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Fruit temperature and ethylene modulate 1-MCP response in 'Bartlett' pears

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ABSTRACT

1-Methylcyclopropene (1-MCP) has been shown to protect 'Bartlett' pears against temperature stress during postharvest handling, and control or reduce incidence of scald and internal breakdown after cold storage. We investigated several factors that can influence pear fruit response to 1-MCP, including temperature during 1-MCP treatment and during storage after 1-MCP application and exposure to ethylene during the 1-MCP treatment to determine factors that might lead to variability in ripening response observed in 1-MCP-treated 'Bartlett' pears. The effect of 1-MCP was significantly reduced when fruit were stored after 1-MCP treatment for 45 d at intermediate temperatures (10 or 5 compared with 0 °C). Ripening of fruit treated with 1-MCP at 0.3 μL/L for 12 versus 24 h at 20 °C was not significantly different, suggesting the 1-MCP response was saturated by a 12 h exposure at 20 °C. However, at 0 °C, treatment with 0.3 μL/L 1-MCP for 24 h was more effective in inhibiting ripening than a 12 h exposure, and 1-MCP treatment for 24 h at 0 °C was less effective than 24 h at 20 °C, suggesting the response takes longer to saturate at lower temperatures. The presence of as little as 0.3 μL/L ethylene during 1-MCP application significantly reduced the efficacy of 1-MCP in ripening inhibition. Ethylene concentration and fruit temperature during 1-MCP application, along with fruit temperature during storage after 1-MCP treatment appear to be important factors regulating 1-MCP efficacy in 'Bartlett pears.

1. Introduction

1-Methylcyclopropene (1-MCP) is an ethylene action inhibitor that delays ripening of many fruit, including European pears (Sisler and Blankenship, 1996; Bai et al., 2006; Calvo and Sozzi, 2009). 1-MCP is registered as SmartFresh^{SM} for postharvest gas application in sealed rooms or tents. The use of 1-MCP for 'Bartlett' pears has been investigated for several years to improve post-storage quality and allow 'Bartlett' pears to be shipped to distant markets (Calvo and Sozzi, 2004, 2009; Calvo, 2004; Ekman et al., 2004; Bai et al., 2006; Villalobos-Acuña and Mitcham, 2008; Villalobos-Acuña et al., 2011).

While results have been promising, a continuing challenge is the balance between storage benefits and eventual ripening of the fruit for marketing (Villalobos-Acuña et al., 2011). Our focus in recent years has been to reduce the fruit's response to 1-MCP through modifications in fruit handling at harvest prior to application. However, some commercial trials of 1-MCP conducted in California during 2008 obtained quite variable fruit response, and in many cases no response was seen or a much higher concentration of 1-MCP was required to see an effect. The reason for these treatment failures was not at all clear, nor for the widely variable results between the laboratory and the field trials. The rate of fruit cooling and the fruit pulp temperature at the time of 1-MCP application could be a factor, as has been shown in other horticultural commodities (Serek et al., 1995; Ku and Wills, 1999; Mir et al., 2001; DeEll et al., 2002; Jiang et al., 2002; Blankenship and Dole, 2003; Liguori et al., 2004).

Recent studies in our laboratory indicate that exposure of fruit to ethylene prior to 1-MCP application results in a moderated effect on fruit ripening, with delayed ripening at harvest, but full ripening after a period of cold storage. Ethylene exposure prior to or during 1-MCP treatment has been shown to induce competition with 1-MCP for binding to the ethylene receptors, reducing the ability of 1-MCP to fully control or reduce ethylene responses (Sisler et al., 2003; Reid and Çelikel, 2008; Apelbaum et al., 2008; Zhang et al., 2009, 2010). Ethylene concentration during 1-MCP treatment in the laboratory or commercial environment in a tent or sealed room can vary from 0.015 to 20 μL/L depending on the fruit maturity stage, amount of fruit per volume in the tent or room, and the time fruit have been stored at intermediate or low temperatures prior to treatment (Villalobos-Acuña, unpublished data). Our objectives in this study were to characterize the influence of pre- and post-treatment temperature exposures and ethylene exposure during 1-MCP treatment on Bartlett pear response to 1-MCP.

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Table 1
Growing region, harvest date, maturity and fruit size of 'Bartlett' pears used in each experiment. All the fruit were packed in cardboard boxes and transported to the Postharvest Pilot Plant in a cargo van the same day of packing.

Experiment	Harvest date	Growing region	Maturity (*N*)	Fruit size
Temperature conditioning prior to 1-MCP	July 13, 2009	Sacramento, CA	82	110
Fruit and room temperature and exposure time during 1-MCP treatment	August 10, 2009	Mendocino, CA	75	110
Fruit temperature after 1-MCP treatment	August 4, 2009	Mendocino, CA	73	110
Ethylene competition during 1-MCP treatment	August 10, 2009	Mendocino, CA	75	110

2. Materials and methods

2.1. Temperature conditioning prior to 1-MCP

Early-season 'Bartlett' pears (*Pyrus communis*) were harvested according to the specifications in Table 1, and packed the following day. Fruit were randomized and divided into groups to be held for 1, 3, 5, or 7 d at 0, 5, 10, 15 or 20 °C. Immediately after each time and temperature combination, half of the fruit from each temperature condition were treated in cardboard boxes with 0.3 μL/L 1-MCP in a 4000 L plastic tent at 0 °C for 12 h while the other half were held as an untreated control. Fruit condition was evaluated during ripening at 20 °C after harvest and after 45 d storage at 0 °C.

