
Effect of carbon monoxide fumigation on the respiration and oxidase activities of postharvest jujube

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Abstract: Postharvest jujubes were fumigated with 5, 10, 20 or 40 $\mu\text{mol/L}$ carbon monoxide (CO) for 1 h and then preserved for 30 d at ambient temperature. The effects of CO fumigation on the respiration and oxidase activities of postharvest jujube were determined. Results showed that low CO concentrations (5–10 $\mu\text{mol/L}$) could restrain the increases of respiration rate, malonaldehyde content, and relative membrane permeability of postharvest jujube. However, high CO concentrations (20–40 $\mu\text{mol/L}$) showed adverse effects on jujube preservation. Jujube fumigated with 10 $\mu\text{mol/L}$ CO demonstrated optimum effects, and this CO concentration effectively reduced the oxidase activities including lipoxygenase, cytochrome oxidase, polyphenol oxidase and ascorbic oxidase during jujube storage time. As for the reduction of respiration rate and oxidase activities, the delay of membrane lipid peroxidation, fumigating jujube with appropriate concentration of CO might be applied to preserve postharvest jujube and other fruits.

Keywords: Jujube, Carbon Monoxide, Respiration, Oxidase Activity, Preservation

1. Introduction

Carbon monoxide (CO) is a small diatomic gas molecule that is toxic to animals and humans. Similar to nitric oxide (NO), CO is produced in many plants (Vreman et al., 2011). It is believed to be an important gaseous signal molecule and appears to play important roles in seed germination (Dulak and Józkwicz, 2003; Liu et al., 2007), adventitious root formation (Xu et al., 2006) and resistance to adverse circumstances, such as salt, heavy metal and water stresses (Xie et al., 2008; Han et al., 2008; Song et al., 2008). Despite the number of available reports on CO, the effect of the molecule on plant senescence has rarely been reported. Ling et al. (2006) found that exogenous CO could restrain the senescence and extend the shelf life of cut rose by adjusting the activities of antioxidant enzymes. In a previous study, we found that CO fumigation could restrain the browning of fresh-cut lotus root and maintain the quality of postharvest jujube (Zhang et al., 2013; Zhang and Li, 2014). CO has also been applied to protect postharvest fresh-cut *Lactuca sativa* from browning; this technology has been certified by the US Food and Drug Administration (Sandhya, 2010).

Jujube, a flavor fruit with abundant nutrients, was easily subject to senescence during storage time, which led to

severe loss for farmers (Zhu et al, 2009). To effectively maintain the commodity value of postharvest jujube, the effects of exogenous CO on the respiration and oxidase activities of postharvest jujube were determined during senescence under ambient temperature in the present work. This study aims to explore the probable mechanism by which CO adjusts the respiration of jujube during storage and expand CO applications in postharvest fruit and vegetable preservation.

2. Materials and Methods

2.1. Materials

Jujubes (*Ziziphus jujuba* Mill. cv. Dongzao) picked at mature-green stage with the white and green peel, the firmness of approximately 9.0 Kg/cm^2 during noon were purchased from an orchard located in Yaodu District, Linfen City, Shanxi Province, China. Jujubes of uniform shape and size and with no defects or mechanical damage were selected and immediately transported to the laboratory in open cartons.

CO gas (99.99% purity) was purchased from Beijing Huaneng Special Gases Co., Ltd., (Beijing, China). All of the reagents used in this study were of analytical grade and

purchased from Alfa Aesar Company (Tianjin, China)

2.2. Treating Jujube

The jujubes were fumigated with CO gas at different concentrations (5, 10, 20 or 40 $\mu\text{mol/L}$) for 1 h under ambient temperature. Approximately 3 kg of jujubes was placed in a glass container (40 cm diameter and 40 cm height), which was then sealed with a lid. CO gas was injected into the glass container through a port in the lid. The jujubes in the container were fumigated with CO for 1 h. Jujubes that had not been fumigated with CO gas were also sealed in a glass container for 1 h; these jujubes were used as control samples. After treatment, all of the samples were placed in plastic bags and stored under ambient temperature with 85% relative humidity. The respiration rate, malonaldehyde (MDA) content, relative membrane permeability and oxidase activities of the jujubes were measured periodically.

