continuous exposure to 20% CO_2 inhibited ethylene production by 80% after the same 5-d period.

Differences were larger in the inner part of the fruit than in the emitted gases making more evident an inhibition of ethylene production by high concentrations of CO_2.

After CO_2 removal, ethylene production remained lower in the treated CO_2 than in the control fruit (Fig. 1), which showed the existence of a residual effect. This effect was more evident in fruit treated with high CO_2 during 5 d.

The inhibition of ethylene production was higher as the CO_2 concentration was increased (Table I).

Repiration was also decreased by high CO_2 treatment (Table I), a residual effect being observed after the removal of the latter.

Effect of CO_2 on C_2H_4 Production in Tissue Slices. The inhibitory effect of CO_2 on ethylene production in apple discs is shown in Figure 2. As in whole fruit, the degree of inhibition increased (Fig. 1) as the CO_2 concentration and exposure period were increased. The CO_2 effect was more evident at 25°C than at 0°C.

By eliminating CO_2 from the medium, either by airing the containers or replacing the medium with an aired incubation medium, it was found that at the end of an incubation period (which was twice the duration of the CO_2 treatment) the reduc-

[1] Member of the Research Career of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

[2] Abbreviations: SAM, *s*-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic.

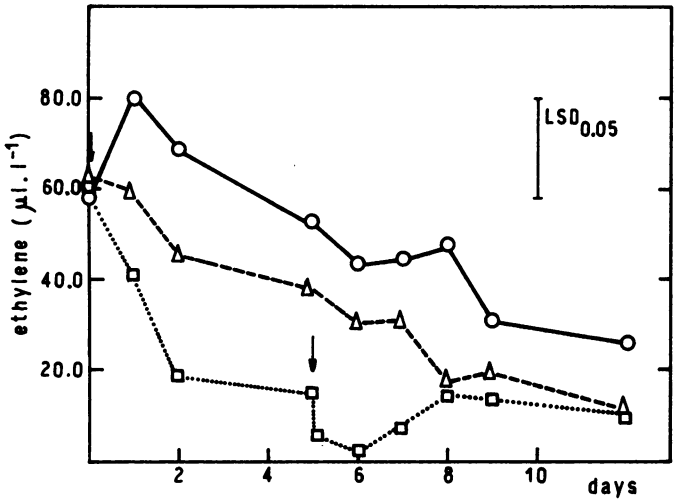

FIG. 1. Internal ethylene production in Granny Smith apples treated with 20% CO_2 during 2 h (Δ- - -Δ) and 5 d (□···□) compared with the ones kept in air (O——O) (the arrows show the end of the treatment). Temperature: 20°C.

Table I. *Ethylene and Respiration Inhibition in Granny Smith Apples with Different Concentrations of CO_2 during 2 Hours and 3 Days at 20°C*

CO_2	Ethylene Inhibition		Respiration Inhibition	
	2 h	3 d	2 h	3 d
%	%		%	
0.03	0.0	0.0	0.0	0.0
10	3.1	31.9		27.2
20	10.8	64.1	11.6	62.6
30	24.3	85.0	17.4	73.0

tion in ethylene production in the CO_2 treated discs still continued (Table II). This result confirmed the residual effect observed in the whole fruit (Fig. 1). The same residual inhibition of respiration was observed (Table II).

CO_2 Effect on Ethylene Production from Exogenous ACC. When ACC was added to the incubation medium at 0.10 mM, a stimulation of ethylene production was observed which was approximately 60 times that of the controls. This ethylene production was also inhibited by the presence of CO_2 (Table III).

The same result was obtained at 0 and 25°C and at both ACC concentrations (4 mM and 100 µM).

CO_2 Effect on the Endogenous ACC Content. The ACC content of tissue slices in air decreased rapidly during the first 6 h of incubation either at 0 or 25°C. For longer periods of time at 0°C it continued to decrease but more slowly. In contrast, at 25°C the ACC content started to increase between the 6th and 10th hour (Table IV), probably due to tissue senescence.

The ACC content in tissue slices did not vary with different temperatures for incubation periods of less than 6 h (Table IV).

Adding 20% CO_2 to discs incubated at 0°C resulted in a slower decline of ACC and this decline started at about 6 h after incubation (Table IV).

At 25°C, with 4 h incubation and in the presence of CO_2, there was an increase in endogenous ACC content with respect to the controls. By the end of 10 h incubation the ACC level started to increase, as in the controls, that level being lower in the CO_2-treated discs (Table IV). This diminution was greater when the percentage of CO_2 in the atmosphere increased.

