Effects of High CO₂ on Respiration in Various Horticultural Crops

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Summary

Under 60% CO₂ + 20% O₂ + 20% N₂, the respiration rates of various fruits and vegetables, based on O₂ uptake, were measured using an automated system aided by a micro-computer. With high CO₂ treatment, O₂ uptake rates of apples and melons declined to approximately one-half of the initial level. Ripening tomatoes and bananas also showed a respiratory reduction by high CO₂, but these fruits, at pre-climacteric stage, showed little change in respiration. Exposure to high CO₂ caused slight or no change in respiration with Natsudaidai (C. natsudaidai Hayata), lemons, potatoes, sweet potatoes and cabbage. Like the ripening climacteric fruits tested in this study, the respiration rate of broccoli was reduced by high CO₂ exposure. In contrast, a stimulation in respiration by high CO₂ was observed in lettuce, eggplants and cucumbers.

These results demonstrate that respiratory responses to high CO₂ were quite different with the kind of horticultural crops and their developmental stage, and raise a doubt as to the inhibitory effects of high CO₂ on respiration, which has been accepted by most researchers.

Introduction

A suppression of respiration in apples by high CO₂ was first recorded by Kidd and West (13) by measuring the weight loss of dry solid constituents due to oxidation to CO₂ and H₂O. This served as basis for the development of Controlled Atmosphere (CA) storage of apples and pears, since a moderate suppression of respiration by CO₂ was associated with a marked retardation of senescence of these fruits.

The idea of respiratory depression by CO₂ has been supported by the facts that CO₂ could have a strong controlling effect on mitochondrial activity(6,18,20), including citrate and succinate oxidation, explaining the reported accumulation of these acids under high CO₂. In addition, higher concentrations of CO₂ especially above 40%, inhibited the NAD-cytochrome c reductase system and cytochrome c oxidase(1).

As information from isolated mitochondria are indirect and from the loss of dry weight are insensitive, evaluation of respiration with CO₂ output or O₂ uptake by intact fruits or vegetables would be needed to establish the precise contribution of CO₂ to the control of respiratory metabolism in CA storage. Under high CO₂ conditions, measurement of respiration rate, based on either CO₂ output or O₂ uptake, has technical difficulties, because small differences in the composition of the gas mixture have to be measured in the presence of a considerable amount of O₂ and CO₂.

In spite of numerous studies on the use of CA conditions on various horticultural products, limited data are available on the respiratory response of some fruits to high CO₂ under consistent O₂ levels, and little was known about vegetables.

Young et al.(25) studied O₂ uptake by avocados, bananas, and lemons in 0, 5, and 10% CO₂ with the combination of 5, 10, and 20% O₂, using a paramagnetic O₂ analyzer. According to their data, CO₂ delayed the onset of the climacteric rise in avocados, and reduced O₂ uptake at the climacteric peak, prolonging storage life. The onset of the climacteric was postponed in bananas, but the rate at the peak was unaffected by CO₂, wherever the climacteric rise occurred. However, CO₂ appeared to have no effect on the rate of respiration in
both fruits before the induction of the climacteric rise. An unexpected and exceptional enhancement of respiration was observed in lemons under high CO₂. Their data suggest the probability that high CO₂ is not necessarily instrumental in depressing respiration, depending on the kind of horticultural products and their developmental stage.

We have been developed an automated system aided by a micro-computer for the simultaneous measurement of the respiration rate of horticultural products even in atmospheres containing a considerable amount of CO₂. The use of this system enabled us to study the effects of CO₂ at constant O₂ levels on respiratory activity. The respiratory response to 60% CO₂ with 20% O₂ in relation to the kind of horticultural products and developmental stage are reported here.

Materials and Methods

Plant materials

Horticultural products of various types were used in this study; Natsudaidai (C. natsudaidai Hayata) and lemons as non-climacteric fruits, melons ‘Prince’, apples ‘Rall’s Janet’, bananas ‘Giant Cavendish’, and tomatoes ‘TVR-2’ as climacteric fruits, eggplants and cucumbers as fruit vegetables, cabbage and lettuce as leaf vegetables, potatoes and sweet potatoes as root vegetables, and broccoli as a flower vegetable. Melons and apples were producing C₂H₄ at the ripening stage. Tomatoes at various stages, including ‘green’ and ‘red’, were obtained from a commercial solution culture house in Okayama. Pre-climacteric bananas imported from the Philippines were supplied by a local market in Okayama. On arrival at the laboratory, fingers in each banana hand were divided into two groups. One group was ripened by treatment with 1,000 ppm of C₂H₄ for 24 h at 25°C. Another group was stored at 15°C to maintain the pre-climacteric stage.

All other materials were obtained from a commercial market in Okayama.

Treatment conditions

Plant materials were sorted for uniformity of size and appearance. Material of about 1 kg was placed in a 5.5-liter chamber. After an air stream humidified by water had passed through the chamber for 12 h or more, the materials were exposed to 60% CO₂ for 24 h at 25°C. The O₂ level was maintained at 21 ~20% throughout the experimental period to measure direct effect of CO₂ on respiration. The controlled atmospheres were obtained by mixing CO₂, O₂, and N₂, as previously described by Inaba et al. (9). Flow rates were maintained at 6 liters per hour.