An experiment was also conducted to verify the homogenous dispersion of CO in the container. Briefly, 5 $\mu\text{mol/L}$ (the lowest concentration) or 40 $\mu\text{mol/L}$ (the highest concentration) CO was injected into the container. After 30 s, the CO concentration of the container was measured online using an infrared CO analyser (GXH-3011A, Beijing Huayun Analysis Instrument Co., Ltd., Beijing, China). The CO concentration tests were performed at the bottom, middle, top, and diagonal areas of the container, and all positions showed the same concentration. Thus, CO may be concluded to have achieved equilibrium in the container.

2.3. Determination of Respiration Rate

The respiration rate was assayed using the method described by Yu *et al.* (2012). Approximately 600 g of jujubes was sealed in a 10 L glass container under ambient temperature for 1 h. The carbon dioxide concentration was measured using an infrared carbon dioxide analyser (GXH-3010F, Beijing Huayun Analysis Instrument Co., Ltd.). The respiration rate was calculated in terms of $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$.

2.4. Determination of Malonaldehyde Content and Relative Electrolyte Leakage

MDA content was measured using the method described by Xing *et al.* (2008). Flesh tissue (2.0 g) from 10 fruits was homogenised with 10 mL of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixture was then heated at 100 °C for 10 min. After rapid cooling of the sample to room temperature and centrifugation at $4000 \times g$ for 15 min at 25 °C, the absorbance of the supernatant was measured at both 532 and 600 nm. MDA concentrations ($\mu\text{mol g}^{-1}$ fresh weight) were calculated using an extinction coefficient of $155 \text{ Mm}^{-1} \text{ cm}^{-1}$ via the formula $(\text{OD}_{532} - \text{OD}_{600}) \times 40 / (0.155 \times \text{fresh weight})$.

Relative electrolyte leakage was determined as described previously (Antunes *et al.*, 2010) with slight modifications. Jujube fruits were sliced into small discs (0.05 cm thick) and

washed three times with de-ionized water to remove surface-adhered electrolytes. After drying with filter paper, 10 discs were incubated in vials containing 40 mL of de-ionized water for 10 min. The water was stirred slowly, and its conductivity was measured as C_1 using a conductivity meter (DDS-307, Shanghai Sophisticated Scientific Instrument, Shanghai, China). This water was then boiled for 10 min, cooled rapidly and mixed with 40 mL of de-ionized water. The conductivity of this solution was measured and recorded as C_2 . Electrolyte leakage was calculated as follows: relative electrolyte leakage (%) = $C_1/C_2 \times 100$.

2.5. Determination of Oxidase Activities

Lipoxygenase (LOX) activity was measured according to the method of Zhong and Xia (2007) with slight modifications. Tissue (2 g) from 10 fruits was homogenised in 10 mL of 50 mmol/L phosphate buffer (pH 7.0) at 4 °C and then centrifuged at $5000 \times g$ for 15 min at 4 °C with an Eppendorf 5417R centrifuge (Germany). The supernatant was collected as the enzyme extract. LOX activity was assayed using 3.0 mL of reaction mixture containing 2.75 mL of 0.1 mol/L phosphate buffer (pH 6.8), 50 μL of 0.1 mol/L sodium linoleate and 0.2 mL of crude enzyme. The absorbance at 234 nm was measured for 1 min using a spectrophotometer (UV-1100, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China). One unit of enzyme activity was defined as the amount of enzyme that caused a change of 0.01 in absorbance at 234 nm per minute.

Cytochrome oxidase (COX) activity was assayed according to the method described by Prasad *et al.* (1994). Fruit tissue (2 g) was homogenised with 15 mL of phosphate buffer (pH 8.0, containing 1 $\mu\text{L/mL}$ mercaptoethanol, 5 mmol/L EDTA and 0.1 mol/L Tris-HCl). The homogenate was centrifuged at $5000 \times g$ for 10 min at 4 °C and the supernatant fractions were collected as the crude enzyme extract. COX activity was determined in a 3 mL reaction mixture containing 2.5 mL of 90 mmol/L phosphate buffer (pH 7.0), 0.2 mol/L sucrose, 20 $\mu\text{mol/L}$ Cyt c (reduced with 3 mg sodium hydrosulfite) and 0.5 mL of crude enzyme extract. The mixture was warmed for 3 min at 40 °C. COX activity was determined as the rate of oxidation of 1 μmol reduced Cyt c measured at A550 per minute.

