

Effect of 1-methylcyclopropene and ethylene on the physiology of peach fruits (*Prunus persica* L.) cv. Dorado during storage

Efecto del 1-metilciclopropeno y el etileno en la fisiología de frutos de durazno (*Prunus persica* L.) cv. Dorado durante el almacenamiento



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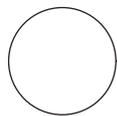
Measurement of color in peaches cv. Dorado.

Photo: K. Africano

ABSTRACT

The peach (*Prunus persica* L., family Rosaceae) is a drupe that is consumed fresh and used in industry; it has a climacteric behavior and, because of its high water content, is highly perishable. This study aimed to evaluate the effect of 1-methylcyclopropene (1-MCP) and ethylene on the postharvest physiology of peach fruits cv. Dorado. Harvested fruits with 100% green, 0% yellow skin color were used in a completely randomized design with four treatments: ethylene, 1-MCP, 1-MCP+ethylene and a control. After treatment, the fruits were stored at room temperature. During storage, the respiration rate, weight loss, firmness, color index of the skin, total soluble solids, total acidity and maturity ratio were evaluated. The results showed the efficiency of 1-MCP in peach cv. Dorado: 1-MCP decreased the respiration rate, color index of the skin, soluble solids and maturity ratio, while the firmness and total acidity were greater. The ethylene application showed an opposite effect, suggesting that it may regulate a large part of peach cv. Dorado ripening.

Additional key words: postharvest technology; ripeners; plant growth substances; peach postharvest; stone fruits.



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RESUMEN

El durazno (*Prunus persica* L., familia Rosaceae) es una drupa apotecada para consumo en fresco y la industrialización; presenta un comportamiento climatérico y tiene alto contenido de agua, por lo cual se considera un fruto altamente perecedero. El objetivo de este estudio fue evaluar el efecto del uso de 1-metilciclopropeno (1-MCP) y etileno sobre la fisiología poscosecha de frutos de durazno, cv. Dorado. Se utilizaron frutos cosechados con color de la epidermis 100% verde, 0% amarillo, se dispusieron en un diseño completamente al azar con cuatro tratamientos que correspondieron a etileno, 1-MCP, 1-MCP+etileno y un control sin aplicación. Luego del tratamiento, los frutos fueron almacenados a temperatura ambiente. Durante el almacenamiento se evaluó la tasa respiratoria, pérdida de peso, índice de color de la epidermis, firmeza, sólidos solubles, acidez total y relación de madurez. Los resultados mostraron la eficiencia de la aplicación de 1-MCP en durazno cv. Dorado, ya que generó significativamente menor tasa de respiración, índice de color de la epidermis, sólidos solubles y relación de madurez, mientras que la acidez total y firmeza fueron mayores. Por su parte, la aplicación de etileno mostró el efecto contrario en estos procesos, indicando que al parecer regula gran parte del proceso de maduración del fruto de durazno cv. Dorado.

Palabras clave adicionales: tecnología poscosecha; maduradores; sustancias de crecimiento vegetal; frutos de hueso.

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INTRODUCTION

The peach (*Prunus persica* L.) cv. Dorado is characterized by a yellow epidermis, with a light red pigmentation, gold-yellow mesocarp with red colorations around the endocarp, trichomes on the exocarp, velvety skin, and mesocarp adnate to the endocarp; additionally, it has a high sugar content and can reach up to 150 g in weight. All of these characteristics make the peach highly desirable for fresh consumption and for industrialization (Campos, 2013). Of the deciduous fruit trees, the peach is very important for Colombian agriculture, particularly in the Department of Boyaca because of its optimal edaphoclimatic characteristics, vocation and productive expertise (Puentes *et al.*, 2015). However, the peach cv. Dorado is highly perishable and has a short postharvest life (11 d in the conditions evaluated by Africano *et al.*, 2016), which limits its commercialization. This may be due to the fact that the peach is a climacteric fruit (Africano *et al.*, 2016), which means there is an accelerated increase in the ethylene production and the respiratory rate associated with ripening (Brackmann *et al.*, 2013).

Ethylene is the hormone responsible for triggering physiological, biochemical and molecular processes that guarantee fruit ripening (Binder, 2008; Balaguera-López *et al.*, 2014a) by bonding to a group of protein receptors mainly located on the membrane of the endoplasmic reticulum (Chen *et al.*, 2005; Serek *et al.*, 2006). Ethylene allows fruits to reach optimal

organoleptic characteristics for consumption; however, it is also in charge of inducing senescence, which causes agricultural products to lose nutritional and commercial value (Bapat *et al.*, 2010; Balaguera-López *et al.*, 2014a).

