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Respiration and C_2H_4 Production in Various Harvested Crops Held in CO_2-enriched Atmospheres

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Abstract. **The respiration rate (O_2 uptake) and the rate of C_2H_4. production were measured before, during, and after 24 hours of treatment with 60% CO_2 (20% O_2) in 18 kinds of fruits and vegetables by use of an automated system connected to a microcomputer. High CO_2 decreased respiration only in climacteric fruit and broccoli, which were producing C_2H_4. Ethylene production decreased with CO, treatment of peaches, tomatoes, and broccoli, but that of bananas increased. In five nonclimacteric fruits (three citrus species, grapes, and Japanese pears) and several vegetables (carrots, onions, cauliflower, and cabbage), in which C_2H_4 production was not detected, high CO_2 affected respiration little, if at all. When eggplants, cucumbers, podded peas, spinach, and lettuce were treated with high CO_2, C_2H_4 production began and respiration increased. These results indicate that the respiratory responses of harvested horticultural crops to high CO_2 might be mediated by the effects of CO_2 on the action and/or synthesis of C_2H_4.**

There have been many studies of the storage of fruit and vegetables since the report of Kidd and West (1927) concerning the beneficial effects of high CO_2/low O_2 storage (controlled atmosphere, CA). Since CO_2 is a product of respiration, it would be expected that respiration rate would decrease as CO_2 concentration in the atmosphere increases (Hewer, 1987). Succinic acid accumulates and the activities of enzymes involved in its metabolism decrease in several fruits stored in high CO_2, which is indirect evidence for this possibility (Biale, 1960; Kader, 1986).

Carbon dioxide inhibits C_2H_4. action competitively and helps regulate C_2H_4 biosynthesis (Burg and Burg, 1967). Therefore, some of the benefits of storage in a high-CO_2 atmosphere arise when C_2H_4 production or C_2H_4-mediated reaction is inhibited (Herner, 1987). It is not well-known how high CO, affects respiration and C_2H_4 synthesis; these two may be related (Sisler and Wood, 1988).

The manner in which horticultural commodities respond to CO_2 depends on the nature of the commodity and on the concentration and length of exposure to the gas (Herner, 1987). Although CO_2 concentrations > 5% to 10% should be avoided in long-term storage, short-term exposure to very high CO_2 (20% to 100'%) has beneficial effects on the storage life of some harvested commodities (Herner, 1987; Higashio et al., 1980).

Using an automated microcomputer system for the measurement of O_2 uptake, we determined that respiratory responses to high CO_2 differed among horticultural crops and among developmental stages (Kubo et al., 1989a, 1989b). In these studies, however, the effects of high CO_2 levels on C_2H_4 production were not measured.

The present study was designed to examine the effects of 60% CO_2 in the presence of 20% 0_2 on the respiratory activity and C_2H_4 production in various horticultural crops.

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Materials and Methods

Eighteen kinds of horticultural crops (Table 1) were used for the high-CO_2 treatments. All plant material was obtained from a commercial market in Okayama, Japan.

Treatment conditions. Plant material weighing =1 kg was placed in a respiration chamber (5.5 liter). Air was passed through the chamber for 12 hr, followed by a gas mixture of 60% CO_2 $\pm$ 0.001%, 20% O_2 $\pm$ 0.001%, and 20% N_2 $\pm$ 0.001% for 24 hr, and by air for another 24 hr. The gas flow rate was 100 $ml \cdot min^{-1}$ and the chamber was at 25 ± lC.

Measurement of respiration and C_2H_4 production. Both O_2 uptake and C_2H_4 production were measured using an automated system connected to a microcomputer. The details of this system were described previously (Inaba et al., 1989). Briefly, the system consisted of a gas flow system, three gas chromatography (GC), a microcomputer, and interface. Two GCS, equipped with a thermal conductivity detector and molecular sieve 5A and Porapack Q column, were used for measurement of O_2, CO_2, and N_2. N_2 was used as an internal standard to improve the accuracy of measurement of 0_2. Another GC, with a flame ionization detector and activated alumina column, was used for measurement of C_2H_4. All operations, including gas sample injection, were regulated by the microcomputer. The measurement of O_2 and C_2H_4 could be reproduced with an accuracy of 0.01% and 0.1 ppm, respectively. Uptake and production were measured every 3 hr during the experimental period. All measurements were repeated at least three times per treatment.

