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Effect of Dips in a 1-Methylcyclopropene-Generating Solution on 'Harrow Sun' Plums Stored under Different Temperature Regimes

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The effect of postharvest dips in a 1-methylcyclopropene-generating solution of the formulation AFxRD-038 (Rohm & Haas) on plum fruit (*Prunus salicina* Lindell cv. 'Harrow Sun') quality and ripening during storage was determined. Fruit weight loss, tissue firmness, soluble solids content (SSC), titratable acidity (TA), ethylene production, respiration, and the activities of the cell wall modifying enzymes polygalacturonase (PG), 1,4-β-D-glucanase/glucosidase (EGase), β-galactosidase (β-gal), and pectin methylesterase (PME) were measured. Fruit reddening, anthocyanin content, and phenylalanine ammonia-lyase (PAL) activity were also analyzed. The 1-MCP-treated fruit showed reduced ethylene production and respiration rate and delayed softening, which was associated with the reduction in the activity of PG, EGase, and β-gal. The immersion in 1-MCP-generating solutions also decreased weight and acidity loss without modifying the fruit SSC. The immersion treatment was particularly effective in the fruit stored at 5 °C, keeping higher overall quality, maintaining lower levels of anthocyanins and PAL activity, and preventing flesh reddening. The present data show that beneficial effects in delaying plum fruit ripening and controlling chilling injury can be obtained by dipping the fruits in a solution of this novel 1-MCP-generating formulation.

KEYWORDS: 1-MCP; chilling injury; flesh reddening; market life; plum ripening; postharvest storage

INTRODUCTION

The plum fruit is highly perishable, and postharvest softening and incidence of chilling injury (CI) symptoms are important factors limiting its shelf life, shipping, and storage (*1*, *2*). Plum cultivars with high rates of ethylene production softened and ripened more rapidly than cultivars that are low ethylene producers (*3*, *4*); therefore, strategies to reduce ethylene sensitivity and/or production would be extremely useful to decrease plum softening and deterioration.

Besides many other advantages, refrigerated storage is a widely used strategy to reduce ethylene production and sensitivity during postharvest storage of horticultural products (*5*). However, the use of low-temperature storage has to be carefully managed in plums because of the fruit susceptibility to CI (*1*). In contrast to other commodities such as peaches, in which the underlying mechanisms of CI have been determined (*6*, *7*), the physiological basis of CI symptoms in plums, including internal browning, gel breakdown, and/or reddening (*1*, *2*, *8*), has not been established. Ethylene has been demonstrated to be involved in the development of CI symptoms in several commodities (*9*−*11*), and reduction of low-temperature storage disorders by reducing ethylene sensitivity and production has also been achieved in products such as avocado (*11*) and pineapple (*12*).

The ethylene action inhibitor 1-methylcyclopropene (1-MCP) has been an excellent tool for studies designed to determine the role of ethylene in biological processes (*13*). 1-MCP treatments also have been extremely useful for delaying the physical−chemical changes related to the ripening process and reducing decay and weight loss (*13*, *14*). Treatments at doses ranging between 0.25 and 0.75 μL L^{-1} delayed ripening in several plum cultivars (*15*−*17*). In these cases, the applications were achieved by mixing the product with water or a buffer solution to release the 1-MCP gas in enclosed areas. However, the availability of the proper facilities to treat the fruit could be a limitation under certain commercial situations. The identification of nonvolatile and nontoxic compounds that will counteract ethylene without requiring a closed system for applications would make ethylene action inhibitors a much more versatile tool for postharvest management (*18*, *19*). The objective of the present study was to evaluate the effect of postharvest dips of 'Harrow Sun' plums in a solution of the 1-MCP-generating formulation AFxRD-038 (Rohm & Haas) on ripening, quality, and incidence of physiological disorders during storage.

