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Research Note

Novel 1-methylcyclopropene immersion formulation extends shelf life of advanced maturity 'Joanna Red' plums (*Prunus salicina* Lindell)

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Abstract

A postharvest application, by immersion, of a new 1-methylcyclopropene (1-MCP) formulation delayed ripening changes and extended the shelf life period of plum fruit (*Prunus salicina* Lindell cv. Joanna Red) harvested at an advanced maturity stage when ripened immediately after harvest or after cold storage. Fruit were either immersed in a water solution (control) or in an aqueous solution of a formulation containing 10, 100, 1000 and 10,000 ng kg⁻¹ of 1-MCP. The fruit were allowed to ripen at 23 °C after 5-m immersion or after immersion and subsequent cold storage (5 °C, RH 90%) for 10 d, prior to being evaluated for quality attributes. 1-MCP immersion treatments reduced firmness loss, skin color changes, fruit weight loss and respiration rate. Furthermore, a pronounced suppression of ethylene production in fruit treated with 1000 and 10,000 ng kg⁻¹ 1-MCP was detected. All fruit ripened normally and did not show any chilling injury (CI) symptoms when ripe fruit were evaluated after cold storage. Overall, 1-MCP concentration of 1000 ng kg⁻¹ was the most effective in controlling fruit ripening changes and extending the shelf life of this advanced maturity (tree ripened), low CI susceptible plum. This is the first study, to the best of our knowledge, reporting the successful application of 1-MCP by immersion on the postharvest performance of fleshy fruit.

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1. Introduction

Plum cultivars are highly perishable, characterized by short postharvest life when exposed to room temperature (shelf life) after cold storage due to decay, incidence of chilling injury (CI) symptoms and fast softening. Furthermore, sensory evaluation studies in plums recommended that in order to increase fruit flavor, and therefore consumption, plums should be harvested at more advanced maturity, when better quality characteristics have developed (Crisosto et al., 2004). So, reduction of fast softening should be avoided during postharvest handling to protect fruit quality and increase shelf life of delayed harvested fruit.

1-Methylcyclopropene (1-MCP), a strong blocker of ethylene receptor, is being used as a tool to further investigate the role of ethylene in ripening and senescence, and as a potential commercial tool to maintain product quality (Sisler and Serek, 2003;

reviewed in Blankenship and Dole, 2003 and Watkins, 2006). 1-MCP has been reported to be particularly effective in delaying plum softening. However, its efficacy and dose is related to plum cultivar (suppressed-climacteric versus climacteric fruit types), as well as to temperature treatment (reviewed in Watkins, 2006).

1-MCP is being distributed commercially as a cyclodextrin powder formulation that needs to be mixed in enclosed areas with warm water or a buffer solution that releases 1-MCP into the air of the storage room, making it quite complicated to apply. The need to explore nonvolatile and nontoxic compounds that will counteract ethylene without a closed system in order for ethylene inhibitors to become a much more versatile tool has been pointed out (Sisler and Serek, 2003; Sisler, 2006) and recently some data regarding the efficacy of sprayable 1-MCP on maturity and quality of apple fruit at harvest and after storage have been reported (Watkins et al., 2006).

In this study, a range of concentrations of a novel 1-methylcyclopropene immersion formulation was tested on 'Joanna Red' plums at an advanced stage of maturity. Ripening related changes were determined in fruit after immersion and

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immediate ripening, and also in fruit ripened after a cold storage period that simulated standard domestic distribution. The selected cultivar represents a group of plums with low susceptibility to CI, but a short shelf life (fast ripening).

2. Materials and methods

Plum fruit (*Prunus salicina* Lindell, cv. Joanna Red) were harvested at advanced maturity (~30N firmness) from a commercial orchard in the Fresno area (Central California, USA). Treatments included immersion for 5 m in water at 20 °C (control) and in water solutions of the formulation AFxRD-038 (Rohm & Haas Co., Spring House, PA) at four 1-MCP concentrations (10, 100, 1000 and 10,000 ng kg⁻¹) with a wetting agent (Nu-Film P, 0.1%, Miller Chemical & Fertilizer Corp., Hanover, PA, USA) for maximum absorption. According to the manufacturer's material safety data sheet the formulation consisted of methylcyclopropene (3.8%), dextrose (≤5.0%), cyclodextrin (88.0–95.0%) and an amino acid salt (1.0–5.0%, trade secret).