Results

Three modes of response to 60% CO_2, in terms of O_2 uptake and C_2H_4 production, were observed among the crops examined (Table 1). Typical responses of 0_2 uptake–decrease, no change, or increase—are shown in Figs. 1, 2, and 3, respectively. Suppression of O_2 uptake by 60% CO_2 was observed in the climacteric fruits and in broccoli, which were producing C_2H_4 before the CO_2 treatment. The rate of 0_2 uptake in peaches decreased to 55% of the control while C_2H_4 production decreased to trace levels during the CO_2 exposure. Both respiration and C_2H_4 production returned to levels close to those of the control when the samples were transferred back to air (Fig. 1).

Table 1. Effects of exposure to 60% CO_2 plus O_2 and 20% N_2 at 25C on the rates of O_2 uptake and C_2H_4 production in various fruits and vegetables.[z]

Respiratory response	Species	O_2 uptake ($ml \cdot kg^{-1} \cdot hr^{-1}$)[y] Control (A)	CO_2-treated (B)	O_2 uptake in (B) as percent of (A)	C_2H_4 production ($\mu l \cdot kg^{-1} \cdot hr^{-1}$)[x] Control	CO_2-treated
Decrease	Peach (*Prunus persica* Batsch)	39.2 ± 1.7	21.5 ± 0.6	55	14.2 ± 3.8	Trace
	Tomato (*Lycopersicon esculentum* Mill.)	17.8 ± 1.2	13.2 ± 2.3	74	10.1 ± 0.3	2.3 ± 0.9
	Banana (*Musa sapientum* L.)	66.8 ± 2.5	49.2 ± 3.3	74	0.8 ± 0.6	8.2 ± 0.3
	Broccoli (*Brassica oleracea* L., italica)	312 ± 18	112 ± 15	35	2.6 ± 0.8	0.5 ± 0.3
No change	Satsuma mandarin (*Citrus unshiu* Marc.)	11.0 ± 0.5	12.5 ± 1.9	114	Trace	Trace
	Iyokan (*Citrus iyo* Hort. ex Tanaka)	17.4 ± 0.9	19.1 ± 0.8	110	Trace	Trace
	Lemon (*Citrus limon* Burm. f.)	10.6 ± 0.6	11.5 ± 1.2	108	Trace	Trace
	Grape (*Vitis labrusca* L. x *Vitis vinifera* L.)	9.0 ± 0.3	9.5 ± 0.4	106	Trace	Trace
	Japanese pear (*Pyrus serotina* Rehd.)	6.0 ± 0.4	6.9 ± 0.8	115	Trace	Trace
	Carrot (*Daucus carota* L.)	15.5 ± 1.1	15.9 ± 0.9	103	Trace	Trace
	Onion, dry (*Allium cepa* L.)	14.0 ± 1.0	14.7 ± 0.8	105	Trace	Trace
	Cauliflower (*Brassica oleracea* L., botrytis)	71.7 ± 4.9	66.7 ± 3.1	93	Trace	Trace
	Cabbage (*Brassica oleracea* L., capitata)	15.5 ± 0.3	15.1 ± 0.9	97	Trace	Trace
Increase	Eggplant (*Solanum melongena* L.)	30.0 ± 0.8	49.6 ± 0.7	165	Trace	0.4 ± 0.2
	Cucumber (*Cucumis sativum* L.)	21.4 ± 1.3	49.2 ± 5.6	230	Trace	2.1 ± 0.6
	Podded pea (*Pisum sativum* L.)	93.0 ± 5.1	120 ± 7.3	129	Trace	2.6 ± 0.3
	Spinach (*Spinacia oleracea* L.)	116 ± 5.1	142 ± 7.4	122	Trace	0.3 ± 0.1
	Lettuce, crisphead type (*Lactuca sativa* L.)	26.8 ± 0.7	75.2 ± 1.2	281	Trace	1.2 ± 0.2

[z]Crops were exposed to 60% CO_2 for 24 hr at 25C.
[y]Rates of O_2 uptake just before the end of CO_2 treatment. Means ± SE. n = 3.
[x]Rates of C_2H_4 production just before the end of CO_2 treatment. Means ± SE. n = 3.