2.2. Fruit and room temperature and exposure time during 1-MCP treatment

Mid-season 'Bartlett' pears were harvested according to the specifications in Table 1, and packed the same day. Fruit were randomized and divided into two groups to be held at either 20 or 0 °C. The following day after all the fruit equilibrated to 20 or 0 °C, half the fruit for each temperature were treated in cardboard boxes with 0.3 μL/L 1-MCP in a 4,000 L plastic tent at the fruit temperature, 20 or 0 °C, for 12 or 24 h, respectively, while the other half of the fruit were held as an untreated control. Untreated fruit kept at 20 and 0 °C had very similar ripening behavior at harvest and after storage; therefore, fruit at 20 °C was selected to represent both controls. Fruit condition was evaluated during ripening at 20 °C immediately after harvest and after 45 d storage at 0 °C. Fruit from all treatments ripened immediately after harvest were warmed to 20 °C and then treated with 100 μL/L ethylene for 24 h to induce uniform ripening capacity at the start of ripening.

2.3. Fruit temperature after 1-MCP treatment

Mid-season 'Bartlett' pears were harvested according to the specifications in Table 1, and packed the same day in Finley, CA. Fruit were randomized, stored for 2 d at 0 °C to equilibrate to the same temperature, and half of the fruit were treated in cardboard boxes with 0.3 μL/L 1-MCP in a 4,000 L plastic tent at 0 °C for 24 h while the other half were held at 0 °C as an untreated control. Fruit were then either warmed to 20 °C and immediately ripened after harvest with 100 μL/L ethylene for 24 h followed by exposure to air at 20 °C or stored for 45 d at 0, 5 or 10 °C prior to ripening in air at 20 °C. Fruit condition was evaluated during ripening at 20 °C immediately after harvest, or every other week during 45 d of cold storage and then during ripening at 20 °C when 45 d storage were completed.

2.4. Ethylene exposure during 1-MCP treatment

Mid-season 'Bartlett' pears were harvested in Finley, CA according to the specifications in Table 1 and packed the same day. After randomization, the fruit were placed at 0 °C for 3 d. Two thirds of the fruit were treated in cardboard boxes with 0.3 μL/L 1-MCP for 24 h at 0 °C in two separate 4000 L plastic tents having either ethylene produced by the fruit only (0.06 μL/L at the end of the treatment) or ethylene artificially injected in the tent using 100% ethylene gas (0.28 μL/L average ethylene concentration throughout the treatment) while the remaining third of the fruit were held as an untreated control sample. Subsequently, fruit were either ripened with ethylene as described in Section 2.2 or stored 45 d at 0 °C prior to ripening at 20 °C and their condition evaluated.

2.5. Experimental designs and fruit evaluations

The experimental design was completely randomized with 3 or 4 replications by treatment (3 replications for the temperature conditioning and 4 replications for the other experiments). Data were analyzed using SAS statistical software (version 9.1, SAS Institute Inc., USA). Means were compared using LSD to calculate the least significant differences for each data set ($\alpha = 0.05$). Power, log or arcsine transformations were used to fulfill ANOVA assumptions of ethylene production, firmness or color data.

Six to eight fruit per replication were assessed for color and firmness at each evaluation time. In addition, six fruit per replication were used to determine ethylene and CO_2 production (respiration rate) daily or every other day during the ripening period at 20 °C. Firmness was measured objectively using a Gűss FTA Penetrometer (Gűss, Strand, Western Cape, South Africa) fitted with an 8-mm probe. CO_2 and ethylene production rates were measured by placing the six fruit from each treatment and replication into a 3.8 L jar and sealing it for 10–90 min, depending on the rate of gas production. The headspace gas was evaluated for the concentration of CO_2 using rapid gas analysis (VIA510, Horiba, Fukuoka, Japan). Ethylene was evaluated using flame ionization gas chromatography (Model 211 Series S, Hach-Carle Co., Fullerton, CA) utilizing two columns (1.22 m and 0.305 m, 8% NaCl on Alumina F-1 80/100 DV) (Chandler Engineering – Carle Chromatography, Tulsa, Oklahoma). Nitrogen was used as the carrier gas at a flow rate 30 mL/min, and the injector port, detector port and oven temperatures were 80 °C. A 10 mL headspace sample was injected into a 2 mL fixed sample volume valve. Color was measured using the Minolta CR 300 Colorimeter (Osaka, Japan). Internal browning and scald (including storage scald and senescent scald) severity were evaluated subjectively using the following scale: 0 = none; 1 = slight; 2 = moderate; 3 = severe.