To reduce the postharvest losses of the peach cv. Dorado, it is necessary to understand the processes associated with ethylene during ripening, as a foundation for various technologies that reduce the biosynthesis and action of ethylene. Zhang *et al.* (2012) stated that the ethylene regulation of peach ripening has been thoroughly studied using biochemical, molecular and genetic techniques. However, several reviews on ethylene biosynthesis suggest that the exact role of ethylene in the ripening process is not yet fully understood (Bapat *et al.*, 2010; Barry and Giovannoni, 2007) and that the knowledge gap is even greater in peach genotypes grown in the high tropics. In this regard, several studies have used the application of ethylene and 1-methylcyclopropene (1-MCP) on fruit (Zhang *et al.*, 2012; Yang *et al.*, 2013; Balaguera-López *et al.*, 2016; Barreto *et al.*, 2017).

1-MCP is the main ethylene inhibitor (Watkins, 2006), taking up the place of the ethylene receptors irreversibly and, thus, blocking the transduction chain of the signals responsible for the genetic expression related to the response to this hormone (In *et al.*, 2013). The effect of 1-MCP on the physiological and biochemical

processes of peach ripening has been evaluated in several studies with conflicting results: in some studies, 1-MCP blocked ethylene biosynthesis, but in others, it was ineffective (Dal Cin *et al.*, 2006; Hayama *et al.*, 2008; Tadiello *et al.*, 2016; Wang *et al.*, 2017). Liu *et al.* (2005, 2015) and Wang *et al.* (2017) found that 1-MCP delayed peach fruit ripening. However, Kluge and Jacomino (2002) and Tonetto *et al.* (2007), among others, suggested that the utility of 1-MCP is limited because it can delay peach ripening only when it is applied during the pre-climacteric state. Therefore, this study aimed to evaluate the effect of 1-MCP and ethylene, both individually and combined, on the postharvest behavior of the peach fruit cv. Dorado in order to understand ethylene-dependent ripening and the commercial potential of 1-MCP for extending the postharvest life and commercial period of this fruit.

MATERIAL AND METHODS

The peach (*Prunus persica* L.) cv. Dorado fruits came from a peach commercial crop located in Cerinza, Boyaca. The fruits were harvested with 100% green epidermis -0% yellow-red, with color values $L^* = 62.54$, $a^* = -4.58$ and $b^* = 40.81$. They had a homogeneous size and good phytosanitary conditions and were taken to the postharvest laboratory of the Universidad Pedagógica y Tecnológica de Colombia (UPTC), Duitama campus, where the experiments were conducted.

Four treatments in a completely random experiment design were evaluated: 1-MCP, 1-MCP+ethylene, ethylene and a control; each treatment was replicated four times, for a total of 16 experiment units, each one composed of approximately 1,000 g of fruit. One day after harvest, the fruits were washed and disinfected with a 1% sodium hypochlorite solution, and the treatments were immediately applied. For the 1-MCP (EthylBloc®) treatment, a 0.35 mg L⁻¹ 1-MCP solution was used, in which the fruits were submerged for 5 min, the adapted methodology of Choi *et al.* (2008). For the ethylene treatment, fruits were submerged in 1000 mg L⁻¹ of ethephon (Ethrel® 48 SL, Bayer Crop-Science) for 5 min and dried at room temperature; for the 1-MCP+Ethylene treatment, the ethylene was applied 7 d after the 1-MCP application. Finally, the fruits were stored on trays of expanded polystyrene at room temperature ($16 \pm 2^\circ\text{C}$; relative humidity of $70 \pm 12\%$ at average). The variables were measured at 1, 4, 7, 12, 16 and 19 days of storage (DOS), until the fruits lost their commercial quality. The respiratory rate (RR; mg CO₂ kg⁻¹ h⁻¹) was estimated by taking

600 g of fruit approximately and placing them in VER BC-2000 2000 cm³ hermetic chambers (Vernier Software & Technology, Beaverton, OR) for 5 min and by determining the concentration of CO₂ with a VER CO₂-BTA sensor and the LabQuest2 interface system (Vernier Software & Technology, Beaverton, OR). The weight loss was calculated by measuring the fresh mass (the same fruits used for RR) in an Acculab VIC 612 electronic scale, 0.01 g precision (Sartorius Spain S.A., Madrid). To determine the color index (CI), the equation (1) reported by Balaguera-López *et al.* (2014a) was used; the system parameters, CIELab "L", "a" and "b", were obtained from three readings in the equatorial zone of two fruits per replication, using a Minolta CR 300 digital colorimeter (Minolta Co., Tokyo).