Responses in tomatoes and broccoli were similar to those observed for peaches. The rates of O_2 uptake in ripening bananas exposed to 60% CO_2 decreased to 74% of those of the control after 24 hr, and C_2H_4 production increased =10-fold.

Oxygen uptake changed little in five of the nonclimacteric fruits (three species of citrus, grapes, and Japanese pears) and in several vegetables (carrots, onions, cauliflower, and cabbage; Table 1, Fig. 2) exposed to high CO_2. Upon transfer back to airs O_2 uptake increased slightly in Japanese pears (Fig. 2) and onions (data not shown), whereas that of the seven other nonclimacteric fruits and vegetables remained at the same levels as those of the controls. Ethylene production by crops classified as "no change" types was not detected during or after exposure to 60% CO_2 (Table 1).

The presence of 60% CO_2 increased O_2 uptake (Table 1, Fig. 3) and slightly stimulated C_2H_4 production (Table 1) in eggplant and spinach. After return to air, both O_2 uptake and C_2H_4 production increased in these two crops. The response of cucumber to high CO_2 was similar to that of eggplant (Table 1). Oxygen uptake and C_2H_4 production by lettuce increased during CO_2 treatment (Table 1), with 0_2 uptake increasing to almost three times that of the control at the end of the CO_2 treatment. After return to air, both rates increased further (Fig. 3). Oxygen uptake and C_2H_4 production of peas increased during the 60% CO_2 treatment. After return to air, O_2 uptake increased further and C_2H_4 production remained high (Fig. 3).

Discussion

The beneficial effects of high CO_2 in the CA storage of horticultural crops are generally thought to arise through a suppression of respiration (Kader, 1986). Our results showed that O_2 uptake was reduced by high CO_2 only in ripening climacteric fruits and in broccoli, all of which were producing C_2H_4.

Kerbel et al. (1988) reported that 'Bartlett' pears maintained in a mixture of air plus 10% CO_2 exhibited reduced respiration and C_2H_4 production. Similar observations have been reported for strawberries (Li and Kader, 1989) and apples (Chaves and Tomas, 1984). Inhibition of C_2H_4 production in high CO_2 environments has been reported for tomatoes (Buescher, 1979) and sweet peppers (Wang, 1977).

Treatment of avocados with 5% to 10% CO_2 does not affect the respiration rate at the preclimacteric stage but delays the onset of the climacteric and reduces the respiration rate at the climacteric peak (Young et al., 1962). Similarly, tomatoes and bananas exhibited reduced respiration with high CO_2 levels at the climacteric stage, but CO_2 had little effect at the preclimacteric stage (Kubo et al., 1989b).

The results reported here show that high CO_2 affected the respiration rate little if at all in the fruits (nonclimacteric) and vegetables that were not producing measurable levels of C_2H_4. High CO_2 levels inhibit the action of C_2H_4 (Biale, 1960). The presence of 10% CO_2 abolishes the biological activity of 1 ppm C_2H_4 (Burg and Burg, 1967). Therefore, suppression of respiration by high CO_2 in crops producing C_2H_4 might occur mainly because of inhibition of the action and/or synthesis of C_2H_4. If so, high CO_2 levels would not suppress respiration that is not accelerated by endogenous C_2H_4.

Other evidence of the suppression of respiration by high CO_2 levels is the inhibition of certain enzymes (e.g., succinic dehydrogenase, cytochrome oxidase, and phosphofructokinase) and disturbed organic acid metabolism, for example, the accumulation of succinic acid (Biale, 1960; Kader, 1986; Kerbel et al., 1988). These phenomena have been found in ripening pears (Frenkel and Patterson, 1973; Kerbel et al., 1988) and apples (Monning, 1983; Shipway and Bramlage, 1973). Further investigation of these enzymes and metabolic intermediates in nonclimacteric fruits is needed.

Our findings show that respiration and/or C_2H_4 production in some vegetables were enhanced during high CO_2 treatment. In some cases, further increases occurred after the treatment ended.