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MATERIALS AND METHODS

Fruit Material, 1-MCP Treatments, and Storage Regime. Plum fruits (*Prunus salicina* cv. 'Harrow Sun') were harvested (Fresno area, CA) at firm-ripe stage during the summer of 2006 and divided into 18 lots of 24 fruits. Twelve lots were immersed for 5 min in solutions of the formulation AFxRD-038 (Rohm & Haas Co., Philadelphia, PA). One hundred and five milligrams or 1.05 g of the AFxRD-038 formulation was dissolved in 40 L of tap water according to the manufacturer's recommendation to generate 0.1 and 1.0 mg L^{-1} of 1-MCP, respectively. Six lots of fruit were dipped in water for 5 min and used as controls. A nonionic surfactant (0.1% Nu-Film P, Miller Chemical & Fertilizer Corp., Hanover, PA) was added to the solutions to increase the effectiveness of the treatments. After immersion, one lot from each treatment was analyzed after 5 and 7 days at 20 °C (shelf life). The remaining lots were stored at 0 °C (simulation of conventional cold storage) or 5 °C (simulation of transportation storage) for 10 days and sampled after both 5 and 7 days of shelf life after removal from cold storage.

Quality Attributes. Weight loss was calculated for each fruit as follows: weight loss % $= 100 \times (W_0 - W_f)/W_0$, W_0 being the initial weight and W_f the final weight of the fruits.

Tissue firmness was initially monitored nondestructively, with a low mass impact Sinclair IQ (Internal Quality) Firmness Tester (SIQ-FT Systems International, LLC, Fresno, CA) and the data were processed by proprietary software (Sinclair IQ version PIQ01-v2.18.01) to obtain measures of fruit firmness indexed from 0 to 100 (arbitrary units), defined as "Sinclair firmness index" (SFI) (*20*). The SFI was monitored on each fruit, and the percentage loss relative to the initial values was calculated. Fruit firmness was also measured on two opposite sides of each fruit with a Fruit Texture Analyzer (FTA). A 7.9-mm-diameter, flat-tipped probe was used to perform compression tests at a speed of 10 mm min^{-1}, and the maximum force during the test was recorded. Twenty-four measurements for each treatment and storage time were made.

For soluble solids concentration (SSC) and titratable acidity (TA) measurements, a longitudinal wedge (from stem end to calyx end) was removed from each fruit and pressed through cheesecloth. Juice from eight fruits was pooled to form a composite sample. SSC of the juice was measured with a temperature-compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan), and the TA was determined by titration of fruit juice with 0.1 N NaOH to pH 8.2 according to an AOAC method (*21*). Triplicate measurements were made per treatment during 5 and 7 days of ripening after harvest or after removal from cold storage.

Each lot of fruits was divided into three eight-fruit sublots. A wedged-shaped slice of mesocarp tissue (6 g) from every fruit in each sublot was removed, pooled at the time of sampling, frozen in liquid nitrogen, stored at −20 °C, and subsequently used for anthocyanin determinations. Fruit tissue was ground in an Ultraturrax (Kinematica, GmbH, Switzerland) with 10 mL of HCl/methanol (1%, v/v) and held at 0 °C for 10 min. The slurry was centrifuged at 1500*g* for 10 min at 4 °C, and the absorbance of the supernatant at 515 nm was measured. Results were calculated using $\epsilon = 29600$ and expressed as micromoles of cyanidin 3-glucoside per kilogram of fresh weight (*22*).

Ethylene and Respiration. Ethylene production and respiration rate were measured during 7 days for each treatment and storage regimen. Individual fruits were weighed and placed in a 0.705 L plastic container, which was then sealed and held at 20 °C and 90% relative humidity. Carbon dioxide concentrations were measured with an infrared gas analyzer (Horiba PIR-2000R, Horiba Instruments Inc., Irvine, CA). Ethylene concentrations were determined using a gas chromatograph equipped with a packed alumina column operated at an isothermal oven temperature of 70 °C, and peak detection was with a flame ionization detector (Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK). Ethylene results were expressed as microliters per kilogram per hour, whereas respiration rates were expressed as milligrams of CO_2 per kilogram per hour. Five independent measurements were made for each storage time and treatment.

Enzyme Extraction and Activity. Approximately 20 g of frozen tissue was homogenized in an Ultraturrax (Kinematica) with 60 mL of buffer containing 50 mM sodium acetate, 1 M NaCl, and 10 g L^{-1} poly(vinylpolypyrrolidone) (PVPP), pH 5.5. The homogenate was stirred for 3 h at 4 °C and then centrifuged (10000*g*, 30 min, 4 °C). The supernatant was collected, dialyzed overnight against 50 mM sodium acetate, pH 5.0, and subsequently used for assaying polygalacturonase (PG, EC 3.2.1.67), 1,4-β-D-glucanase/glucosidase, and β-galactosidase (β-gal, EC 3.2.1.23) activities.