Polyphenol oxidase (PPO) activity was determined according to the method of Zhu *et al.* (2009) with slight modifications. Tissue (2 g) from 10 fruits was homogenised in 15 mL of 0.1 mol/L potassium phosphate buffer (pH 7.0) containing 2 mmol/L EDTA and 1% PVPP. The homogenate was centrifuged at $8000 \times g$ for 15 min at 4 °C, and the supernatant fraction were collected as the crude enzyme extract. A 3 mL reaction mixture contained 0.5 mL of enzyme extract and 2.5 mL of 10 mmol/L phosphate buffer (pH 7.0). Catechol (as the substrate) was added to this mixture at a final concentration of 20 mmol/L, and the mixture was aerated for 4 min in a small test tube. PPO activity was determined from the initial rate of quinone formation as indicated by the absorbance at 420 nm. PPO

activity (U) was expressed as an increase in absorbance of 0.001 per min per gram of fresh weight.

Ascorbic acid oxidase (AO) activity was assayed using the method of Leong and Oey (2012) with slight modifications. Tissue (2 g) from 10 fruits was homogenised in 15 mL of sodium phosphate buffer (pH 6.0) containing 1 mmol/L EDTA and 2% PVPP. The homogenate was centrifuged at $8000 \times g$ for 15 min at 4 °C, and the supernatant fractions were collected as the crude enzyme extract. The reaction assay included a mixture of 2.8 mL of sodium phosphate buffer (pH 5.6) containing 0.6 mmol/L EDTA, 0.05 mL of crude enzyme extract and 0.1 mL of 0.25 mmol/L ASA. The mixture was warmed for 15 min at 40 °C, and the assay was performed using a UV/visible spectrophotometer. AO activity was defined as the amount of enzyme that caused a 0.01 change in absorbance at 265 nm per minute.

2.6. Statistical Analysis

Each treatment was repeated three times and the data were analysed (ANOVA) using DPS7.05 statistical software (Refine Information Tech. Co., Ltd., Hangzhou, China). Treatments were compared using Tukey's test at $P = 0.05$ to determine multi-comparison values in each case. Values are expressed as mean \pm standard deviation ($n = 3$).

3. Results and Analysis

3.1. Respiration Rate

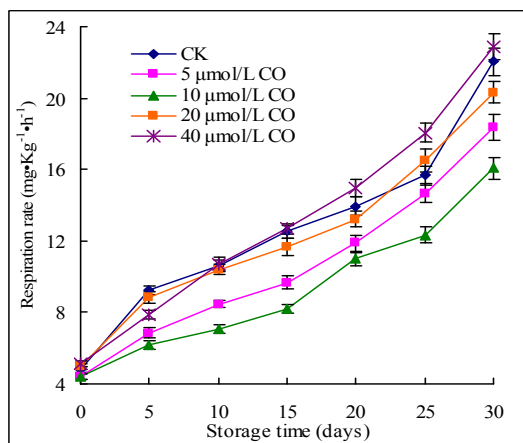


Fig. 1. Effect of CO fumigation on respiration rate of postharvest jujube. Each point represents the mean value \pm SD.

As shown in Fig. 1, the respiration rate of postharvest jujube increased during storage but no apparent respiration peak was observed. CO-treated and control samples showed the same patterns of respiration. The respiration rates of jujube fumigated with 5 or 10 $\mu\text{mol/L}$ CO were lower than that of the control sample. The respiration rate of jujube treated with 40 $\mu\text{mol/L}$ was higher than that of the control sample after 10 d. In addition, treatment with 20 $\mu\text{mol/L}$ CO showed no positive effect to the respiration rate of postharvest jujube. The respiration rate of jujube treated

with 10 $\mu\text{mol/L}$ CO showed the slowest increase during storage and was 27% lower than that of the control sample after 30 d ($P < 0.05$).