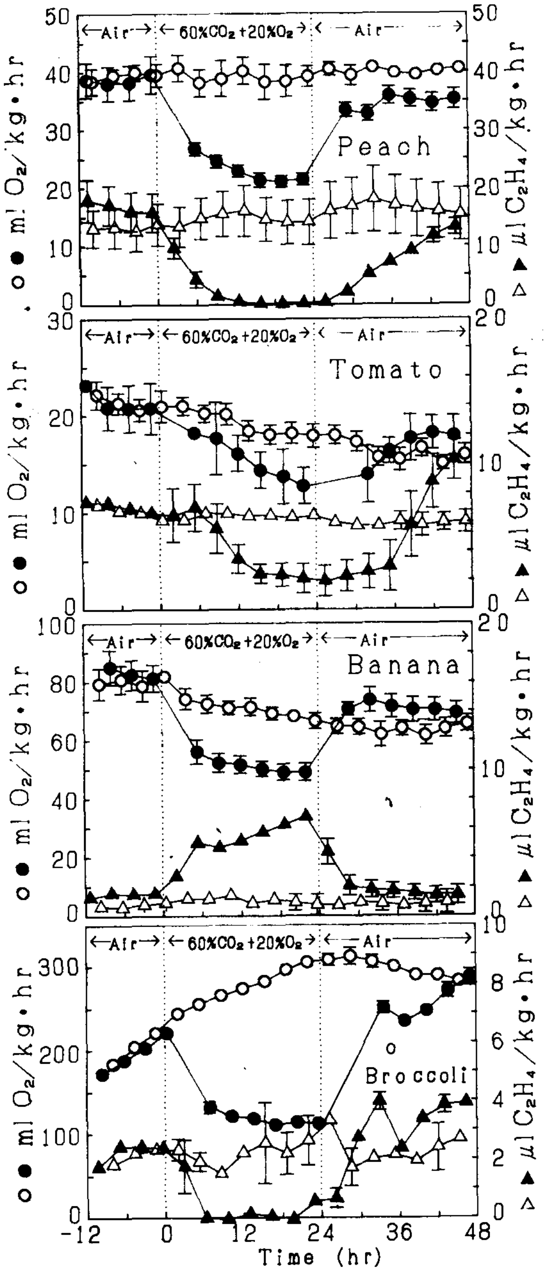

Fig. 1. Effects of exposure to 60% CO_2 plus 20% O_2 and 20% N_2 on the rates of O_2 uptake (●, ○) and C_2H_4 production (▲, △) at 25 ± 1C in three species of climacteric fruits and in broccoli (●, ▲ CO_2-treated, ○, △ control). Vertical bars are the standard error. n = 3.

This suggests that CO_2 might act simultaneously as an inducer and suppressor of C_2H_4 synthesis or action in some vegetables. Enhanced respiration during CO_2 exposure has been reported for lemons (Young et al., 1962) and potatoes (Perez-Trejo, 1981). Siriphanich and Kader (1985) found that high CO_2 levels promoted respiration and C_2H_4 production in lettuce. These increases in respiration and C_2H_4 production by exposure to excess CO_2 maybe related to physiological injury (Kader, 1986). We observed the development of brown stain, a CO_2 disorder, on lettuce after the CO_2 treatment. No signs of CO_2-induced disorders were seen in the other crops. Further investigation is needed to determine the mechanism by which high CO_2 influences C_2H_4 production in fruit and vegetable crops.