$$IC = (1000 \times a^*) / (L^* \times b^*) \quad (1)$$

The fruit firmness was estimated using a PCE-PTR200 digital penetrometer (PCE-Ibérica SL, Albacete, Spain) with 0.05 N approximation, and the soluble solids (SS) were measured using a Hanna HI 96803 digital refractometer (Hanna Instruments, Woonsocket, RI) with a 0-85% range and 0.1 °Brix precision. For the total acidity (TA), which was expressed as the percentage of malic acid, the volume data of NaOH, using 1 g of fruit juice, taken to 25 mL with distilled water, and adding two drops of phenolphthalein in a base-acid titration, were used. The maturity ratio (MR) was determined as the quotient between SS and TA. For the destructive measurements (firmness, SS, TA, MR), one fruit was used in each sample for experimental unity.

The obtained data were analyzed with normality (Shapiro-Wilk) and homogeneity (Levene test) tests; subsequently, an analysis of variance and a Tukey range test ($P \leq 0.05$) were conducted; the statistics software R v 3.4.2 was used.

RESULTS AND DISCUSSION

All sampling points showed statistically significant differences ($P \leq 0.05$) in the respiration rate; the treatment that resulted in the highest respiratory rate was ethylene, followed by the control, although they were not significantly different. These two treatments were significantly different from the treatments with 1-MCP. Additionally, the treatment with ethylene and the control increased the respiration rate notably after 12 d of storage, reaching values of 60.89 and

59.01 mg CO₂ kg⁻¹ h⁻¹, respectively. On the contrary, the application of 1-MCP resulted in a smaller respiration rate over the storage period, showing a prolong increase from day 7 to 19, with a value of 50.13 mg CO₂ kg⁻¹ h⁻¹; this result was similar to that observed in the fruits treated with 1-MCP+ethylene (Fig. 1).

The respiratory rate in climacteric fruits, including peach, depends on ethylene. Zhang *et al.* (2012) found that ethephon can accelerate energy production, glycolytic metabolism and ethylene biosynthesis, which may accelerate respiration in the tissue. In apple fruits, Yang *et al.* (2013) noted that the application of ethylene increased the respiratory rate, whereas 1-MCP decreased it, similar to that observed in this research.

The results showed that the application of 1-MCP decreased the respiration rate, perhaps because it blocks ethylene cellular receptors (Watkins, 2006). Similar studies have suggested that peach fruits reduce their respiratory rate in the presence of 1-MCP, thus, decreasing the intensity of physiological processes (Steffens *et al.*, 2009; Wang *et al.*, 2017). Likewise, Valero *et al.* (2004) showed that applications of 1-MCP delayed the climacteric increase in plums, whereas treatments with ethylene accelerated it. Therefore, the respiratory rate is closely related to the climacteric rate; hence, the application of 1-MCP is a

viable alternative to prolong peach fruit quality. On the other hand, the increase in the respiratory rate shown by the fruits treated with 1-MCP may have been due to a rise in the amount of ethylene receptors synthesized by the tissue (Serek *et al.*, 2006). Despite this, the addition of ethylene after 1-MCP did not increase the respiration, as compared to the fruits treated with 1-MCP, possibly because the low levels of ethylene in the tissues were sufficient to bind to the few existing receptors (through the effect of the 1-MCP) and induce an increase in the respiratory rate of the peach fruits.

For firmness, the peach cv. Dorado fruits showed a constant decrease in firmness during storage, which agrees with Africano *et al.* (2016) for the same cultivar. Statistically significant differences ($P \leq 0.05$) were observed starting at 7 DOS, when the fruits treated with 1-MCP had the highest firmness, and the fruits treated with ethylene had the lowest. The control fruits did not have differences from those treated with ethylene; the fruits under these two treatments lost their organoleptic quality at 16 DOS, showing a final firmness of 25.2 ± 2.95 N for the control and 22.23 ± 2.06 N for the ethylene treatment; the fruits treated with 1-MCP+ethylene became soft rapidly toward the end of the storage period (Fig. 2).