PG was measured in a mixture containing 400 μL of 50 mM sodium acetate buffer, pH 5.0, 400 μL of 0.15% w/v polygalacturonic acid, and 800 μL of the enzyme extract. The mixture was incubated at 40 °C, aliquots (200 μL) were taken at regular intervals up to 8 h, and the amount of galacturonic acid reducing ends generated was measured according to the method of Gross (*23*). Results were expressed as micromoles of galacturonic acid per kilogram per second.

EGase was assayed in a mixture containing 400 μL of 50 mM sodium acetate buffer, pH 5.0, 400 μL of 0.2% w/v carboxymethylcellulose, and 800 μL of enzyme extract. The mixture was incubated at 40 °C, and aliquots (200 μL) were taken up to 8 h and assayed for reducing sugars according to the method of Gross (*23*). Enzyme activity was expressed as micromoles of glucose per kilogram per second.

β-Gal activity was assayed in a mixture containing 400 μL of 50 mM sodium acetate buffer, pH 5.0, 200 μL of 10 mM *p*-nitrophenyl-β-D-galactopyranoside, and 400 μL of enzyme extract. The reaction mixture was incubated at 40 °C, and aliquots (200 μL) were taken at different times and discharged into 600 μL of 0.4 M Na_2CO_3. The change of optical density at 400 nm was followed. Results were expressed as micromoles of *p*-nitrophenol released per kilogram per second.

For pectinmethylesterase (PME, EC 3.1.1.11), approximately 20 g of frozen tissue was homogenized in an Ultraturrax with 60 mL of buffer containing 50 mM sodium acetate and 10 g L^{-1} PVPP, pH 5.5. The homogenate was stirred for 3 h at 4 °C and centrifuged (10000*g*, 30 min, 4 °C). The supernatant was extensively dialyzed against water, adjusted to pH 7.5 with 0.01 M NaOH, and used for assaying the enzyme activity (*24*). The activity was assayed in a mixture containing 2.6 mL of 0.5% w/v pectin (72% methoxyl content), 800 μL of 0.01% bromothymol blue in 0.003 M phosphate buffer, pH 7.5, and 2.6 mL of enzymatic extract. The mixture was incubated at 40 °C, and the absorbance changes at 620 nm were measured. The enzymatic activity was expressed as change in OD_{620} per kilogram per second.

PAL activity was determined as follows: about 5 g of frozen fruit was homogenized in an Omnimixer at 4 °C with 2 volumes of buffer of the following composition: 0.1 M $Na_2B_4O_7 \cdot 10H_2O$, 5 mM 2-mercaptoethanol, 2 mM EDTA, and 10 g L^{-1} of PVPP, pH 8.8. The mixture was stirred for 1 h at 4 °C and then centrifuged at 10000*g* for 20 min at 4 °C. The enzymatic activity was measured in the supernatant according to the method reported by Zucker (*25*), using the following reaction mixture: 0.03 M $Na_2B_4O_7 \cdot 10H_2O$, pH 8.8, 0.01 M L-phenylalanine, and 2 mL of enzymatic extract, in a total volume of 6 mL. The reaction mixture was incubated at 30 °C, and the reaction was evaluated through the increase in optical density at 290 nm, caused by production of *trans*-cinnamic acid. For EGase, β-gal, PME, and PG, three independent extracts per 1-MCP treatment and storage condition were prepared, and measurements were done in duplicate. For PAL, two independent extracts per 1-MCP treatment and storage condition were prepared, and measurements were done in duplicate for each independent replication.