3.2. Malonaldehyde Content and Relative Membrane Permeability

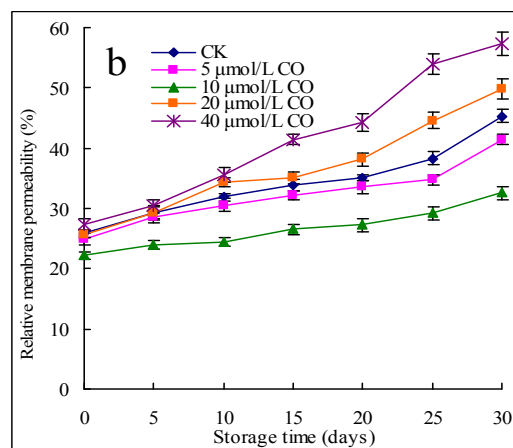
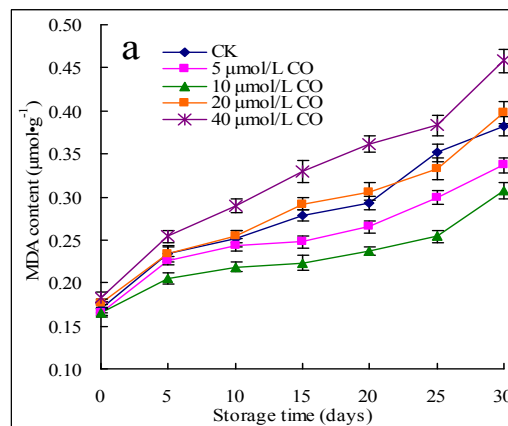


Fig. 2. Effect of CO fumigation on MDA content (a) and relative membrane permeability (b) of postharvest jujube. Each point represents the mean value \pm SD.

As shown in Fig. 2a, the MDA content of treated and control samples showed an increasing trend during storage. The increase rates of MDA content of jujube fumigated with 5 or 10 $\mu\text{mol/L}$ CO were lower than those of the control sample or other treatments. Jujubes treated with 10 $\mu\text{mol/L}$ CO showed the lowest MDA content during storage. The MDA content of jujube treated with 20 $\mu\text{mol/L}$ CO was higher than that of the control sample, although no significant difference was found between them ($P > 0.05$). In addition, the MDA content of jujube fumigated with 40 $\mu\text{mol/L}$ CO was the highest amongst all of the samples after 10 d.

Similar to MDA content, the relative membrane permeability of jujube also increased during storage (Fig. 2b). Compared with the control sample, jujubes fumigated with 5 or 10 $\mu\text{mol/L}$ CO showed lower relative membrane permeability than the control sample, whereas jujubes fumigated with 20 or 40 $\mu\text{mol/L}$ CO showed high relative

membrane permeability. The relative membrane permeability of jujubes fumigated with 10 $\mu\text{mol/L}$ was 32% lower than that of the control sample after 30 d. This treatment also showed the slowest increase rate during storage. Significant difference of relative membrane permeability was found between the 10 $\mu\text{mol/L}$ treated jujube and other samples after 30 d ($p < 0.01$).

The results above indicate that fumigating postharvest jujube with low concentrations of exogenous CO (5 or 10 $\mu\text{mol/L}$) could restrain increases in respiration rate, MDA content, and relative membrane permeability during jujube preservation. In particular, the samples treated with 10 $\mu\text{mol/L}$ CO demonstrated optimum effects in view of its lower respiration rate, MDA content, and relative membrane permeability. In our previous study, jujubes treated with 10 $\mu\text{mol/L}$ CO showed the best appearance quality such as firmness, decay incidence (Zhang and Li, 2014). However, fumigating jujubes with high CO concentrations (20 or 40 $\mu\text{mol/L}$) showed adverse effects on postharvest jujube. Thus, only jujubes fumigated with 10 $\mu\text{mol/L}$ CO were used for further investigation.

3.3. Lipxygenase Activity

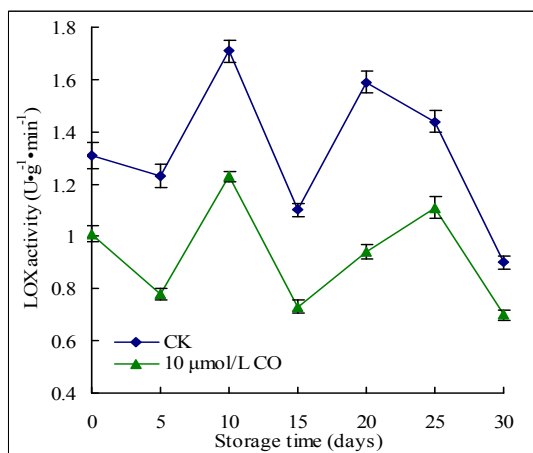


Fig. 3. Effect of CO fumigation on the LOX activity of postharvest jujube. Each point represents the mean value \pm SD.