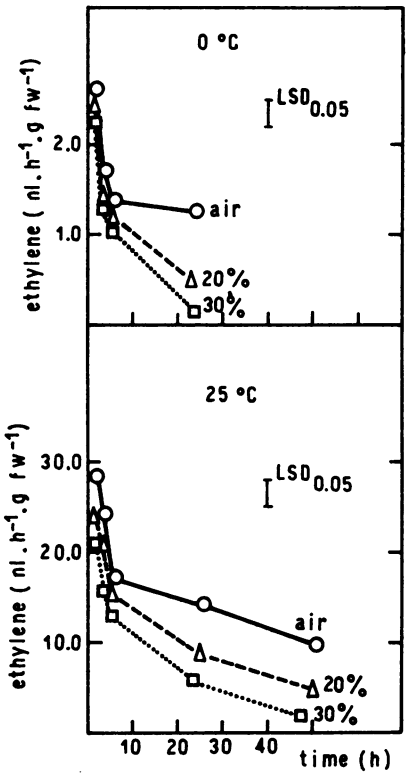

FIG. 2. Ethylene production of cortex tissue slices of Granny Smith apples incubated in air, 20 and 30% CO_2 during the indicated periods at 0 and 25°C.

Table II. *Ethylene and CO_2 Production of Apple Cortex Slices*

The slices were incubated in air or in 20% CO_2 during 2 or 24 h and then in air 4 or 48 h at 25°C.

Time	Ethylene Production		Respiration Rate	
	Air	20% CO_2	Air	20% CO_2
h	*nl/h·g fresh wt*		*µl CO_2/h·g fresh wt*	
2 + 4	9.34	3.23	31.35	27.02
24 + 48	4.32	2.79	24.76	20.30
$LSD_{0.05}$	0.62		0.97	

After airing the discs, the ACC level was slightly higher in the ones incubated in CO_2 at both temperatures (Table V).

Whole fruit were treated during 3 d with 20% CO_2 at 0°C for determination of the ACC content; after being analyzed it was found that the ACC content was higher in the treated fruit (4.83 *versus* 5.92 nmol ACC/g in controls and treated fruit, respectively). This situation remained the same after 7 d of aeration at 25°C (2.01 and 4.72 nmol ACC/g, respectively). In both the controls and treated fruit stored at 25°C, the ACC content increased when the treatment period increased and the difference between them tended to disappear (for 30 d at 25°C: 91.86 *versus* 98.76 nmol ACC/g, respectively).

DISCUSSION

As reported, CO_2 at high concentrations of 10 to 30% inhibited ethylene production in apple cortex slices and in whole fruit (Figs. 1 and 2). Likewise, respiration is decreased due to an alteration of mitochondrial activity (20).

In 1981, Apelbaum *et al.* (4) reported that apple discs convert

Table III. *Effect of ACC on Ethylene Production of Granny Smith Apple Discs*

The discs were incubated either in absence or presence of 4 μM and 100 μM ACC in air or in 20% CO_2 during the indicated periods at 25°C.

Time	Ethylene Production		Inhibition
	Air	CO_2	
h	*nl/h·g*		%
Control			
6	8.40	7.65	8.9
24	6.95	3.33	52.1
$LSD_{0.05}$	0.81		
ACC 4 μM			
6	11.90	10.72	9.9
24	9.18	4.27	53.5
$LSD_{0.05}$	0.60		
ACC 100 μM			
6	982.96	340.26	65.38
24	555.00	248.06	55.30
$LSD_{0.05}$	64.22		

Table IV. *Endogenous ACC Content of Granny Smith Apple Discs*

The discs were incubated in air or in the presence of 20% CO_2 during the indicated periods of time at 0 and 25°C.

Time	ACC Content in	
	Air	CO_2
h	*nmol/g*	
Temperature 0°C		
0	4.00	4.00
2	1.11	1.08
4	0.82	0.75
6	0.45	0.67
10	0.40	0.65
24	0.27	0.57
Temperature 25°C		
0	4.00	4.00
2	2.03	1.84
4	0.62	1.11
6	0.39	0.66
10	0.75	0.77
24	2.30	1.46
48	5.66	2.83
$LSD_{0.05}$ between treatments		0.27

Table V. *Endogenous ACC Content of Granny Smith Apple Discs*

The discs were incubated in air during the indicated periods of time or in 20% CO_2 during 2 or 24 h and then in air during 4 or 48 h at 25°C.

Time	ACC Content in	
	Air	CO_2
h	*nmol/g*	
2 + 4	0.84	0.98
24 + 48	0.88	1.07
$LSD_{0.05}$	0.13	

exogenous ACC to ethylene through the natural physiological system. In order to establish how CO_2 acts, we studied its effect on the conversion of ACC to ethylene in tissue slices. A 20% CO_2 treatment inhibited this conversion indicating that CO_2 influences C_2H_4 biosynthesis by affecting the last step in the ethylene biosynthesis sequence.

Adams and Yang (2) reported ACC to be the immediate precursor of C_2H_4. The oxidation of the latter would produce C_2H_4 as well as CO_2 (1). Thus, CO_2 could act through a mass action effect in the inhibition of this reaction. However, when discs were treated with CO_2 and then aired, they continued to produce less ethylene. This clearly indicates that the CO_2 effect is not readily reversible. Therefore, the inhibition of ethylene production could not be due to a simple mass effect.