Treated and untreated fruit were allowed to drain for 1 h and were separated into two lots of 60 fruits. One lot was placed at 23 °C for immediate ripening after the treatment and the other lot was cold stored (5 °C, 90% RH) for 10 d and then allowed to ripen at 23 °C. Sub-lots of 30 fruits were used to determine flesh firmness, total soluble solids (TSS) and titratable acidity (TA) after 5 and 11 d ripening after harvest and after 5 and 7 d ripening after removal from cold storage. For each treatment, fruit skin color, weight loss, nondestructive measurement of fruit texture, ethylene (C₂H₄) and carbon dioxide (CO₂) production were monitored daily up to 11 d after harvest and up to 7 d after removal from cold storage.

Thirty independent fruit per treatment after harvest and after removal from cold storage were analyzed for fruit skin color with a colorimeter (Model CR-200, Minolta USA Co., Ramsey, NJ, USA) and weight loss.

A longitudinal wedge (from stem end to calyx end) was removed from 10 fruit to obtain a composite sample, pressed with a hand press (Hamilton Beach®, model no. 932, Proctor-Silex Inc., Washington) through cheesecloth, and total soluble solids (TSS) and titratable acidity (TA) of the juice were determined with a temperature compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan) and an automatic titrator (Radiometer, Copenhagen, Denmark), respectively. TA was expressed as H⁺ mol L⁻¹. Triplicate measurements per treatment during ripening after harvest or after removal from cold storage were made.

Nondestructive fruit texture changes were monitored daily on 15 fruits per each replication from all treatments during ripening, using the low-mass impact bench top Sinclair IQ (Internal Quality) Firmness Tester (SIQ-FT Systems International, LLC, Fresno, CA, USA) and data was processed by proprietary software (Sinclair IQ version PIQ01-v2.18.01) and expressed as a number indexed from 0 to 100 (arbitrary units), defined as 'Sinclair firmness index' (SFI). Destructive flesh firmness was measured on 30 fruits per treatment using a fruit texture analyzer (FTA) interfaced to a personal computer equipped with the appropriate software (FTAWin). Skin from opposite cheeks

on each fruit was removed and flesh firmness calculated as an average of two measurements per fruit. A 7.9-mm diameter, flat-tipped probe was driven into the flesh with a cross-head speed of 10 mm m⁻¹, and the peak force was recorded in N.

Five individual fruit from each treatment after harvest or cold storage were weighed and placed in 0.705-L plastic containers in an ambient atmosphere room at 23 °C. Air samples were taken daily from each container and CO₂ and C₂H₄ concentrations measured with an infrared gas analyzer (Horiba PIR-2000R, Horiba Instruments Inc., Irvine, CA, USA) and a gas chromatograph equipped with a flame ionization detector (Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK, USA), respectively.

Fruit were evaluated for CI symptoms after the 10 d cold storage and then allowed to ripen at room temperature (23 °C). Gel breakdown, flesh browning, translucency, and mealiness were evaluated according to our published protocol (Crisosto et al., 1999).

Data were subjected to analysis of variance (ANOVA) and LSD means separation with significance level $P < 0.05$. A correlation analysis and slope comparison was applied to some variables to compare fruit changes over time. Statistical analysis was performed using the statistical software SPSS 14.0 (SPSS Inc., Chicago, USA).

3. Results and discussion

Skin color change from red to dark black during ripening was delayed in all fruit treated with 1-MCP concentrations equal or higher to 100 ng kg⁻¹ (Table 1), as has been previously reported by other researchers in plums (Martínez-Romero et al., 2003; Valero et al., 2003). A similar situation occurred on fruit cold stored prior to ripening. However, in this case all fruit were darker than fruit immediately ripened, indicating some fruit darkening occurred during the 10 d cold storage period prior to ripening.

