

Endogenous and Exogenous Ethylene Modulates the Response of 'Bartlett' Pears to 1-Methylcyclopropene

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Keywords: ethylene, 1-methylcyclopropene, pears, postharvest, *Pyrus communis*, ripening

Abstract

The capacity of the ethylene binding inhibitor, 1-methylcyclopropene (1-MCP; SmartFresh™ technology) to delay ripening of European pears reportedly decreases with advancing fruit maturity. In the present study, the influence of endogenous and exogenous ethylene on the efficacy of 1-MCP to delay 'Bartlett' pear fruit ripening was determined. Physiologically mature-green fruit were harvested at an early-, mid-, and late-season maturity. They were treated with 0 or 600 nl L⁻¹ 1-MCP alone or in combination with 12, 30, 60 or 600 nl L⁻¹ ethylene for 24 hours at 0°C. The fruit were then exposed to 100 µl L⁻¹ ethylene for 24 hours at 20°C or stored at 1°C for 5 weeks. Treatment of early-season fruit with 600 nl L⁻¹ 1-MCP for 24 hours at 0°C delayed ethylene-mediated ripening by 9 days at 20°C. Mid- and late-season fruit were less responsive to this treatment and shelf life was only extended by 3 days. The reduction in treatment efficacy was associated with increased ethylene production and accumulation in treatment chambers by mid- and late-season fruit. Similarly, including exogenous ethylene in the treatment atmosphere modulated 1-MCP efficacy. For early-season fruit, the benefits of 1-MCP were maintained even when 12 and 30 nl L⁻¹ ethylene was initially added to chambers. Inclusion of 60 nl L⁻¹ ethylene in chambers; however, reduced 1-MCP efficacy. For mid- and late-season fruit, the addition of 12, 30 and 60 nl L⁻¹ ethylene did not reduce 1-MCP effects although the delay in ripening was modest even without added ethylene. 1-MCP benefits were completely negated when 600 nl L⁻¹ ethylene was included in the treatment atmosphere for all fruit maturity stages. 1-MCP responses were also slightly diminished after storage at 1°C for 5 weeks following treatment compared to the response at harvest. These findings highlight the competitive nature of 1-MCP and ethylene for fruit binding sites, and underscore the importance of monitoring ethylene concentrations in treatment atmospheres.

INTRODUCTION

European pears (*Pyrus communis*) ripen in association with a climacteric rise in ethylene biosynthesis. The capacity of pears to produce ethylene and ripen is related to their physiological maturity at harvest (Chen and Mellenthin, 1981). Pears picked early in the season produce very little ethylene at harvest and ripen slowly, while fruit harvested later in the season synthesize higher rates of ethylene and ripen rapidly. Exposure to exogenous ethylene can accelerate and coordinate uniform ripening of pear fruit regardless of the maturity (Agar et al., 1999).

Maintenance of European pears at low temperature (Mitchell, 1990) and under controlled atmospheres (Yoshida et al., 1986) can extend the storage life of fruit. Similarly, pre-treatment of climacteric fruit with the ethylene action inhibitor, 1-methylcyclopropene (1-MCP) gas can also delay ripening responses (Watkins, 2008). 1-MCP molecules bind to the ethylene protein receptors in fruit tissues and thereby prevent ethylene perception (Sisler et al., 1996). There are numerous reports in the literature describing the efficacy of 1-MCP to extend the shelf life of pears (e.g., Lelievre

et al., 1997; Argenta et al., 2003; Trincherro et al., 2004; Calvo and Sozzi, 2009; Gamrasni et al., 2010; Villalobos Acuna et al., 2011). 1-MCP preparations, including SmartFresh™, are now registered for use on fruit crops (Watkins, 2008).

The efficacy of 1-MCP to delay ethylene-mediated ripening of some climacteric fruits can be variable and depends in part on the physiological state of fruit. For example, 1-MCP treatment of avocado and banana is highly effective when applied to preclimacteric fruit, but is less efficacious when administered after ripening has commenced (Joyce et al., 1999; Adkins et al., 2005). The increase in endogenous ethylene concentrations within ripening fruit tissues presumably reduces the competitive ability of 1-MCP molecules to bind to available receptors (Zhang et al., 2009, 2010). Similarly, the presence of exogenous ethylene in the treatment atmosphere can moderate 1-MCP efficacy (Villalobos Acuna et al., 2011).