In general, CO₂ bulkup to concentrations above 5~10% or more causes various problems in CA storage (7). The development of CO₂ disorder depends on the kind of products, concentration and length of CO₂ exposure and temperature. Recent reports showed that a brief exposure to considerable CO₂ (20~100%) had beneficial effects on the shelf-life of some fruits and vegetables (8, 22, 24). In a preliminary experiment, as the CO₂ concentration increased, the respiratory response became clearer, with exposure times up to 24 h. Our purpose was to intensify the response of horticultural crops to the treatment. For these reasons, 60% CO₂ treatment was selected in this study. No CO₂ injury was observed except with lettuce in this short-term exposure lasting 24 h. Moreover, climacteric fruit treated with 60% CO₂ ripened normally when they were returned to air.

Measurements of respiration

Respiratory activity was measured by the automated system for measurement of the O₂ uptake rate using a micro-computer and gas chromatograph, equipped with TCD and molecular sieve column (9). This system could measure respiratory activity based on both O₂ uptake and CO₂ output by horticultural products under various gaseous conditions. As CO₂ may be produced by anaerobic as well as aerobic pathways of metabolism, CO₂ evolution is not a sufficient index for establishing the nature of biological oxidation. Therefore, respiratory activity was evaluated only with the O₂ uptake rate in this study.

O₂ uptakes were measured with one-hour intervals throughout the experimental period. All measurements were repeated at least three times.

Results and Discussion

Responses of fruits to 60% CO₂

Figure 1 shows the effects of 60% CO₂ on
the changes in the respiratory gas exchange of two climacteric fruits. \( \text{O}_2 \) uptake rates of melons decreased by approximately one-half of the initial level within 9 h of \( \text{CO}_2 \) exposure and then remained constant until the end of treatment. In apples, the first 8 h exposure to 60% \( \text{CO}_2 \) did not change \( \text{O}_2 \) uptake rate, but a further exposure gradually reduced it and then put on steady-state at a low level.

The respiratory responses to elevated \( \text{CO}_2 \) of tomatoes and bananas at different ripening stages are shown in Fig. 2 and Fig. 3, respectively. Under 60% \( \text{CO}_2 \), \( \text{O}_2 \) uptake rates of

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**Fig. 1.** Effects of 60% \( \text{CO}_2 \) on \( \text{O}_2 \) uptake rates in melons and apples (25°C).

Dotted line means the period during which \( \text{O}_2 \) uptake rate was not accurately measured.

**Fig. 2.** Effects of 60% \( \text{CO}_2 \) on \( \text{O}_2 \) uptake rates in tomatoes at different stages (25°C).

Green stage: Entirely green, having not developed to full size. Mature green stage: Pale green. Pink stage: Pink color in approximate one-third of fruit surface. Red stage: Red color in approximate two-thirds of fruit surface.

**Fig. 3.** Effects of 60% \( \text{CO}_2 \) on \( \text{O}_2 \) uptake rates in bananas at different stages (25°C).

**Fig. 4.** Effects of 60% \( \text{CO}_2 \) on \( \text{O}_2 \) uptake rates in 2 species of citrus fruit (25°C).

**Fig. 5.** Effects of 60% \( \text{CO}_2 \) on \( \text{O}_2 \) uptake rates in potatoes, sweet potatoes and broccoli (25°C).
ripening tomatoes at pink and red stages declined and reached about 12–13 ml/kg-h, which was equal to the rate at the mature green stage. In contrast, tomatoes at pre-climacteric stages, green and mature green, had no respiratory changes with 60% CO₂ treatment. Similarly, the O₂ uptake rate of ripening bananas continued to decline with increasing time of exposure to 60% CO₂, while that of pre-climacteric bananas remained at the initial level.

Figure 4 shows the respiratory responses of 2 species of citrus to 60% CO₂. A slight reduction of O₂ uptake rate was observed in Natsudaidai fruit exposed for more than 12 h. Respiratory activity of lemons recovered to the initial level after a temporary increase.

Generally, the effects of elevated CO₂ on reducing respiration rate has been assumed to be the primary reason for the beneficial effects of CA storage on fruits and vegetables (11). According to measurement just after CO₂ treatment by Chaves and Tomas (4), CO₂ output rate by ‘Granny Smith’ apples was inhibited by the treatment with 20% CO₂ for 2 h or 5 days. Wang and Mellenthin (22) reported with pears that treatment with 12% CO₂ for 2 or 4 weeks prolonged storage life, retarded C₂H₄ production, and delayed the onset of respiratory climacteric rise after 5 months of storage. McGlasson and Wills (16) indicated that 5% CO₂ exposure to green bananas increased the total O₂ uptake over the period before the beginning of respiratory climacteric. However, there is little data concerned with respiration during CO₂ treatment in horticultural crops, especially in vegetables and non-climacteric fruits.