TSS and TA values ranged from 14.7 to 15.4% and 0.052 to 0.078 H⁺ mol L⁻¹, respectively for all of the treatments, without any differences between the control and 1-MCP-treated fruit during ripening (data not shown). These TSS and TA levels are typical quality attributes of 'Joanna Red' and these TSS

Table 1

Skin color changes expressed as hue angle (h°), on 'Joanna Red' plums, without and with 10 d cold storage (5 °C), during ripening for 5 and 11 and 5 and 7 d at 23 °C, respectively

1-MCP (ng kg ⁻¹)	Hue angle (h°)			
	Without cold storage		With cold storage	
	5 d	11 d	5 d	7 d
0	18.0 c ^a	15.6 a	15.3 b	12.9 a, b
10	18.4 c	16.9 a	15.3 b	11.7 b
100	25.8 b	16.5 a	14.5 b	12.0 b
1000	33.0 a	18.4 a	20.2 a	15.0 a
10,000	35.7 a	19.1 a	19.9 a	14.0 a, b

^a Values within columns within each attribute followed by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

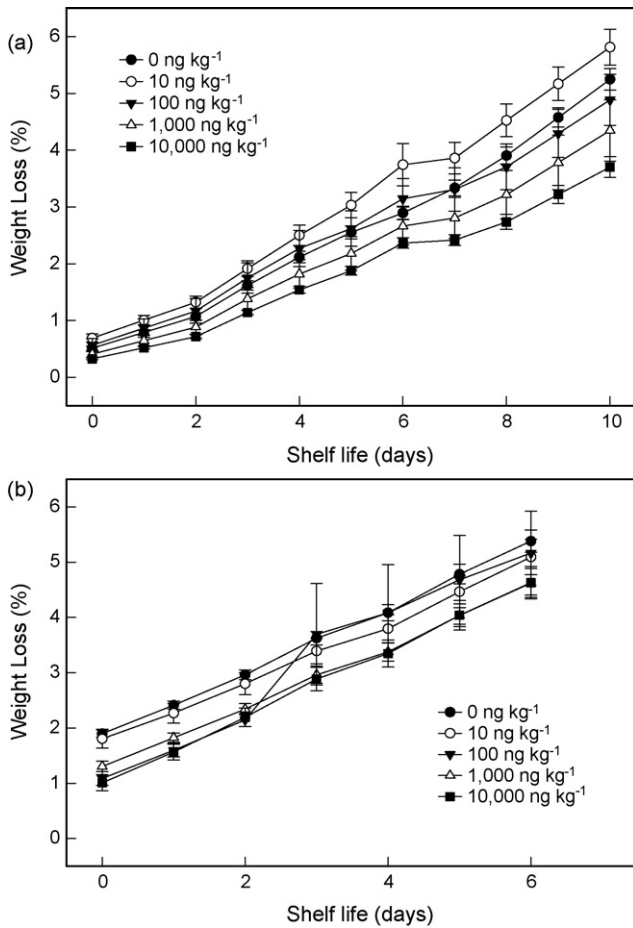


Fig. 1. Weight loss (percent of initial fresh weight) of 'Joanna Red' plums, (a) without cold storage and (b) with 10d cold storage (5 °C), during ripening at 23 °C. The lines represent the standard error of mean (±S.E., n=5).

values are well accepted by plum consumers (Crisosto et al., 2004).