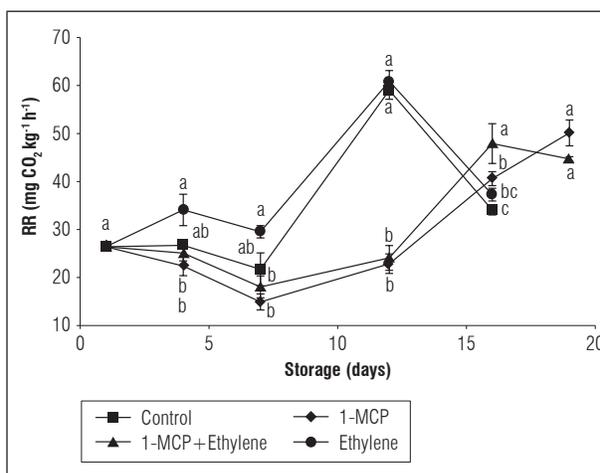


Figure 1. Effect of ethylene and 1-methylcyclopropene on respiratory rates (RR) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P \leq 0.05$). Vertical bars on each mean indicate the standard error ($n = 4$).

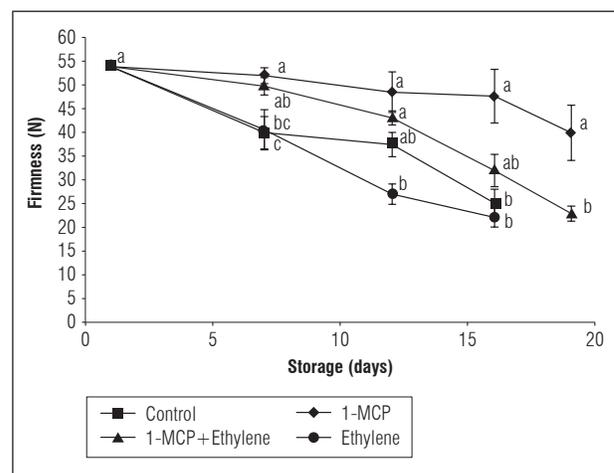


Figure 2. Effect of ethylene and 1-methylcyclopropene on the firmness of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P \leq 0.05$). Vertical bars on each mean indicate the standard error ($n = 4$).

The loss of firmness in a fruit may be related to an increase in the activity of enzymes that degrade the cell wall in peaches, such as polygalacturonase, pectin-methylesterase, endo- β -1,4-glucanase, α -arabinosidase and β -galactosidase. The activity of these enzymes may increase in the presence of ethylene because the depolymerization of pectins and xyloglucans is regulated by ethylene (Nishiyama *et al.*, 2007; Pech *et al.*, 2008) and it is expected to decrease as a result of the effect of 1-MCP, which blocks ethylene activity at the cellular level; this suggests that, in peach cv. Dorado fruits, firmness may also be regulated by ethylene, even after the application of 1-MCP. Özkaya *et al.* (2016) found a reduction in the activity of pectin methyl esterase and polygalacturonase in apricot fruits. Liu *et al.* (2015), in peach cv. Yahuala, and Wang *et al.* (2017), in peach cv. CN13, found that the application of 1-MCP increased firmness in comparison with non-treated fruits. Similarly, Khan and Singh (2007) found that 1-MCP delayed the loss of firmness in *Prunus salicilina* Lindl fruits by reducing the activity of polygalacturonase, pectin esterase and endo-1,4-d-glucanase, whereas Zhang *et al.* (2012), when using proteomics on peach fruits, found that 1-MCP decreased the loss of Ca ions, suppressed the degradation of the cell wall and maintained the mechanical properties of the cellular structure.

The color index (CI) of the skin increased with storage time. There were significant differences ($P \leq 0.05$) between all treatments starting at day seven of storage. The changes were smaller in the fruits treated with 1-MCP ($P \leq 0.05$), showing a CI of 2.63 ± 0.59 at the end of the storage period. The fruits treated with ethylene showed a higher index color (3.87 ± 0.48), despite the use of ethylene 7 d after 1-MCP; the control treatment showed a CI increase during storage (Fig. 3). Lelievre *et al.* (1997) stated that color changes may be dependent or independent of ethylene, as was later suggested by Dong *et al.* (2002), who observed changes in color independent of ethylene in *Prunus armeniaca*. However, these studies indicated that color in the peach cv. Dorado is dependent on ethylene; in fact, Barreto *et al.* (2017) found that, by applying ethylene to 'Chiripá' peach fruits, the color changed more than when applying 1-MCP.