LOX catalyses the oxygenation of polyunsaturated fatty acids to form fatty acid hydroperoxides, promoting the senescence of postharvest fruit and vegetable (Baysal and Demirdöven, 2007). The effect of CO fumigation on the LOX activity of jujubes is summarised in Fig. 3. Two apparent peaks in LOX activity of the postharvest jujube were found during storage. The first peak appeared after 10 d in all samples, and the second peak appeared on day 25 in treated jujube and day 20 in control samples. The appearance of the second LOX activity peak showed a 5 d delay in fumigated jujube. During the entire storage period, the LOX activity of jujubes fumigated with 10 $\mu\text{mol/L}$ CO was significantly lower than that of the control sample ($P < 0.01$).

3.4. Activity of Cytochrome Oxidase

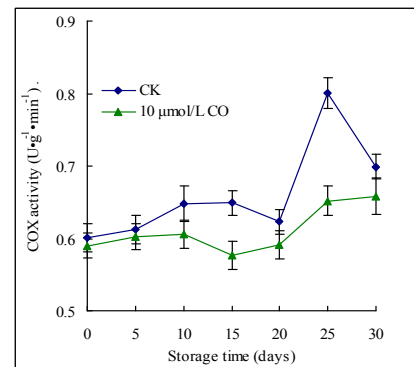


Fig. 4. Effect of CO fumigation on the COX activity of postharvest jujube. Each point represents the mean value \pm SD.

Cytochrome oxidase is one of a superfamily of proteins which act as the terminal oxidases of respiratory chains, playing a crucial role in aerobic respiration (Arnold, 2012). As shown in Fig. 4, the COX activity of jujube treated with 10 $\mu\text{mol/L}$ CO first decreased until day 15 and then increased over the rest of the storage period. The COX activity of the control sample initially increased before day 10 and then decreased from days 10 to 20. Afterward, a COX activity peak appeared in the control sample on day 25. During the entire storage period, the COX activities of the treated jujube were about 8% and 25% lower than those of the control sample on days 10 and 25, respectively.

3.5. Activities of Polyphenol Oxidase and Ascorbic Acid Oxidase

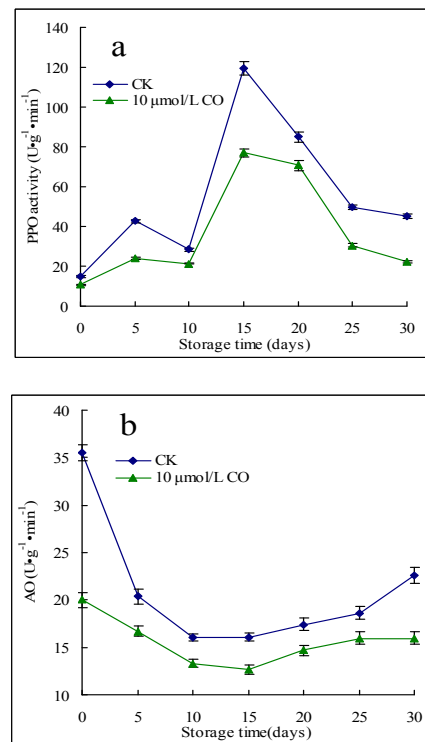


Fig. 5. Effect of CO fumigation on PPO (a) and AO (b) activities of postharvest jujube. Each point represents the mean value \pm SD.

PPO catalyses the oxidation of the diphenol to the corresponding quinone, and molecular oxygen is used in the reaction (Mayer, 2006). PPO activity of jujube slightly increased before day 5 and then decreased between days 5 and 10 (Fig. 5a). The PPO activity again markedly increased, reached a peak on day 15 and then decreased from days 15 to 30. The PPO activity of treated jujube, which was 36% and 50% lower than that of the control sample on days 15 and 30, respectively, was always lower than that of the control sample during the entire storage period.