Suppression of the O₂ uptake rate during high CO₂ exposure, accompanied with a decrease of C₂H₄ evolution, was reported in ripening pears by Kervel et al. (12). High CO₂ level can reduce, promote or have no effect on C₂H₄ production rates by fruits and vegetables, depending on the commodity, concentration, and period of CO₂ exposure (11). Suppression of C₂H₄ production was also observed in tomatoes (2), pears (12), and apples (4, 5) during and following high CO₂ exposure. In addition to that, CO₂ has appeared to be a competitive inhibitor of C₂H₄ action (3). However, it has not been recognized how the inhibition of C₂H₄ production and action by high CO₂ influenced respiration in fruits and vegetables.

Our results suggested that non-climacteric fruits and climacteric fruits at pre-climacteric stages which were not producing C₂H₄, showed no or slight reduction in respiration as the response to high CO₂. On the contrary, a marked reduction in respiration rate was observed in every fruit at the climacteric rise stage, at which fruits were producing C₂H₄. These date led us to the idea that respiratory suppression, observed in ripening climacteric fruits, might be mainly involved in the effects of CO₂ via its suppression of C₂H₄ production and action rather than a direct effect of CO₂ on respiratory metabolism.

Response of vegetables to 60% CO₂

Figure 5 shows the respiratory responses of 2 species of root vegetables, and broccoli. The
O₂ uptake rates of potatoes and sweet potatoes were not affected by high CO₂ treatment. A reduction in respiration of broccoli exposed to 60% CO₂ was observed, just like that of ripening climacteric fruit. Broccoli is one of the most perishable commodities and has been responsive to CA conditions. Lieberman et al. (14) reported that respiratory reduction of 41% in broccoli was attained with 20% CO₂ + 2% O₂ compared with ambient atmosphere. Broccoli has been classed among crops producing a certain amount of C₂H₄. Lipton and Harris (15) attributed the color retention observed with CA storage to a reduced concentration or inhibited action of C₂H₄ by the CA with a subsequent reduction in the rate of senescence. Similarly to the case of climacteric fruits, a marked suppression of respiration observed in broccoli exposed to 60% CO₂ might be an effect of CO₂ on C₂H₄. The insensitiveness of sweet potatoes and potatoes to high CO₂ was worth notice in considering the effect of CO₂.

Figure 6 shows the respiratory responses of 2 species of leaf vegetables. While CO₂ exposure slightly reduced the respiration of cabbage, an enhancement in respiration was observed in lettuce with increasing time of exposure. This enhancement may partly involve in CO₂ injury becoming visible after transfer to air.

Figure 7 shows the respiratory responses of 2 species of fruit vegetables. Following a temporary decrease in O₂ uptake rates, a gradual increase occurred in both vegetables without any visible symptom of CO₂ injury. The enhancement of respiration by CO₂ has been shown for lemons (25) and carrots (23). Perez-Trejo (17) reported CO₂-stimulated respiration in potatoes, accompanied by a pronounced increase in the content of ATP. Kerbel et al. (12) thought that above a level of 10% CO₂ can increase the rates of respiration, most likely reflecting injury to the tissue, depending on tissue type, cultivar, and duration of exposure. Lettuce was shown to be sensitive to CO₂ and had CO₂ injury, called "brown stain" (10), as observed in our experiment. A large increase in C₂H₄ production was noted in lettuce tissue exposed to high CO₂, indicating that injury had occurred (21). CO₂ stimulated both basal and ACC-dependent C₂H₄ production in excised photosynthetic tissue (19). Further investigation is necessary with CO₂-stimulated respiration in the light of C₂H₄ synthesis.

In conclusion, respiratory responses of horticultural crops to high CO₂ were quite different with the kind of commodities and their stages. Measuring respiratory responses to high CO₂ by this method might provide a good index to evaluate the sensitivity of horticultural crops to CA conditions.

**Literature Cited**


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高濃度炭酸ガスが種々の青果物の呼吸活性に及ぼす影響

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摘 要

60% CO₂ + 20% O₂ + 20% N₂ 環境下での各青果物の呼吸活性をコンピュータ制御による自動ガス代謝計測装置を用いて O₂ 吸収量について測定した。成熟中の中梅とリンゴでは、60% CO₂ 下で呼吸活性が空気中の約半分まで抑制された。成熟トマトとバナナでは、高 CO₂ による顕著な呼吸活性の抑制がみられたが、プレクライマクテック型果実では高 CO₂ による呼吸活性の変動はほとんどみられなかった。ブロッサリーでも成熟したクライマクテック型果実の場合と同様に高 CO₂ による顕著な呼吸活性の抑制がみられた。高 CO₂ 処理はジャガイモ、サツマイモ及びキャベツの呼吸活性には全く影響を与えず、ナツダイダイ、レモンにもわずかな変化しか起さなかった。レタス、ナス及びキュウリでは、高 CO₂ 処理中に顕著な呼吸活性の促進がみられた。

以上の結果から高 CO₂ に対する呼吸反応は、青果物の種類や果実の成熟段階により全く異なることが明らかとなり、従来考えられている高 CO₂ の呼吸抑制作用には疑問があるように思われた。