In the same way, a higher level of endogenous ACC was found in apple discs incubated for more than 4 h at 0°C and during 4 and 6 h at 25°C. For longer incubation periods at this temperature we must take into account the tissue senescence and/or the tissue alteration effect on the conversion of ACC, which is strongly diminished. Mayak *et al.* (17) reported that the conversion of ACC to ethylene becomes rate limiting with tissue senescence, resulting in an accumulation of ACC, as it is shown in Table IV. When delaying senescence, CO_2 probably produces a lower accumulation of ACC, and even a lower one when the percentage of CO_2 is higher.

Since the enzyme or enzymes involved in the conversion of ACC to ethylene is or are linked to membranes (3, 4, 11), the CO_2 action could be due to an effect on them. The higher content of ACC found in discs treated with CO_2 and then aired confirms the partial reversibility of the effect of CO_2. The results obtained with whole fruit were the same as the ones found for tissue slices.

To be able to elucidate the action of CO_2 at high concentrations it will be necessary to study the enzyme or enzymes involved in ethylene biosynthesis.

Acknowledgments—The authors thank Dr. María Cristina Añón for having read and criticized this work.

LITERATURE CITED

1. ABELES FB 1972 Biosynthesis and mechanism of action of ethylene Annu Rev Plant Physiol 23: 259–292
2. ADAMS DO, SF YANG 1979 Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76: 170–174
3. ANDERSON DJ, M LIEBERMAN, R STEWART 1979 Ethylene production by apple protoplasts. Plant Physiol 63: 931–935
4. APELBAUM A, AC BURGOON, JD ANDERSON, T SOLOMOS, M LIEBERMAN 1981 Some characteristics of the system converting 1-aminocyclopropane-1-carboxylic acid to ethylene. Plant Physiol 67: 80–84
5. BEYER EM JR 1979 Effect of silver ion, carbon dioxide and oxygen on ethylene action and metabolism. Plant Physiol 63: 169–173
6. BEYER EM JR 1979 ^{14}C-Ethylene metabolism during leaf abscission in cotton. Plant Physiol 64: 971–974
7. BRAMLAGE WJ, PH BAREFORD, GD BLANPIED, DH DEWEY, S TAYLOR, SW PORRITT, EC LOUGHEED, WH SMITH, FS MCNICHOLAS 1977 Carbon dioxide treatments for "Mc Intosh" apples before C.A. storage. J Am Soc Hortic Sci 10: 658–662
8. BUESCHER RW 1979 Influence of carbon dioxide on postharvest ripening and deterioration of tomatoes. J Am Soc Hortic Sci 104: 545–547
9. BURG SP, EA BURG 1967 Molecular requirements for the biological activity of ethylene. Plant Physiol 42: 144–152
10. BURG SP, EA BURG 1969 Interaction of ethylene, oxygen and carbon dioxide in the control of fruit ripening. Qual Plant Mater Veg XIX: 185–200
11. IMASEKI H, A WATANABE 1978 Inhibition of ethylene production by osmotic shock. Further evidence for membrane control of ethylene production. Plant Cell Physiol 19: 345–348
12. LIEBERMAN M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
13. LIZADA MC, SF YANG 1979 A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100: 140–145
14. LOONEY NE 1975 Control of ripening in "Mc Intosh" apples II. Effect on growth regulators and CO_2 on fruit ripening, storage behaviour and shelf life. J Am Soc Hortic Sci 100: 332–336
15. MARCELLIN P, AR CHAVES 1982 Effets d'un traitement intermittent in atmosphere fortement enrichie en gaz carbonique sur la maturation de tomates vertes a 20° et 12°C. CR Acad d'Agric de France Seánce 13/1/82 pp 112–123
16. MARCELLIN P, AR CHAVES 1983 Effects of intermittent high CO_2 treatment on storage life of avocado fruits in relation to respiration and ethylene production. Acta Hortic 138:
17. MAYAK S, L RAYMOND, JE THOMPSON 1981 Ethylene formation from 1-aminocyclopropane-1-carboxylic acid by microsomal membranes from senescing carnation flowers. Planta 153: 49–55
18. RANSON SL, DA WALKER, ID CLARKE 1957 Effects of CO_2 on mitochondrial enzymes from Ricinus. Biochem J 66: 57P

19. RANSON SL, DA WALKER, ID CLARKE 1960 Effects of CO_2 on mitochondrial enzymes from Ricinus. Biochem J 76: 216–220
20. SHIPWAY MR, WJ BRAMLAGE 1973 Effects of carbon dioxide on activity of apple mitochondria. Plant Physiol 51: 1095–1098
21. UMBREIT WW, RH BURRIS, JT STAUFFER 1949 Manometric Techniques and Tissue Metabolism, Chap 3. Burgess Publ. Co., Minneapolis
22. WANG CY, WM MELLENTHIN 1975 Effect of short term high CO_2 treatment on storage of "d'anjou" pear. J Am Soc Hortic Sci 100: 492–495