AO catalyses the oxidation reaction of ascorbate to dehydroascorbic acid with the concomitant reduction of molecular oxygen to water (Leong and Oey, 2012). As demonstrated in Fig. 5b, the AO activity of postharvest jujube decreased to a minimum after approximately 15 d and then gradually increased. The AO activity of postharvest jujube fumigated with 10 $\mu\text{mol/L}$ CO was significantly lower than that of the control sample ($P < 0.05$). In particular, the AO activity of treated jujube was 44% lower than that of the control on day 0. On day 30, the AO activity of the treated jujube was 29% lower than that of the control sample.

4. Discussion

Similar to NO, CO is an important gaseous signal molecule that is widely found in organisms and performs many physiological responses. CO and NO protect plants by inducing antioxidase gene expression, enhancing antioxidase activity, adjusting the balance of active oxygen species and scavenging systems and effectively inhibiting various stresses (Xuan et al., 2008; Chen et al., 2009). CO with low concentration demonstrated positive effects in decay incidence, red index, firmness, and weight loss (Zhang and Li, 2014). Despite the number of available reports on CO, however, studies on the effect of CO fumigation on fruit senescence are limited. Our experiment results suggested that CO showed concentration effects. The MDA content, relative membrane permeability and respiration rate of postharvest jujube were restrained under low CO concentrations and enhanced under high CO concentrations during storage.

MDA is the main product of lipid peroxidation, and its content indicates the degree of membrane system damage (Suda et al., 1994). MDA accumulation is an important index of cell damage and physical senescence of postharvest fruit (Long et al., 2006). In present study, the MDA content and relative membrane permeability of jujube gradually increased as the storage time postponed. Low CO concentrations (5 or 10 $\mu\text{mol/L}$) could reduce MDA accumulation in fruit tissue, restrain LOX activity and delay the increase of relative membrane permeability of jujube.

Respiratory metabolism is a main physiological metabolic process in postharvest fruits and vegetables. Fumigating jujube with low CO concentrations ($<10 \mu\text{mol/L}$ CO) could restrain the increases of respiration rate. The respiratory chains of aerobic organisms use molecular oxygen as the

final electron acceptor. Respiratory metabolic pathways differ according to disparities in growth stages and the external environment (Leprince et al, 1992). The respiration rate increase of postharvest jujube during storage was probably related to the cooperative regulation of several respiratory pathways involving the participation of oxidase including COX, PPO and AO. Before day 5, the COX activity of treated jujube slightly increased whereas the PPO activity rapidly increased. Although the activity of AO promptly decreased before day 5 of storage, AO showed the highest activity in this time compared with the whole storage period. Therefore, the respiration rate of jujube was mainly regulated by AO and PPO during the early stages of storage. From days 5 to 20, PPO activity increased to approximately 6 times of the initial PPO activity; at the same time, COX activity slightly increased and AO activity remained very low. Thus, PPO is dominant in the respiration of jujube during moderate periods of storage. Though showing a rapidly decreasing trend on day 30, PPO activity was still 3 folds higher than the initial PPO activity. Meanwhile, AO activity slightly increased and COX activity reached maximum levels (only approximately 30% higher than the initial COX activity). Thus, respiration might be cooperatively regulated by PPO and COX. Obviously, PPO obviously served as the main factor to regulate respiration of postharvest jujube at the storage later period. CO could restrain the respiration rate of postharvest jujube by reducing the oxidase activities of COX, PPO and AO, and these results were similar to those of Ling et al. (2006), who treated cut rose flowers with CO to postpone senescence.

5. Conclusions

Fumigating jujube with low CO concentrations (5–10 $\mu\text{mol/L}$) for 1 h could restrain increases in respiration rate, MDA content and relative membrane permeability. By contrast, fumigation with high CO concentrations (20–40 $\mu\text{mol/L}$) showed adverse effects on postharvest jujube preservation. In addition, fumigating jujube with 10 $\mu\text{mol/L}$ CO effectively reduced oxidase activities of LOX, COX, PPO and AO of postharvest jujube. Thus, Treatment with 10 $\mu\text{mol/L}$ CO for 1 h is an effective method to delay the senescence of postharvest jujube.

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