The lower CI that resulted from the application of 1-MCP in the peach fruits may have been due to the fact that this compound delays color change since it can decrease chlorophyll degradation by reducing the activity of the chlorophyllase enzyme (Watkins, 2006; Sun *et al.*, 2012), which is up-regulated by ethylene.

Likewise, 1-MCP also decreased color changes in the peach cv. Aurora-1 (Kluge and Jacomino, 2002) and in the peach 'Roubidoux' (Steffen *et al.*, 2009).

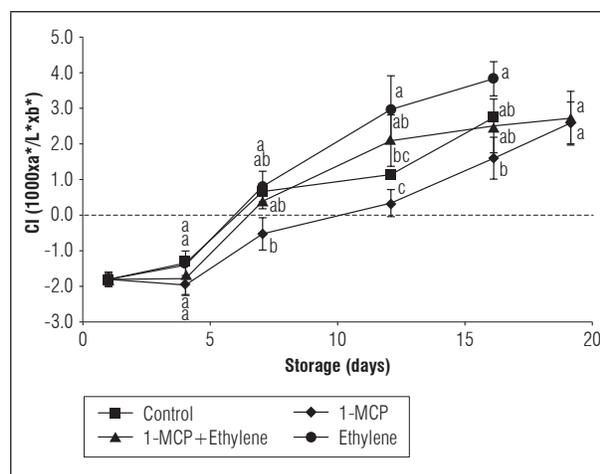


Figure 3. Effect of ethylene and 1-methylcyclopropene on color index (CI) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P \leq 0.05$). Vertical bars on each mean indicate the standard error ($n = 4$).

The soluble solids (SS) increased gradually with storage. There were significant differences ($P \leq 0.05$) between all sampling points. The fruits treated with 1-MCP accumulated less SS in every measurement, showing a value of 13.7 ± 0.21 °Brix at the end of storage, whereas the fruits treated with ethylene, alone or in combination with 1-MCP, showed a higher concentration of SS (16.1 ± 0.35 °Brix; Fig. 4).

The higher concentration of SS at the end of storage may be explained by the hydrolysis of starch and polysaccharides in the cell wall (Kays and Paull, 2004); in peach cv. Dorado fruits, ethylene may play a role in accelerating hydrolysis processes, while 1-MCP may delay them, which supports the hypothesis that ethylene regulates a large part of peach fruit ripening in cv. Dorado. Likewise, Zhang *et al.* (2012) found that, in peach cv. Huiyulu, 1-MCP decreased starch degradation, decreasing the glycolytic metabolism and, therefore, ripening.

Our results agree with Barreto *et al.* (2017) who, in peach cv. Chiripá, found lower SS values for fruits treated with 1-MCP than for fruit treated with ethylene; on the contrary, Tonetto *et al.* (2007) and

Steffens *et al.* (2009) did not observe any effect on SS after applying 1-MCP in the peaches 'Eldorado' and 'Roubidoux'.

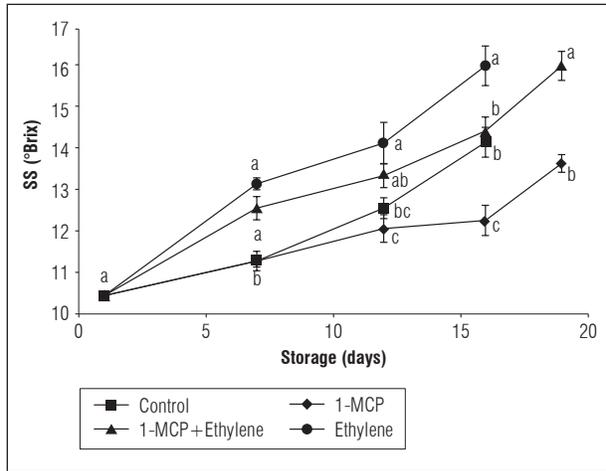


Figure 4. Effect of ethylene and 1-methylcyclopropene on soluble solids (SS) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P \leq 0.05$). Vertical bars on each mean indicate the standard error ($n = 4$).

The total acidity (TA) showed statistically significant differences ($P \leq 0.05$) between all sampling points during storage, except at 19 DOS. For all treatments, there was a gradual decrease in acidity, which was higher for the ethylene and control treatments and lower for the 1-MCP treatment; at 19 DOS, the fruits with 1-MCP showed $0.95 \pm 0.11\%$ TA (Fig. 5).