Fruit weight loss increased over time, but the rate was treatment dependent only for fruit ripened without being cold stored (Fig. 1a). By 11 d on fruit immediately ripened, 1-MCP-treated fruit reached between 2 and 4% weight loss, while untreated fruit had ~6%. A comparative analysis (*t*-test) of the regression equation slopes indicated a significantly ($P < 0.05$) lower weight loss on fruit treated with 100, 1000 and 10,000 ng kg⁻¹ 1-MCP than untreated and 10 ng kg⁻¹ 1-MCP treated fruit. This data suggests that 1-MCP applied at concentrations higher than 100 ng kg⁻¹ could have modified epicuticular wax metabolism during ripening as the cuticle acts as a barrier to vapor movement from inside the cuticle to the environment or could simply delay changes in epicuticular waxes that occur during senescence thereby reducing water loss. Our results on fruit ripening without cold storage agreed with some previous reports that 1-MCP applied in enclosed areas reduced weight losses in plum fruit (Martínez-Romero et al., 2003; Valero et al., 2003). On fruit previously stored at 5 °C for 10 d, fruit weight loss increased over time but it was not affected by the 1-MCP treatments. On these cold stored fruit, weight loss measured immediately after cold storage was significantly different between treatments based on

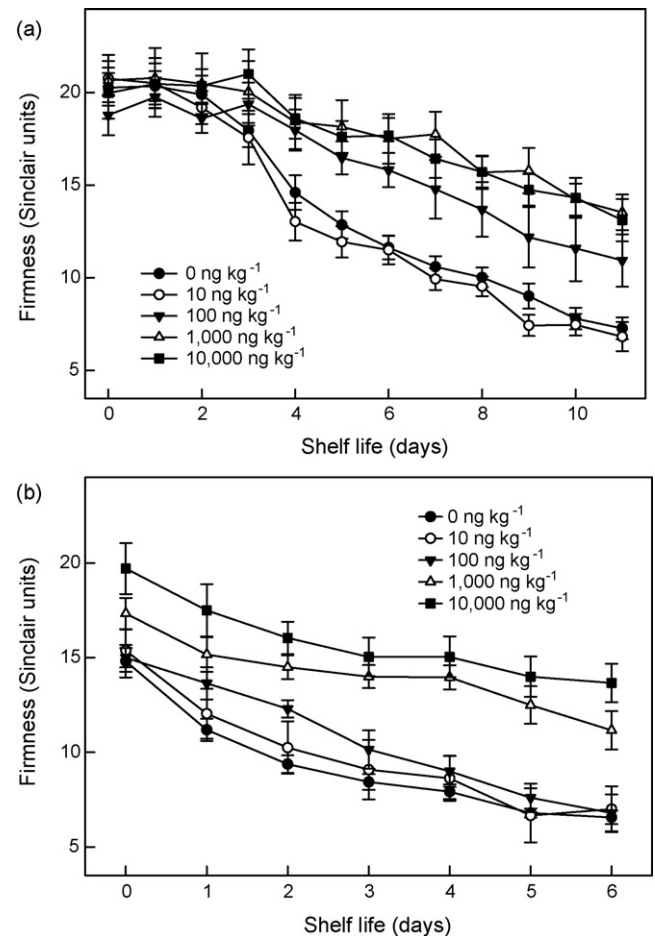


Fig. 2. Sinclair values for 'Joanna Red' plums, (a) without cold storage and (b) with 10d cold storage (5 °C), during ripening at 23 °C. The lines represent the standard error of mean (±S.E., n=5).

their interception point on the regression equation. Fruit treated with 100, 1000 and 10,000 ng kg⁻¹ 1-MCP had lower weight loss than fruit from the other treatments, however the rate of weight loss during the 7 d ripening period was the same (Fig. 1b). The effect of cold storage on weight loss response may explain the different published results about 1-MCP and weight loss (reviewed in Blankenship and Dole, 2003; Watkins, 2006). In our test, despite the two- to threefold difference in weight loss between untreated and 1-MCP-treated fruit, we did not observe any fruit shriveling symptoms within this ripening period. Shriveling symptoms would probably have been visible earlier on untreated fruit than on 1-MCP-treated if fruit had been kept for longer periods or under more adverse environmental conditions. In peaches and nectarines, shriveling occurs when fruit weight loss exceeded 6%; thus, as plums are less susceptible to weight loss these 4–6% losses should not be affecting the cosmetic appearance of the fruit.