Statistical Analysis. The experimental design was a randomized block, and data were analyzed by ANOVA, followed by Duncan's multiple-range test at a significance level of $P = 0.05$. Data in percentages were subjected to arcsine transformation prior to statistical analysis. ANOVA was performed using the statistical software SPSS 14.0 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Tissue Firmness and Activity of Cell Wall Degrading Enzymes. Fruit firmness measured by a tissue failure test decreased in control fruit during storage, and dips in the 1-MCP-generating liquid solution at both concentrations (0.1 and 1 mg

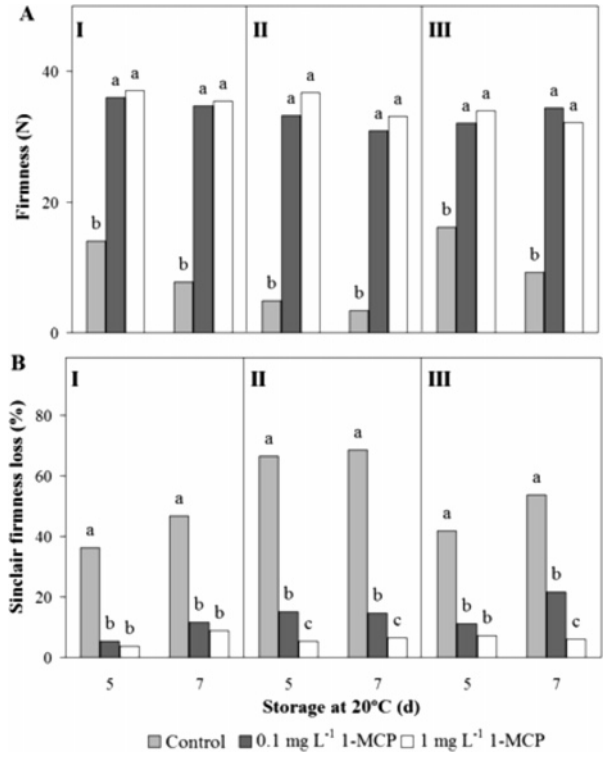

Figure 1. Firmness of 'Harrow Sun' plums dipped in a 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control). Fruit was ripened at 20 °C (I) or stored at 5 °C (II) or 0 °C (III) for 10 days and subsequently transferred to 20 °C: (**A**) tissue firmness; (**B**) Sinclair firmness index (SFI) loss. Letters on each column show differences among treatments for a given storage time at a level of significance of $P = 0.05$.

L^{-1} 1-MCP) were effective in delaying softening under all of the storage regimens (**Figure 1A**). Firmness determinations using the low mass impact Sinclair Tester provided data consistent with those from the FTA, showing that both MCP treatments reduced firmness loss under all storage treatments (**Figure 1B**). Excessive softening is a major factor limiting plum shelf life. Firmness loss in fruits has been associated with cell wall modifications (*26*), caused by the action of several polysaccharide-degrading proteins (*27*). The dips for 5 min at either concentration substantially reduced the activity of PG, EGase, and β-gal (**Figure 2**). No differences in extracted PME activity were observed in the fruit stored directly at 20 °C after harvest or maintained at 5 °C for 10 days and subsequently transferred to 20 °C, but the MCP treatments of fruits that were subsequently stored at 0 °C before transfer to 20 °C reduced extractable PME activity significantly. Khan and Singh (*28*) reported that 1-MCP significantly suppressed fruit-softening enzymes in plum fruit, including PME, EGase, exo-PG, and endo-PG. Previous studies have reported that gassing the fruit with the commercially available formulation of 1-MCP controls plum softening (*15*, *28*, *29*), demonstrating 1-MCP's value as an effective tool in maintaining quality and extending the postharverst life in plums. The data show that the simpler mode of application, that is, dipping the fruit in solutions of the formulation AFxRD-038, is also effective in delaying plum fruit softening.

Soluble Solids Content, Titratable Acidity, and Weight Loss. Sugar and acid contents are two major determinants of flavor characteristics in many fruits, including plums. TA was higher in 1-MCP-treated plum fruit stored directly at 20 °C or