Given that treatment rooms can be contaminated with biologically active concentrations (0.01-0.18 $\mu\text{l L}^{-1}$) of ethylene (D. Holcroft, unpublished data), we determined the ethylene concentration that would reduce the efficacy of 1-MCP treatment to extend the postharvest life of 'Bartlett' pears. The 1-MCP response of fruit at different harvest maturities was also examined.

MATERIALS AND METHODS

Plant Material

Mature-green 'Bartlett' pear (*Pyrus communis*) fruit were obtained on the day of harvest from a packinghouse near Sacramento, California. Fruit were collected every 8-11 days during the harvest season to capture three (early, mid, late) stages of maturity (81, 79, 68 N flesh firmness, respectively). Fruit in cardboard boxes were transported to the laboratory in 1 hour.

1-MCP and Ethylene Co-Treatments

Fruit of uniform quality were randomly assigned to treatments. They were repacked into cardboard boxes and held at 0°C for 16 hours to equilibrate to treatment temperature. Fruit were enclosed into 300 L stainless steel chambers at a fill ratio (50 kg fruit per 300 L volume) consistent with a marine container fully loaded with palletized fruit. Fruit were treated with 0 or 600 nl L^{-1} 1-MCP (as SmartFresh™; AgroFresh, Inc.) alone or in combination with 12, 30, 60 or 600 nl L^{-1} ethylene for 24 hours at 0°C on the day after harvest (Table 1). Half of the fruit were then exposed to 100 $\mu\text{l L}^{-1}$ ethylene for 24 hours at 20°C. Remaining fruit were stored at 1°C for 5 weeks to simulate a marine shipment from California to South America. After the ethylene or storage treatment, fruit were held at 20°C for ripening and shelf life evaluation.

Assessments

The internal ethylene concentration was determined for ten fruit from each harvest after cooling to 0°C. Unbound ethylene was extracted from internal fruit tissues with vacuum. Ethylene concentrations were quantified using a Carle model 211 gas chromatograph (GC) (Carle Instruments, Inc., Anaheim, CA, USA) fitted with a flame ionization detector. Ethylene concentrations inside chambers at the beginning and end of each 1-MCP treatment were measured using the same GC. We also measured rates of ethylene production and respiration by fruit at harvest and during shelf life using the Carle GC and a Horiba VIA-510 gas analyzer (Horiba Instruments Co., Irvine, CA, USA), respectively. The flesh firmness and skin color of 24 fruit in each treatment were evaluated at harvest and every 3 days during ripening at 20°C. Flesh firmness was measured on the peeled surface on opposite sides of each fruit using a Güss FTA GS-14 penetrometer (Güss Manufacturing Ltd, Strand, South Africa) fitted with an 8 mm probe. Skin color was determined using a Minolta CR-300 Chroma Meter (Minolta Ltd., Osaka, Japan).

Experiment Design

Fruit were arranged in a randomized complete block design. Four replicate boxes containing fruit were used for each treatment. Six fruit were removed at random from every box at each sampling time for evaluation of firmness and color. A separate set of fruit were maintained for ethylene production and respiration for the duration of the experiments. Data are presented as means \pm standard errors. Data were analyzed as one-way ANOVAs using the generalized linear model procedure of SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA).

RESULTS

Early-, mid- and late-season 'Bartlett' fruit ripened to an eating firmness of 13.4 N in 6, 3 and 3 days, respectively, at 20°C following exposure to ethylene after harvest (Fig. 1). Pre-treatment with 600 nl L⁻¹ 1-MCP at 0°C reduced the sensitivity of fruit to ethylene to varying degrees depending upon the harvest maturity (Fig. 1). For early-season fruit, 1-MCP treatment extended the shelf life (time to eating firmness) by 9 days at 20°C. For mid- and late-season fruit, 1-MCP treatment extended shelf life by 3 days. 1-MCP treatment also delayed the coloration of fruit skin (Fig. 2) and peak ethylene production and respiration rates (data not shown).