Nondestructive fruit firmness measured on fruit with and without cold storage decreased over time during ripening in all of the treatments, but its rate was treatment dependent (Fig. 2a and b). On fruit immediately ripened, regression analysis using time [days (d)] as a predictor of fruit firmness (N) showed that slopes for 100, 1000 and 10,000 ng kg⁻¹ 1-MCP were significantly

different ($P < 0.05$) than fruit from untreated and 10 ng kg^{-1} 1-MCP. Only fruit treated with 1000 and $10,000 \text{ ng kg}^{-1}$ 1-MCP were significantly different ($P < 0.05$) from other fruit in the group that had been cold stored. Similarly, destructive measurements of fruit firmness, measured twice during the ripening period, indicated that fruit treated with 1000 and $10,000 \text{ ng kg}^{-1}$ 1-MCP were the firmest, fruit treated with 100 ng kg^{-1} 1-MCP had an intermediate firmness and, untreated and 10 ng kg^{-1} 1-MCP-treated fruit were the softest. For example, by 5 d (immediately ripened group) untreated plums and 10 ng kg^{-1} treated fruit had firmness values of $\sim 4 \text{ N}$, corresponding to fruit that was too soft to handle and even too soft to consume (Crisosto et al., 2004) while fruit treated with 100, 1000 and $10,000 \text{ ng kg}^{-1}$ 1-MCP were firm enough ($\sim 30 \text{ N}$) to tolerate postharvest handling (Table 2). By 11 d on fruit without cold storage, all of the 1-MCP-treated fruit were able to soften to 3–23 N. Fruit treated with 100 ng kg^{-1} were soft ($\sim 3 \text{ N}$) and fruit from the 1000 and $10,000 \text{ ng kg}^{-1}$ 1-MCP treatments had average firmness values of $\sim 20 \text{ N}$ which is considered in the high range of ideal for consumption for most Californian cultivars (Crisosto et al., 2004). Cold stored plums gave fruit of acceptable flesh firmness up to 5 (treated with 100 ng kg^{-1} 1-MCP) and 7 d (treated with 1000 and $10,000 \text{ ng kg}^{-1}$ 1-MCP) shelf life.

Table 2

Flesh firmness of ‘Joanna Red’ plums, without and with 10 d cold storage (5°C), during ripening for 5 and 11 and 5 and 7 d at 23°C , respectively

1-MCP (ng kg^{-1})	Flesh firmness (N)			
	Without cold storage		With cold storage	
	5 d	11 d	5 d	7 d
0	3.6 c ^a	1.9 c	2.6 c	2.4 b
10	3.7 c	1.9 c	2.6 c	2.1 b
100	27.3 b	3.2 c	11.9 b	2.8 b
1000	29.6 a, b	20.0 b	29.5 a	18.5 a
10,000	31.9 a	23.4 a	29.6 a	18.6 a

^a Values within columns within each attribute followed by the same letter are not significantly different at $P < 0.05$ (Duncan’s multiple range test).

Based on our destructive and the nondestructive firmness data, we calculated a 5–7 d longer market life for cold stored and immediately ripened fruit treated with 100 ng kg^{-1} 1-MCP, and even longer for fruit treated with 1000 and $10,000 \text{ ng kg}^{-1}$. It is well documented that 1-MCP applied in enclosed areas retarded plum softening (Menniti et al., 2006) and significantly extended shelf life of plum (Valero et al., 2003). Our data demonstrated that the shelf life for ‘Joanna Red’ plum was extended when