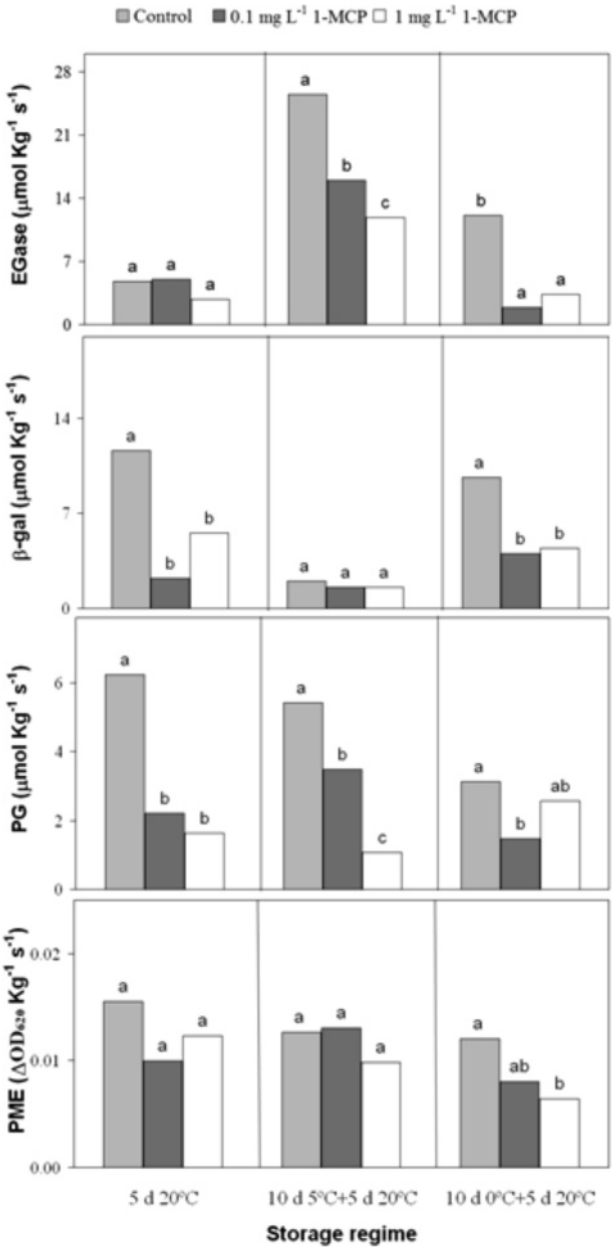

Figure 2. EGase, β-gal, PG, and PME activities in 'Harrow Sun' plums dipped in a 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control) and then ripened at 20 °C or stored at 5 or 0 °C for 10 days and subsequently transferred to 20 °C. Letters on each column show differences among treatments for a given storage time at a level of significance of $P = 0.05$.

at 0 °C for 10 days and subsequently transferred to 20 °C (**Figure 3A**). However, when the fruits were stored at 5 °C, an increase in TA was detected in the control fruit. This could be partly due to CI damage in the nontreated tissues. No differences in SSC content between control and 1-MCP-treated plum were observed (**Figure 3B**). Finally, fruit weight loss was clearly reduced in the 1-MCP-treated fruit (**Figure 3C**). Overall, the data show that the immersion treatments might be sufficient to control plum fruit ripening.

Ethylene Production and Respiration Rate. Ethylene production and responses in plums depend on the cultivar considered. Abdi et al. (*3*, *4*) reported that some cultivars show a typical climacteric behavior, whereas others are considered to have a suppressed climacteric phenotype. For 'Harrow Sun' plums, control fruit stored at 20 °C without refrigeration showed