According to Alves *et al.* (2009), the effect of 1-MCP on the TA of peach fruits may be explained by a lower acid consumption during the tricarboxylic acid cycle through the reduction of respiration. As observed in this study, TA decreases over the storage period because organic acids are part of the respiration substrates (Kays and Paull, 2004). Similar results have been shown by Barreto *et al.* (2017) in the peach, who found higher acidity in fruits treated with 1-MCP than in fruits treated with ethylene, which accelerates the loss of TA. Liu *et al.* (2015) also observed a higher TA with 1-MCP in peaches. On the contrary, Tonetto *et al.* (2007) did not observe effects from 1-MCP on the TA of the peach 'Eldorado', similar to Steffens *et al.* (2009) for the peach cv. Roubidoux. This suggests that the effect of 1-MCP on acidity depends on the cultivar, as indicated by Ligouri *et al.* (2004).

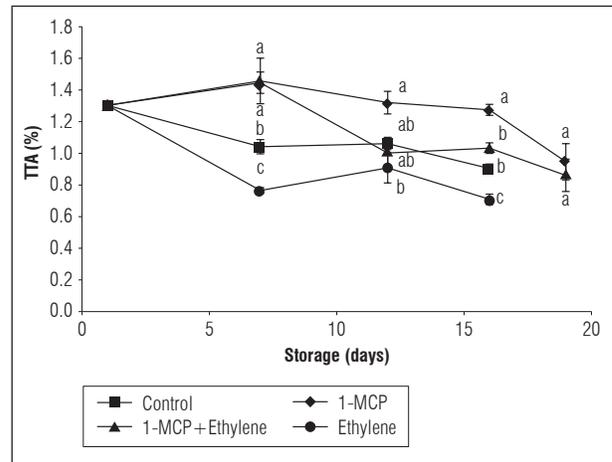


Figure 5. Effect of ethylene and 1-methylcyclopropene on total titratable acidity (TTA) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P \leq 0.05$). Vertical bars on each mean indicate the standard error ($n = 4$).

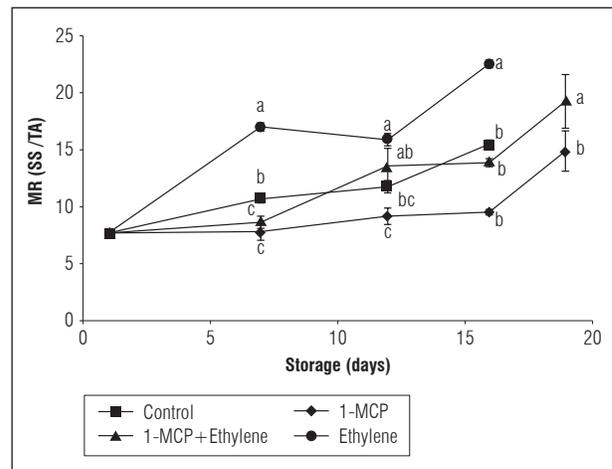


Figure 6. Effect of ethylene and 1-methylcyclopropene on maturity ratio (MR) of peach cv. Dorado fruits during storage. Means followed by different letters on the same sampling day showed statistically significant differences according to Tukey's test ($P \leq 0.05$). Vertical bars on each mean indicate the standard error ($n = 4$).

The maturity ratio (MR) increased gradually throughout storage and showed significant differences ($P \leq 0.05$) between the treatments in all sampling points. The lowest values were found in the fruits treated with 1-MCP and with 1-MCP+ethylene, indicating a delay in MR (Fig. 6). The fruits treated

with ethylene ripened faster, showing higher MR values over the storage period, which is a typical behavior for this variable, perhaps because of the increase in SS and decrease in TA (Ferrer *et al.*, 2005; Africano *et al.*, 2015). Accordingly, the exogenous application of ethylene accelerates ripening in peach cv. Dorado fruits, whereas 1-MCP delays it; however, Tonnetto *et al.* (2007) did not find any effect from 1-MCP on the MR of the peach 'Eldorado'.

CONCLUSIONS

Our results suggest that 1-MCP decreases the respiration rate, loss of firmness, epidermis color index, total soluble solids, total titratable acidity, and maturity ratio in the peach cv. Dorado, whereas ethylene has an opposite effect. Our results demonstrate that several ripening processes in peach cv. Dorado fruits are associated with the presence of ethylene and that 1-MCP may delay ripening throughout the storage of this fruit.

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Conflict of interests: this manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts the validity of the presented results at risk.

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