The inclusion of ethylene in the treatment atmosphere modified the efficacy of 1-MCP in a concentration-dependent manner that also varied with fruit harvest maturity (Figs. 1 and 2). In early-season fruit, increasing the initial concentration of ethylene in chambers from 0 nl L⁻¹ to 12 and 30 nl L⁻¹ (1-MCP:ethylene ratio of 50:1 and 20:1, respectively) did not interfere with the capacity of 1-MCP to extend shelf life (Fig. 1). However, inclusion of 60 nl L⁻¹ ethylene (10 1-MCP: 1 ethylene) in chambers reduced 1-MCP treatment efficacy slightly. For mid- and late-season fruit, the inclusion of 12, 30 and 60 nl L⁻¹ ethylene in the chamber did not reduce the benefits of 1-MCP although the delay in fruit ripening of 3 days was modest (Fig. 1). A simultaneous exposure to 600 nl L⁻¹ ethylene and 600 nl L⁻¹ 1-MCP (1:1 ratio) abolished any benefits of 1-MCP treatment for fruit at all maturity stages.

Levels of internal ethylene at harvest were lowest for early- and mid-season fruit (0.04 \pm 0.01 and 0.16 \pm 0.02 μ l L⁻¹, respectively) and highest (3.98 \pm 1.18 μ l L⁻¹) for the late maturity stage. While ethylene was carefully administered to give the desired 1-MCP:ethylene ratios at the beginning of treatment, ethylene produced by the fruit accumulated in the closed chambers (Table 2). This build-up of ethylene was low for early-season fruit, and higher for late-season fruit that produced higher concentrations of ethylene. Curiously, the accumulation of ethylene was greatest for mid-season fruit despite their low initial internal ethylene concentration. Nevertheless, there was a general association between a decrease in 1-MCP treatment efficacy with an increase in ethylene concentrations within fruit and/or chambers.

Treatment responses for fruit that were stored at 1°C for 5 weeks following the 1-MCP and ethylene co-treatments were diminished compared to the response at harvest (Figs. 1 and 2). The benefits of 1-MCP pre-treatment were largely maintained during storage for early-season fruit. Pre-treatment with 1-MCP did not extend the shelf life of mid- and late-season fruit after storage.

DISCUSSION

In the present study, the capacity of endogenous and exogenous ethylene to modulate 1-MCP treatment efficacy for 'Bartlett' pears was determined. Treatment with 600 nl L⁻¹ 1-MCP was found to delay ripening at harvest by up to 9 days at 20°C (Figs. 1 and 2). However, treatment efficacy decreased with advancing fruit maturity in association with increasing levels of endogenous ethylene within fruit and the treatment atmosphere (Fig. 1, Table 2). Similarly, Gamrasni et al. (2010) reported that treatment of 'Spadona' pears with 200 nl L⁻¹ 1-MCP delayed ripening of early-season fruit but was less effective for late-season fruit. Moreover, delaying 1-MCP application until after climacteric ethylene production and ripening has been initiated has previously been

reported to reduce treatment efficacy for several fruit (Joyce et al., 1999; Adkins et al., 2005; Zhang et al., 2009; Gamrasni et al., 2010).

The addition of increasing concentrations of exogenous ethylene to the treatment atmosphere also steadily decreased the benefits of 1-MCP (Figs. 1 and 2). These data highlight the competitive nature of 1-MCP and ethylene for fruit binding sites, and are consistent with similar observations by Zhang et al. (2009) and Villalobos Acuna et al. (2011) for tomato and 'Bartlett' pear, respectively. A relatively high initial 1-MCP:ethylene concentration ratio (e.g., 20-50:1) was necessary for maximum ripening inhibition in early-season 'Bartlett' fruit. However, this initial ratio did not greatly extend the shelf life of mid- and late-season pears because ethylene produced by these fruit accumulated more substantially during the treatment than for early-season fruit (Table 2). Increasing the initial ratio of 1-MCP:ethylene may therefore be necessary to improve treatment efficacy for fruit harvested later in the season. Methods to reduce ethylene contamination of treatment areas may also help maintain a more favorable 1-MCP:ethylene ratio. Conversely, decreasing the 1-MCP:ethylene ratio might provide an opportunity to specifically target a range of ripening responses for different market scenarios.