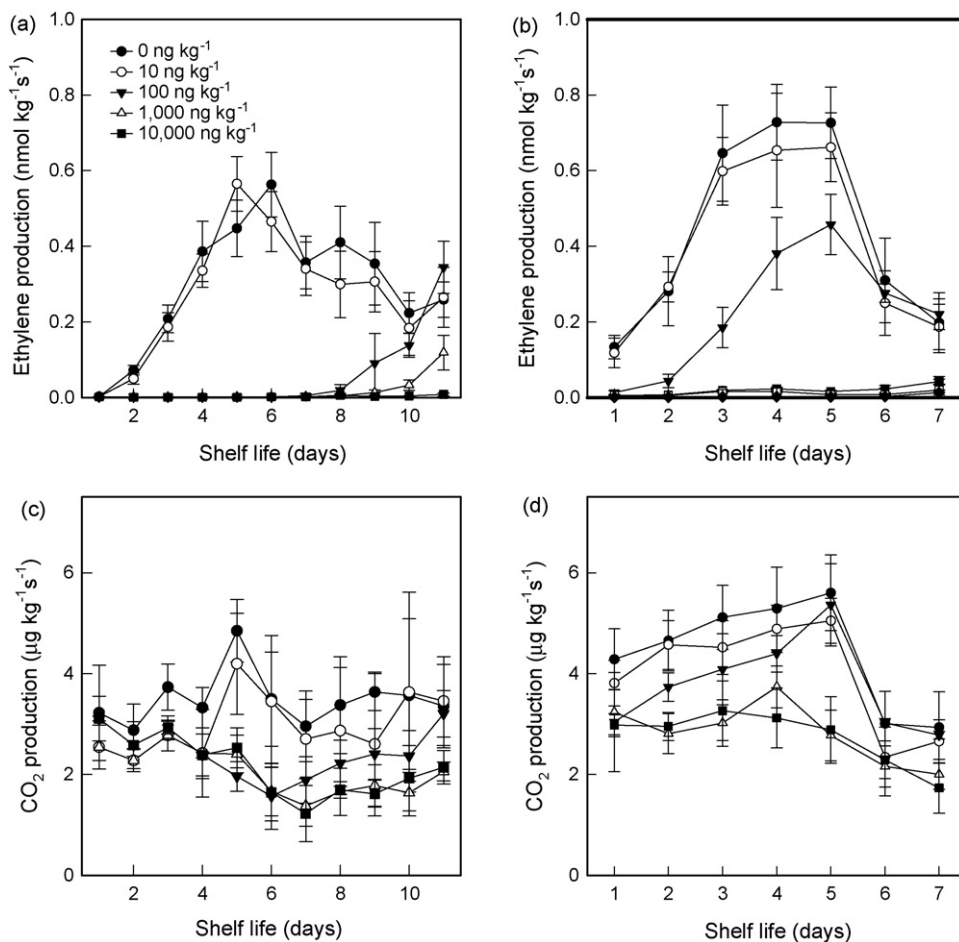


Fig. 3. Ethylene production ($\text{nmol kg}^{-1} \text{ s}^{-1}$) (a, b) and respiration rate (evolution of CO_2 in $\mu\text{g kg}^{-1} \text{ s}^{-1}$) (c, d) of ‘Joanna Red’ plums during ripening at room temperature (23°C). Ethylene production (a) during ripening without cold storage, (b) during ripening after 10 d cold storage (5°C). Respiration rate, (c) during ripening without cold storage and (d) during ripening after 10 d cold storage (5°C). The lines represent the standard deviation of mean ($\pm \text{S.D.}$, $n = 5$).

treated with this new 1-MCP formulation and ripened properly with and without cold storage period; therefore it can be used as an effective tool for extension of shelf life in plums harvested at advanced maturity stage.