3. Results

3.1. Temperature conditioning prior to 1-MCP

After storage, 1 d conditioned, untreated-fruit ripened normally after 6 d at 20 °C while the 1-MCP-treated fruit had lower ethylene production, took additional days to soften and presented higher hue angles (less yellow skin), reflecting slower ripening rates (Fig. 1). The relationship of flesh temperature during 1-MCP treatment and treatment efficacy was only analyzed after storage because of the differences between treatments in the rate of warming to 20 °C during ripening at harvest. After storage, there was significantly lower firmness after 6 d at 20 °C in the 1-MCP treated

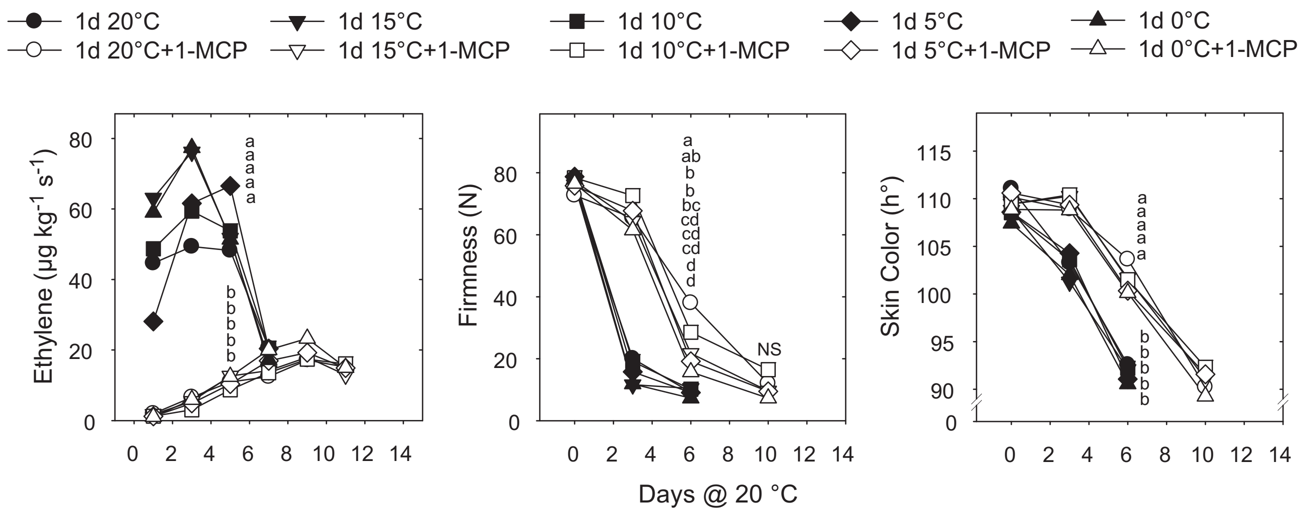

Fig. 1. Ethylene production ($\mu g\,kg^{-1}\,s^{-1}$), firmness (N) and color (hue angle) during ripening after 45 d storage at 0 °C of fruit temperature-conditioned for 1 d at 20, 15, 10, 5, and 0 °C then treated with 0.3 μL/L 1-MCP or maintained as untreated control. Different letters within each evaluation represent means with statistically significant differences using LSD ($\alpha \leq 0.05$). Ethylene concentration in the treatment tent was 40 and 100 nL/L at the beginning and end of the 1-MCP treatment, respectively.

fruit that were held at colder temperatures during conditioning at harvest. This firmness difference was only observed in the 1 d conditioned fruit, and such a difference was not clearly detected in other ripening parameters such as ethylene production or skin color (Fig. 1).

1-MCP-treated fruit that had been conditioned for 3 d showed minor or no differences in ripening relative to their untreated counterpart. Distinct ripening behavior among treatments observed at harvest was likely due to a combination of distinct warming rates after the exposure to conditioning temperatures and some ripening delay provided by 1-MCP (Fig. 2A, C and E). After storage, however, conditioned 1-MCP treated fruit showed no differences in firmness or external color compared with the untreated counterpart after 3 d of ripening (Fig. 2D and F). This minor to absent effect provided by 1-MCP was reproduced in fruit conditioned for 5 and 7 d (data not shown). Ethylene concentrations in the tent during 1-MCP treatments were 1.6, 5.8, and 12 μL/L at the end of the 1-MCP treatments of fruit previously conditioned for 3, 5, and 7 d, respectively, compared with 0.10 μL/L ethylene at the end of the 1-MCP treatment of the fruit conditioned 1 d.

3.2. Fruit and room temperature and exposure time during 1-MCP treatment

Regardless of the fruit temperature or exposure time during 1-MCP treatment, 1-MCP treated fruit had less ethylene production, softening, and yellow color development at harvest and after 45 d cold storage relative to the untreated fruit. Fruit treated with 1-MCP at 0 °C had a significantly faster induction of ripening parameters than the fruit treated with 1-MCP at 20 °C, including ethylene production, softening and yellow skin color development (Fig. 3). The magnitude of ripening delay provided by 1-MCP was also dependent on the duration of the treatment when the fruit were treated at 0 °C, with a significantly stronger effect from the 24 h treatment compared with the 12 h treatment; however, there was no difference between 12 and 24 h when fruit were treated at 20 °C (Fig. 3).