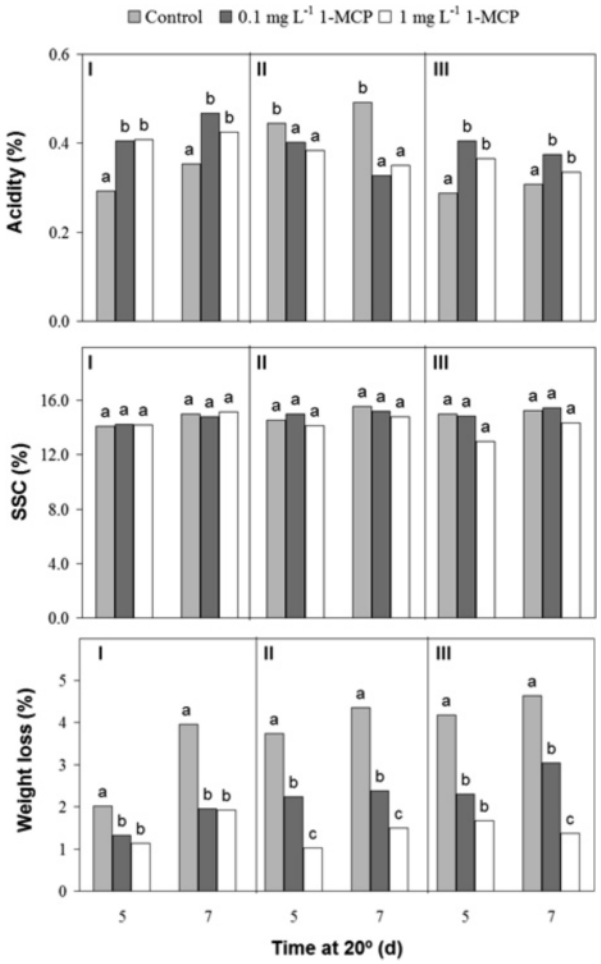

Figure 3. Titratable acidity, soluble solids content (SSC), and weight loss in 'Harrow Sun' plums dipped in a 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control) and then ripened at 20 °C (I) or stored at 5 °C (II) or 0 °C (III) for 10 days and subsequently transferred to 20 °C. Letters on each column show differences among treatments for a given storage time at a level of significance of $P = 0.05$.

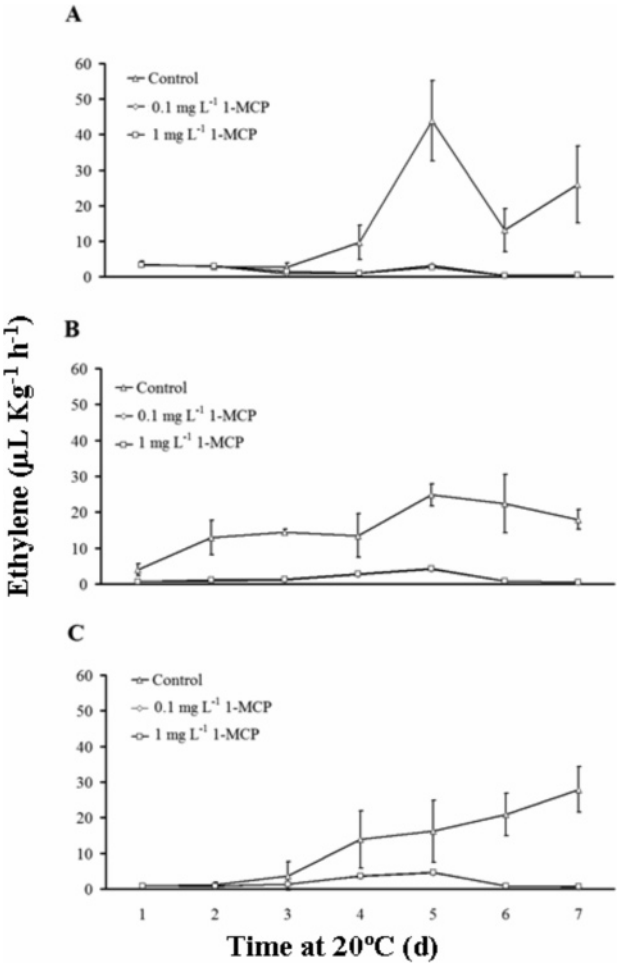

Figure 4. Ethylene production in 'Harrow Sun' plums dipped in a 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control): during ripening at 20 °C after (**A**) harvest, (**B**) a 10-day cold storage at 5 °C, or (**C**) a 10-day cold storage at 0 °C. Error bars are shown.

a peak of ethylene production after 4 days (**Figure 4A**). Interestingly, when the fruit was stored at 0 or 5 °C and then transferred to 20 °C, ethylene production increased, but did not peak, as storage time progressed (**Figure 4B**,**C**), showing that the ethylene production pattern of 'Harrow Sun' plums is highly dependent on the fruits' thermal history. Under all of the storage regimens analyzed in this work ethylene production was clearly reduced in the 1-MCP-dipped fruit (**Figure 4**), and no differences between the two applied 1-MCP concentrations were observed. 1-MCP-treated fruit also displayed decreased fruit respiration rate (**Figure 5**), with both inhibitor dosages having equal effectiveness. These results also confirm that the dips using the new 1-MCP-generating formulation were effective in controlling plum fruit ripening.