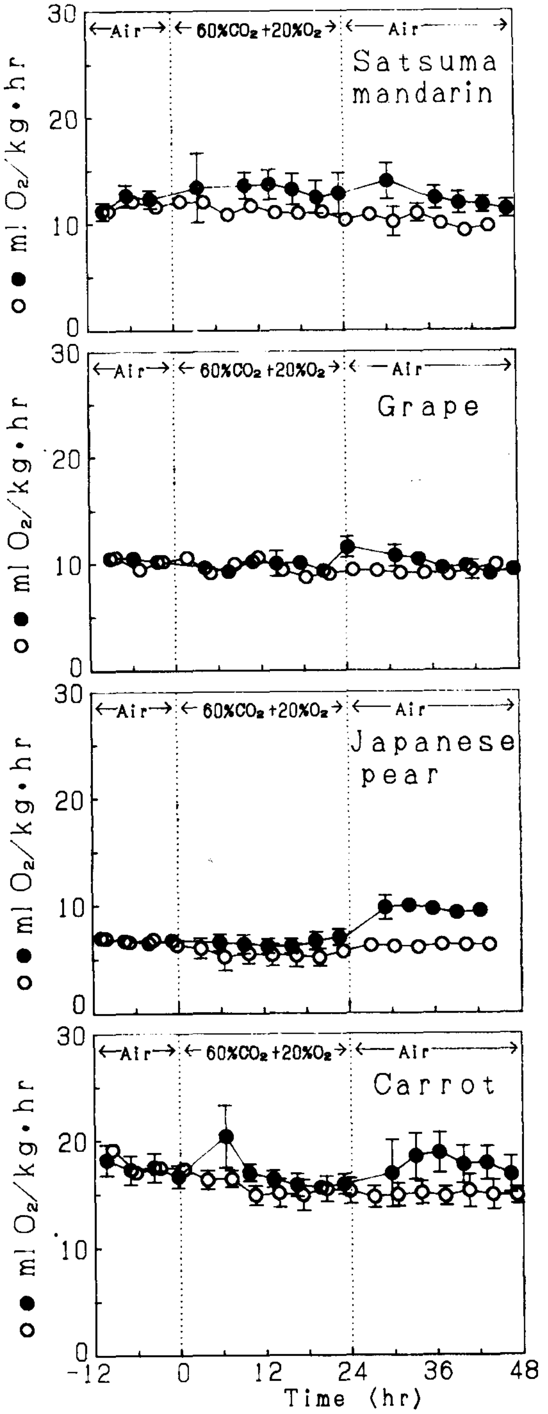

Fig. 2. Effects of exposure to 60% CO_2 plus 20% O_2 and 20% N_2 on the O_2 uptake rate at 25 ± 1C in three nonclimacteric fruits and in carrot (● CO_2-treated, O control) Vertical bars are the standard error. n = 3.

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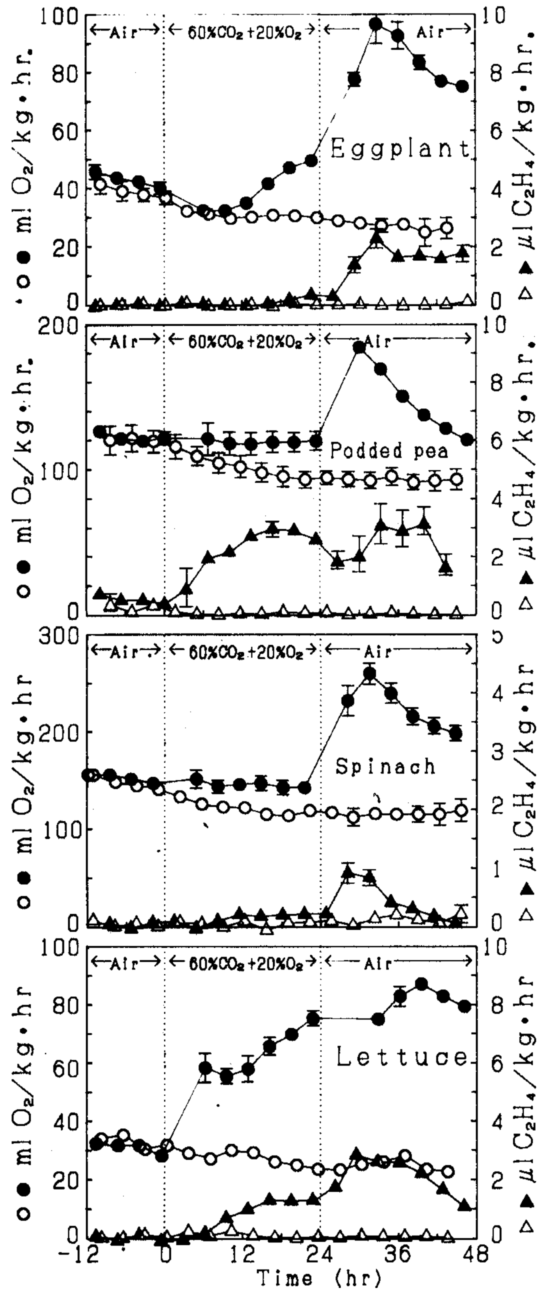

Fig. 3. Effects of exposure to 60% CO_2 plus 20% O_2 and 20% N_2 on the rates of O_2 uptake (●, ○) and C_2H_4 production (▲, △) at 25 ± 1C in four species of vegetables (●, ▲ CO_2-treated, ○, △ control). Vertical bars are the standard error. n = 4.

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