Ethylene production of untreated and 10 ng kg⁻¹ 1-MCP-treated fruit increased during ripening, reaching a maximum after 5 d for fruit without cold storage (Fig. 3a), but peaked about 2 d earlier on fruit previously cold stored for 10 d at 5 °C (Fig. 3b). Ethylene production was very low on fruit treated with 100, 1000, 10,000 ng kg⁻¹ 1-MCP during ripening. Only for 100 ng kg⁻¹ 1-MCP-treated fruit previously cold stored, ethylene started to increase after 8 d and produced similar amounts of ethylene as untreated and 10 ng kg⁻¹ 1-MCP-treated fruit by day 11. These results suggest that 1-MCP significantly suppressed ethylene production in plums in a dose-dependent manner, reaching the saturation point at 1000 ng kg⁻¹ concentration. On fruit ripened after the cold storage period, the 100 ng kg⁻¹ 1-MCP-treated fruit had an ethylene climacteric rise at the same time with control and 10 ng kg⁻¹ 1-MCP-treated fruit (day 5). This treatment suppressed the ethylene production, without, however, delaying the onset of the climacteric rise. Baseline levels of ethylene production throughout the 7-d ripening period were recorded when 1000 and 10,000 ng kg⁻¹ 1-MCP concentrations were applied. It has been demonstrated that 1-MCP applications delay rather than totally inhibit ethylene production in a range of fleshy fruit, including plum (Dong et al., 2002; Valero et al., 2003), while Martínez-Romero et al. (2003) reported that 1-MCP applications completely inhibit ethylene production in other low ethylene producing plum cultivars. Our data indicates that application by immersion in this 1-MCP formulation both delayed and reduced ethylene production in 'Joanna Red' plums. In cold stored fruit, only 100 ng kg⁻¹ 1-MCP-treated fruit led to a reduction of ethylene production without affecting the onset of climacteric rise. Furthermore, there was even very low ethylene production of fruit treated with 1000 and 10,000 ng kg⁻¹ 1-MCP, but these low ethylene levels did not stop the softening process (Fig. 3a and b and Table 2).

The CO₂ production rate was less affected by 1-MCP immersion treatments compared with ethylene production. A decrease in CO₂ production rate after immersion in fruit treated with 100, 1000 and 10,000 ng kg⁻¹ 1-MCP compared with control fruit was detected (Fig. 3c and d). Differences were more intense after 5 d shelf life, where a reduction in CO₂ production up to 60% was observed. The 100 ng kg⁻¹ 1-MCP-treated fruit had similar CO₂ production rates to the controls after 11 d shelf life, when loss of firmness was evident. Fruit previously cold stored and treated with 10 and 100 ng kg⁻¹ 1-MCP exhibited CO₂ production rates similar to untreated fruit while fruit treated with 1000 and 10,000 ng kg⁻¹ showed low CO₂ production rates. A decrease in respiration rate in 1-MCP-treated plums has also been reported by other researchers (Dong et al., 2002; Martínez-Romero et al., 2003). Our results reinforce the hypothesis that respiration rate is affected by 1-MCP in a dose dependent manner, like Valero et al. (2003) reported with the application of 1-MCP in enclosed areas.

After the 10 d cold storage at 5 °C followed by 7 d ripening at 23 °C, ripe fruit from all of the treatments did not show any

cold storage related CI symptoms. This agreed with our previous storage evaluation that this cultivar is highly resistant to any CI symptoms such as gel breakdown, flesh browning, mealliness or translucency. This observation also confirms our recommendation for this cultivar as a good candidate for delayed harvest to assure high flavor quality combined with 1-MCP treatment to reduce softening rate during postharvest handling. In cultivars with low susceptibility to chilling injury (Crisosto et al., 1999), postharvest life is limited by their rate of softening rather than CI symptoms expression.

4. Conclusions

Concentrations of 100–1000 ng kg⁻¹ 1-MCP were sufficient for extending the shelf life of advanced maturity 'Joanna Red' up to 5 d, whereas 1000–10,000 ng kg⁻¹ 1-MCP may extend it even longer including cold storage without inducing CI symptoms. Delayed harvest (tree ripened) combined with immersion in 1-MCP is suggested as an excellent technique to assure tasty fruit and extend plum shelf life. As cold storage period did not affect 1-MCP performance, this 1-MCP immersion formulation treatment in addition to delayed harvesting can be applied on low CI susceptible cultivars for domestic and overseas markets. To the best of our knowledge, this is the first study reporting the successful application of this new 1-MCP immersion formulation on fleshy fruit. Future experiments should be conducted on a range of fleshy fruits in order to investigate the possibility of commercializing this formulation.

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