Incidence of Flesh Reddening, Anthocyanin Accumulation, and PAL Activity. Ethylene has been demonstrated to be involved in the development of CI symptoms in different commodities (*9*, *10*). The hormone seems to be involved in the process in some, but not all, cases. In pear (*30*), avocado (*11*), and pineapple (*12*), CI-associated disorders have been reduced by 1-MCP treatments. The nature of the CI syndrome in plums has not been studied in detail. The disorder is usually manifested as internal browning, gel breakdown, and/or flesh reddening (*8*). In the present work anthocyanin content increased in control fruit as storage time at 20 °C progressed (**Figure 6**), and pigment accumulation was clearly exacerbated in the fruit stored at 5 °C and subsequently transferred to 20 °C. The intense flesh reddening and pigment accumulation observed in the control fruit stored at 5 °C did not occur in 1-MCP-treated fruit (**Figure 7**), showing that this is an ethylene-dependent disorder. In addition, the control fruit also showed increased PAL, which catalyzes the first step of anthocyanin biosynthesis (**Figure 8**). Treatment of strawberry fruit with 1-MCP has been reported to cause a reduction in PAL activity and the normal ripening-associated increases in fruit anthocyanin content (*31*). Taking into account that flesh reddening is regarded as a CI symptom in stone-fruits (*2*, *7*), results of the present study suggest that 1-MCP dips could be a promising strategy to prevent or reduce the occurrence of flesh discoloration during transportation and storage.

Several studies have shown that exposure of fruits to gaseous 1-MCP can be a useful tool for fruit handlers to maintain quality and extend the postharvest life of several plum varieties, delaying softening while not causing unfavorable effects on fruit quality. The results of the present work show that dipping the fruits in a water solution of the new 1-MCP-generating formulation (Rohm & Haas) is effective in counteracting ethylene responses and extending the postharvest life of plums and eliminates the need for a closed system for treating the fruit. This mode of application could make 1-MCP a much more

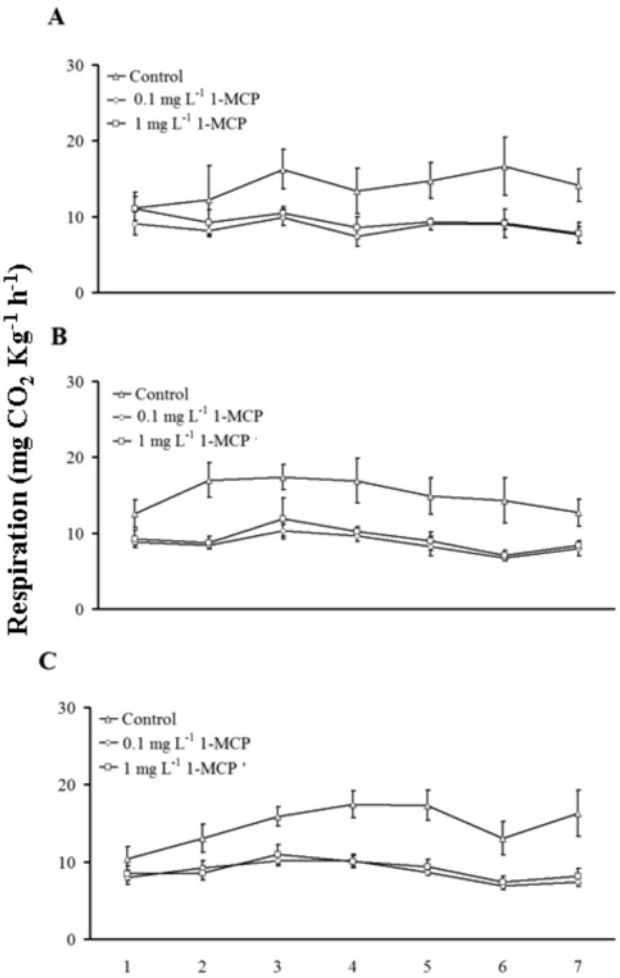

Figure 5. Respiration rate in 'Harrow Sun' plums dipped in 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control) during ripening at 20 °C after (**A**) harvest, (**B**) a 10-day cold storage at 5 °C (B), or (**C**) a 10-day cold storage at 0 °C. Error bars are shown.