3.3. Fruit temperature after 1-MCP treatment

When 'Bartlett' pears were stored at 0, 5, or 10 °C for 45 d after harvest, all fruit treated with 1-MCP before storage exhibited reduced ethylene production, softening, and yellow color development relative to the untreated fruit. Throughout storage, 1-MCP treated fruit held at 0 °C after treatment had very low ethylene production and only slight changes in firmness and color (Fig. 4). 1-MCP treated fruit held at 5 °C after treatment produced similarly low amounts of ethylene as the untreated fruit held at 0 °C, but slightly higher than the 1-MCP treated fruit held at 0 °C (Fig. 4A). Firmness of 1-MCP treated fruit held at 5 °C after treatment remained high, similar to both untreated and 1-MCP treated fruit stored at 0 °C, but development of yellow color was more pronounced in 1-MCP treated fruit held at 5 °C (Fig. 4B). Untreated fruit held at 10 °C ripened within 14 d at 10 °C, while 1-MCP treated fruit held at 10 °C were fully ripe after approximately 37 d, and were unmarketable by the end of the 45 d cold storage period.

After harvest and 45 d storage at 0 or 5 °C, fruit were transferred to 20 °C for ripening (Fig. 5). 1-MCP treated fruit exhibited significantly lower ethylene production, and slower softening and yellow skin color development during ripening at harvest (Fig. 5A–C). 1-MCP treated fruit stored 45 d at 0 °C after 1-MCP treatment took approximately 20 d to ripen, while fruit stored at 5 °C after 1-MCP treatment softened after 6 d at 20 °C, similar to the untreated fruit stored at 0 °C, but with lower ethylene production (Fig. 5B, D and F).

3.4. Ethylene exposure during 1-MCP treatment

The degree of ripening inhibition provided by 1-MCP was dependent on the amount of ethylene in the tent during 1-MCP treatment. Adding 0.28 μL/L ethylene during the 1-MCP treatment reduced softening variability sometimes present during ripening of 1-MCP treated fruit, and significantly decreased the effect of 1-MCP on reduction of ethylene production, softening rates and skin color changes relative to the 1-MCP-treated fruit exposed only to ethylene produced by the fruit (0.06 μL/L) during treatment (Fig. 6).

4. Discussion

Recovery of ripening capacity in 1-MCP-treated California 'Bartlett' pears has been challenging under some conditions (Villalobos-Acuña et al., 2011). However, our results demonstrate that ripening after 1-MCP treatment can be modulated under a variety of conditions. Ethylene concentration and fruit temperature during 1-MCP treatment together with storage temperature after 1-MCP treatment appear to be important factors that regulate 1-MCP response.