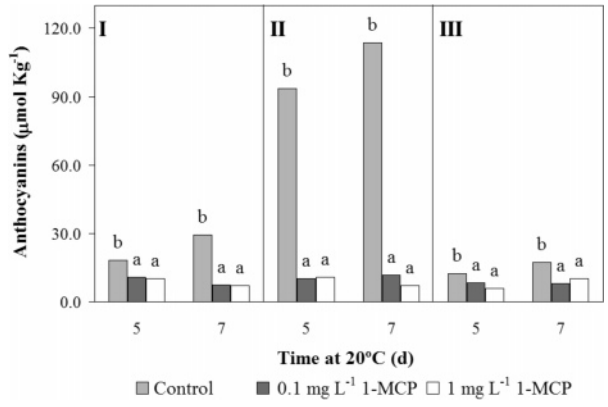

Figure 6. Anthocyanin content in 'Harrow Sun' plums dipped in 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control) during ripening at 20 °C (I) or stored at 5 °C (II) or 0 °C (III) for 10 days and subsequently transferred to 20 °C for ripening. Letters on each column show differences between treatments for a given storage time at a level of significance of $P = 0.05$.

versatile tool for postharvest management of fruits. Future experiments on a range of fleshy fruits, particularly those harvested at an advanced maturity stage when the most desirable organoleptic attributes have already developed on the tree,

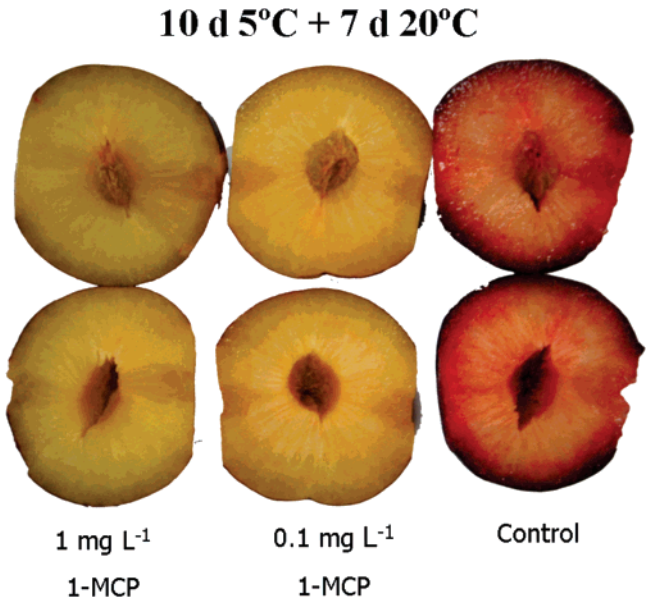

Figure 7. Visual appearance of 'Harrow Sun' plums dipped in a 1-MCP-generating liquid solution at two different concentrations (0.1 or 1 mg L^{-1} 1-MCP) or water (control), stored at 5 °C for 10 days and subsequently transferred to 20 °C for 7 days.

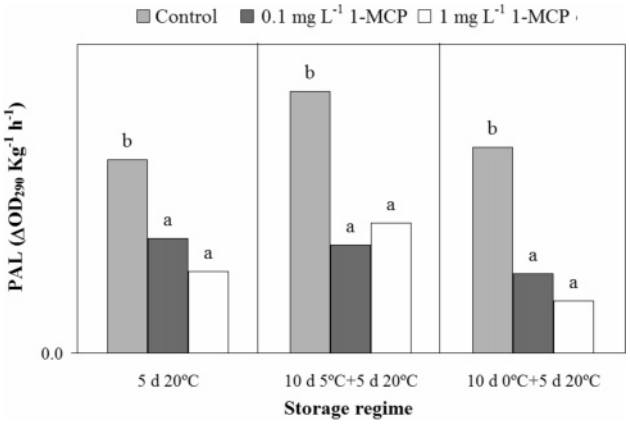

Figure 8. PAL activity in 'Harrow Sun' plums dipped in 1-MCP-generating liquid solution or water (control) and ripened at 20 °C or stored at either 5 or 0 °C for 10 days and subsequently transferred to 20 °C. Letters on each column show significant differences among treatments for a given storage time at a level of significance of $P = 0.05$.

should be carried out. In addition, the minimum treatment times required to modulate the chemical effects on the postharvest lives of fruits should be determined to increase the utility of this kind of application.

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