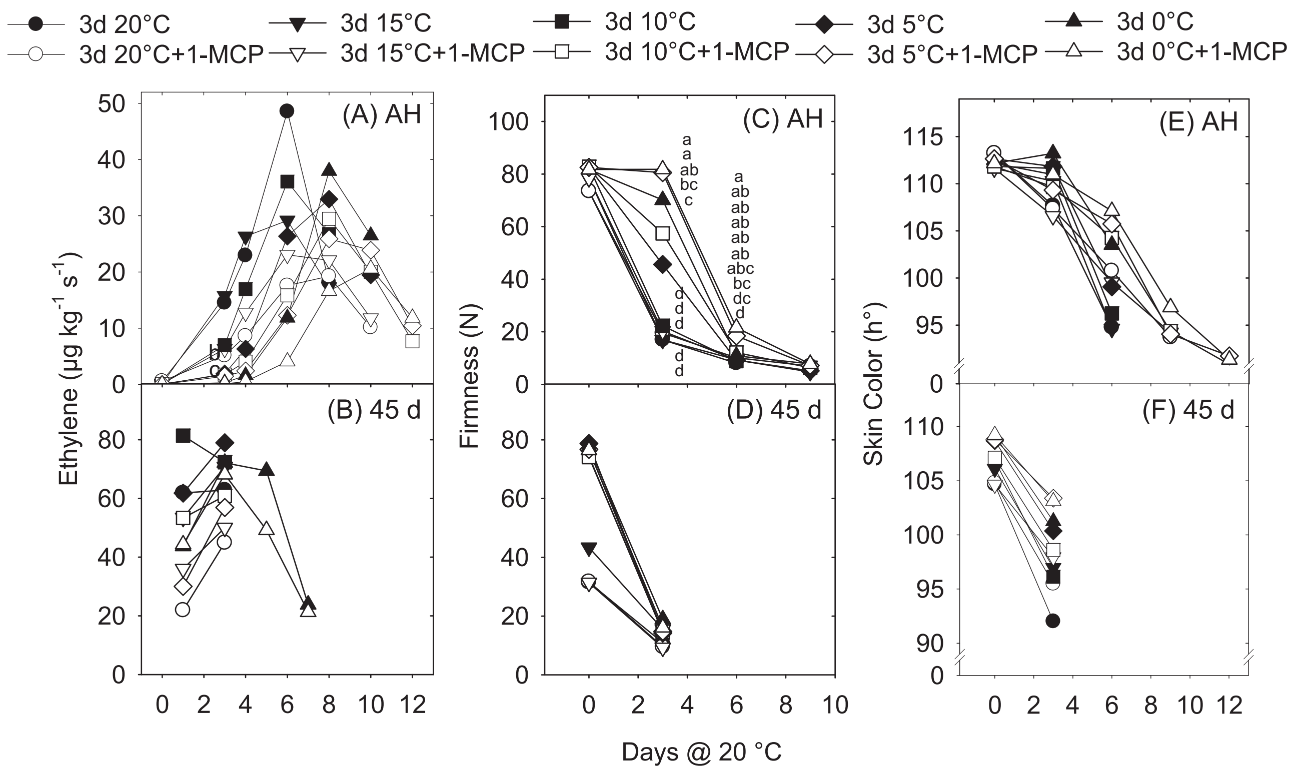

Fig. 2. Ethylene production ($\mu g\ kg^{-1}\ s^{-1}$), firmness (*N*) and color (hue angle) during ripening immediately after harvest (AH) and after 45 d storage at 0 °C of fruit temperature conditioned for 3 d at 20, 15, 10, 5, and 0 °C then treated with 0.3 μL/L 1-MCP or maintained as untreated control. Different letters within each evaluation represent means with statistically significant differences using LSD ($\alpha \leq 0.05$). Ethylene concentration in the treatment tent was 0.12 and 1.6 μL/L at the beginning and end of the 1-MCP treatment, respectively.

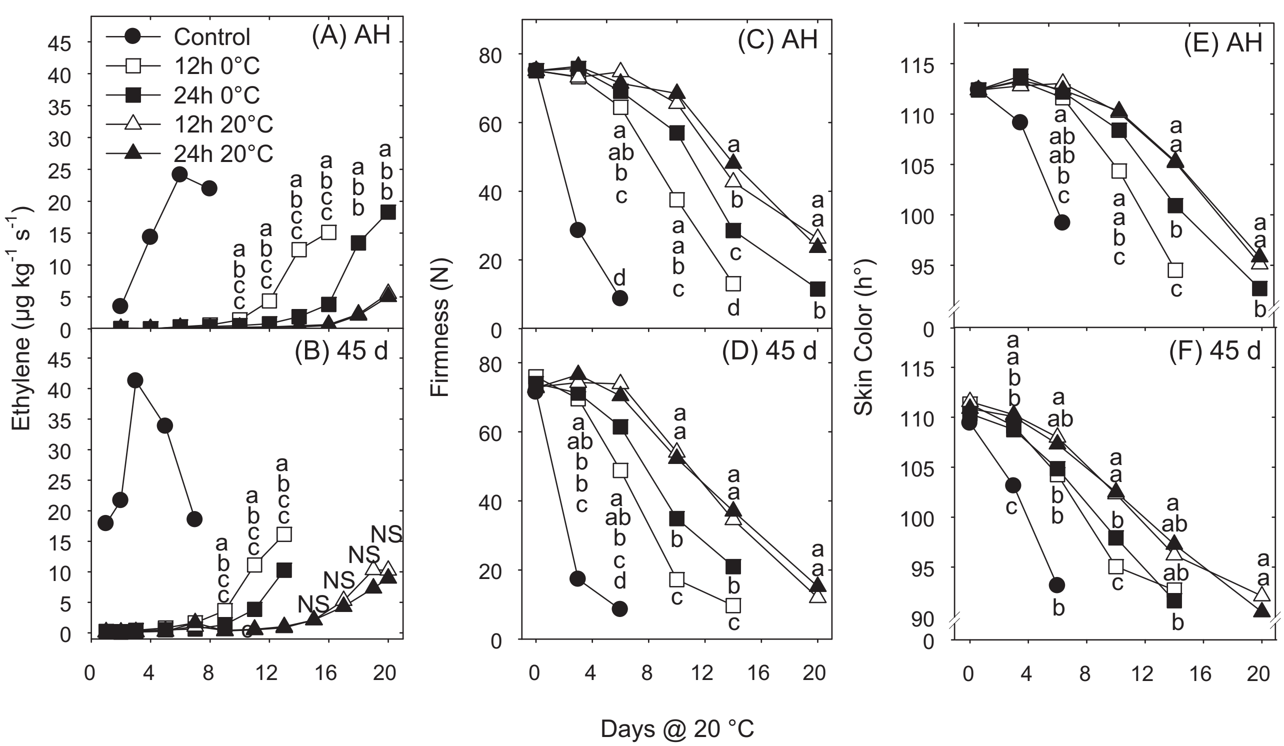

Fig. 3. Ethylene production ($\mu g\ kg^{-1}\ s^{-1}$), firmness (*N*) and color (hue angle) during ripening immediately after harvest (AH) and after 45 d storage at 0 °C of fruit treated with 0.3 μL/L 1-MCP 12 or 24 h at 0 or 20 °C. Different letters within each evaluation represent means with statistically significant differences using LSD ($\alpha \leq 0.05$). Ethylene concentration in the tent at the beginning and end of the 1-MCP treatment ranged from 60 to 51, 39 to 26, 9 to 23, and 13 to 16 nL/L for 12 h and 24 h at 0 °C and 12 h and 24 h at 20 °C, respectively.

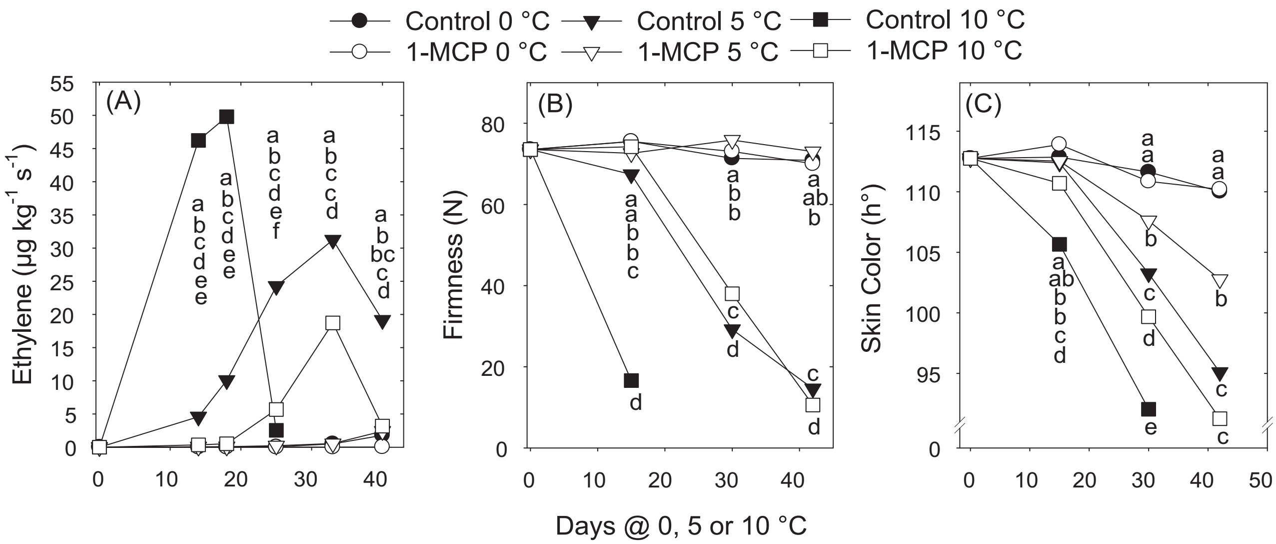

Fig. 4. Ethylene production ($\mu g\,kg^{-1}\,s^{-1}$), firmness (*N*) and color (hue angle) during 45 d storage at 0, 5, or 10 °C of untreated and 0.3 μL/L 1-MCP-treated fruit stored after treatment. Different letters within each evaluation represent means with statistically significant differences using LSD ($\alpha \leq 0.05$). Ethylene concentration was 12 and 44 nL/L in the tent at the beginning and end of the 1-MCP treatment, respectively.

Ethylene competition with 1-MCP for binding to the receptors has been shown to modulate 1-MCP responses in other plant tissues (Sisler et al., 2003; Reid and Çelikel, 2008; Apelbaum et al., 2008; Zhang et al., 2009, 2010). Zhang et al. (2009) showed that ethylene remains bound to the ethylene receptors for some hours after ethylene treatment in tomato fruit, and this can influence the ability of 1-MCP to bind to the ethylene receptors, changing the efficacy of the latter on ripening inhibition.

Ethylene produced by the fruit once ripening is initiated has been suggested as one reason for variations in 1-MCP response among cultivars, delays after harvest, and harvest dates in some climacteric fruit producing high levels of ethylene during ripening (Zhang et al., 2009, 2010). Our results agree with those obtained by Zhang et al. (2009, 2010) and show that as little as 0.3 μL/L in the treatment room during 1-MCP exposure considerably decreases its effect on 'Bartlett' pear ripening. Pears produce high levels of ethylene during ripening; therefore, monitoring ethylene concentration during 1-MCP treatment might provide useful information to predict 1-MCP responses. Changes in the relative amount of 1-MCP and ethylene might be used to modulate the response to fit commercial needs.

Storage temperature after 1-MCP treatment also had a significant effect on the longevity and strength of ripening inhibition. At higher temperatures, 1-MCP treated fruit recovered the capacity

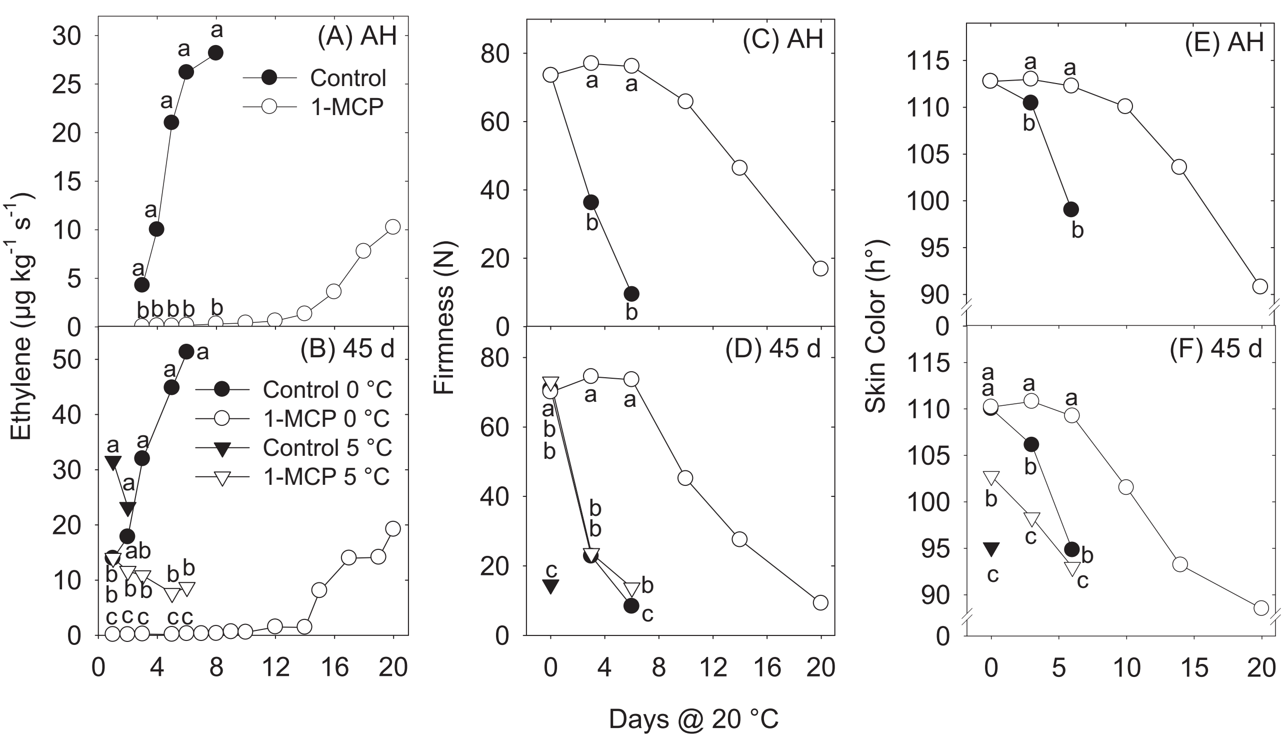

Fig. 5. Ethylene production ($\mu g\,kg^{-1}\,s^{-1}$), firmness (*N*) and color (hue angle) during ripening immediately after harvest (AH) and after 45 d storage of fruit untreated and treated with 0.3 μL/L 1-MCP and then stored at 0, 5 or 10 °C for 45 d. Different letters within each evaluation represent means with statistically significant differences using LSD ($\alpha \leq 0.05$). Fruit stored at 10 °C ripened before the end of storage and thus are not shown in this graph. Ethylene concentration was 12–44 nL/L in the tent after 1-MCP treatment.

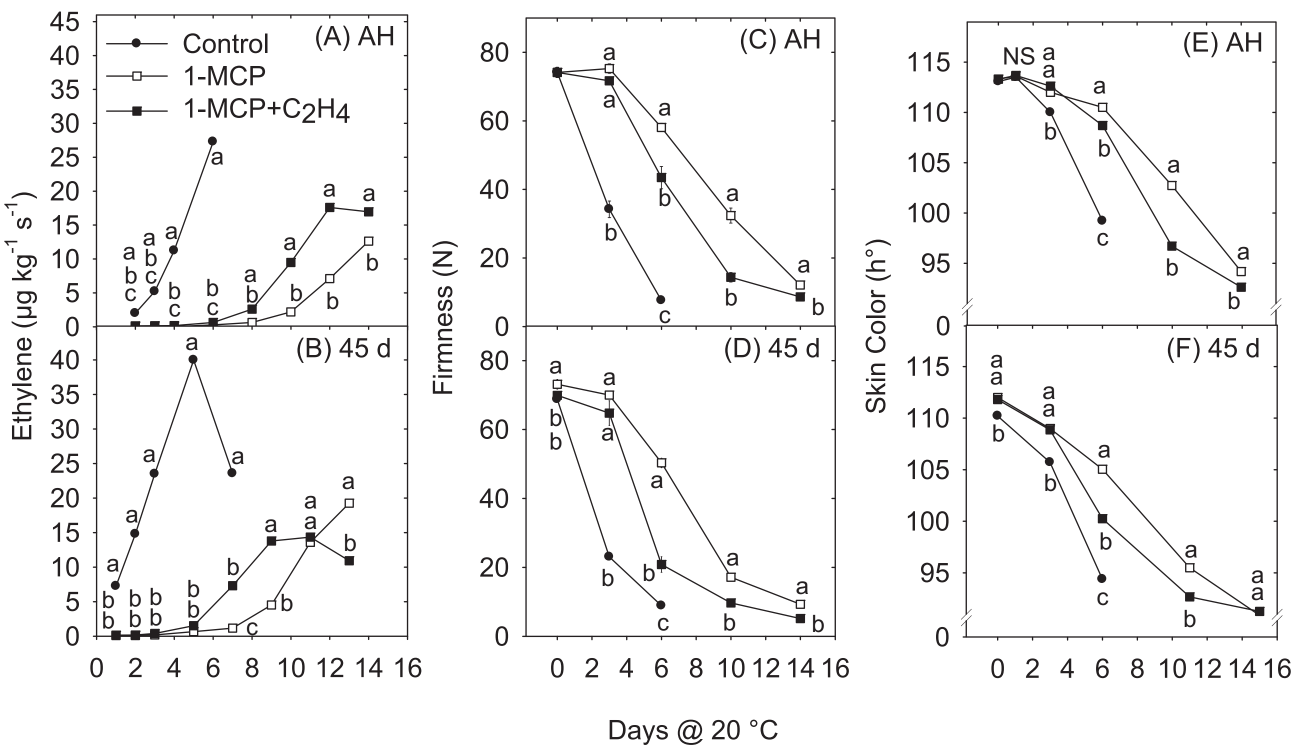

Fig. 6. Ethylene production ($\mu g\ kg^{-1}\ s^{-1}$), firmness (N) and color (hue angle) during ripening immediately after harvest (AH) and after 45 d cold storage of untreated and 0.3 μL/L 1-MCP-treated fruit. 1-MCP was applied with low ethylene competition (1-MCP; 58–81 nL/L ethylene at the beginning and end of the treatment) and high ethylene competition (1-MCP + C_2H_4; 280 nL/L ethylene throughout treatment). Different letters within each evaluation represent means with statistically significant differences using LSD ($\alpha \leq 0.05$).

for ripening more quickly. This result parallels previous findings by Bai et al. (2006) who tested different temperatures to induce ripening after storage at low temperatures. Further investigations are required to understand whether temperature favors the release of 1-MCP from the receptors or synthesis of new ethylene receptors or an interaction of these factors.

When 1-MCP treatments were applied at lower room and fruit temperatures, the treatments tended to be less effective to prevent ethylene responses in a variety of plants including apples, peaches and nectarines (Serek et al., 1995; Ku and Wills, 1999; Mir et al., 2001; DeEll et al., 2002; Jiang et al., 2002; Blankenship and Dole, 2003; Liguori et al., 2004). Our study found similar results with 'Bartlett' pears. The response to 1-MCP might be easier to regulate at 0 °C since more moderate effects on ripening inhibition were obtained at 0 °C in this study. Low temperatures may affect a variety of factors influencing the interaction of 1-MCP with the receptors. Enzymatic 1-MCP metabolism has been shown to be important in plant tissues and would be slowed at lower temperatures (Huber et al., 2010). Lower temperatures might decrease 1-MCP affinity or interaction with the ethylene receptors as suggested previously (Mir et al., 2001) and/or decrease the solubility of 1-MCP in the cytoplasm. Furthermore, 1-MCP has a boiling point of 4.7 °C (Pesticide Properties Database, 2010) and, therefore, its conversion from the cyclodextrin-1-MCP complex into a gaseous state available for movement through the plant tissue to interact with the ethylene receptors might be slowed when treatment is performed at 0 °C. In this experiment; however, there were slightly different ethylene concentrations in the plastic tent at the beginning and end of the 1-MCP treatments, ranging from 60 to 51, 39 to 26, 9 to 23, and 13 to 16 nL/L for 12 h and 24 h at 0 °C and 12 h and 24 h at 20 °C, respectively. It is unknown what effect this might have had on the results, but as shown above, ethylene competition decreases the 1-MCP effect. This fact could make it difficult to compare between 1-MCP treatments applied in different tents and at different temperatures.

Since 'Bartlett' pears have high ethylene production rates, ethylene competition is likely to be a major factor affecting 1-MCP sensitivity. Interestingly, important differences have been found among European pear varieties in 1-MCP sensitivity. For instance, 'd' Anjou' is much more sensitive to 1-MCP than 'Bartlett' (Bai et al., 2006). We have tested ethylene treatments of 100 μL/L for 4 and 8 h (data not shown) prior to 1-MCP treatment and have not observed important effects on the effectiveness of 1-MCP treatment as compared with fruit not treated with ethylene prior to 1-MCP application. This suggests that ethylene receptor degradation, triggered by ethylene binding to the receptors (Chen et al., 2007; Kevany et al., 2007, 2008), may not be an important factor for 1-MCP efficacy in 'Bartlett' pears, at least for 4 and 8 h exposures. Whether the amount of ethylene receptors plays a more important role in 1-MCP sensitivity among pear cultivars remains an area for future research and illustrates the complexity of ethylene perception in fruit and the variety of factors that can influence 1-MCP efficacy in 'Bartlett' pears.

5. Conclusions

Our results suggest that 1-MCP treated 'Bartlett' pear fruit can be stimulated to ripen effectively under a variety of conditions. In fact, fine tuning of ethylene and 1-MCP concentrations and temperature during treatment, together with fruit temperature during storage or transport to final markets after treatment is important to assure beneficial effects and eventual ripening. Treatments performed with low ethylene concentrations in the atmosphere, at warmer pulp temperatures (20 °C), and followed by fruit storage at low temperatures (0 °C) had strong ripening inhibition, while the presence of ethylene, cold fruit pulp temperatures during treatment and/or storage or transport at warmer temperatures (≥5 °C) after treatment reduced ripening inhibition. These various factors possibly interact to determine the final response of the fruit. Further research is required to determine the specific combination of these factors with a variety of fruit maturities and growing regions to consistently deliver pears with the capacity to ripen and with good flavor attributes.

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