1-MCP treatment effects were diminished for fruit stored at 1°C for 5 weeks relative to responses at harvest (Figs. 1 and 2) in line with the findings of Ekman et al. (2004) and Calvo and Sozzi (2009). Since 'Bartlett' pears are harvested commercially at different stages of maturity whereby ethylene production rates and ripening capacity can vary (Chen and Mellenthin, 1981), the results of the current study highlights the challenge in devising a consistently reliable 1-MCP treatment protocol. The different 1-MCP response of fruit at each maturity stage may also explain some of the variable ripening responses (e.g., no inhibition vs. irreversible suppression) previously reported in pears (Ekman et al., 2004).

In conclusion, this study highlights the capacity of endogenous and exogenous ethylene to modulate the ripening response of 'Bartlett' pears to 1-MCP. This response underscores the importance of monitoring ethylene concentrations in treatment rooms before applying 1-MCP. Establishing a higher ratio of 1-MCP:ethylene within the treatment atmosphere is recommended to extend the shelf life of pears harvested later in the season.

ACKNOWLEDGEMENTS

We acknowledge AgroFresh, Inc., the California Pear Advisory Board, and the USDA NIFA Award #2009-51181-05783 for funding of this study. We also thank Greene and Hemly, Inc. for donating fruit for these experiments.

Literature Cited

- Adkins, M.F., Hofman, P.J., Stubbings, B.A. and Macnish, A.J. 2005. Manipulating avocado fruit ripening with 1-methylcyclopropene. *Postharvest Biol. Technol.* 35:33-42.
- Agar, I.T., Biasi, W.V. and Mitcham, E.J. 1999. Exogenous ethylene accelerates ripening responses in Bartlett pears regardless of maturity or growing region. *Postharvest Biol. Technol.* 17:67-78.
- Argenta, L.C., Fan, X. and Mattheis, J.P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. pear fruit. *J. Agric. Food Chem.* 51:3858-3864.
- Calvo, G. and Sozzi, G.O. 2009. Effectiveness of 1-MCP treatments on 'Bartlett' pears as influenced by the cooling method and the bin material. *Postharvest Biol. Technol.* 51:49-55.
- Chen, P.M. and Mellenthin, W.M. 1981. Effects of harvest date on ripening capacity and postharvest life of 'd' Anjou' pears. *J. Amer. Soc. Hort. Sci.* 106:38-42.
- Ekman, J.H., Clayton, M., Biasi, W.V. and Mitcham, E.J. 2004. Interactions between 1-MCP concentration, treatment interval and storage time for 'Bartlett' pears.

- Postharvest Biol. Technol. 31:127-136.
- Gamrasni, D., Ben-Arie, R. and Goldway, M. 2010. 1-Methylcyclopropene (1-MCP) application to Spadona pears at different stages of ripening to maximize fruit quality after storage. *Postharvest Biol. Technol.* 58:104-112.
- Joyce, D.C., Macnish, A.J., Hofman, P.J., Simons, D.H. and Reid, M.S. 1999. Use of 1-methylcyclopropene to modulate banana ripening. p.189-190. In: A.K. Kanellis, C. Chang, H. Klee, A.B. Bleecker, J.C. Pech and D. Grierson (eds.), *Biology and Biotechnology of the Plant Hormone Ethylene II*, Kluwer Academic Publishers, The Netherlands.
- Lelievre, J.-M., Tichit, L., Dao, P., Fillion, L., Nam, Y.-W., Pech, J.-C. and Latche, A. 1997. Effects of chilling on the expression of ethylene biosynthetic genes in Passe-Crassane pear (*Pyrus communis* L.) fruits. *Plant Mol. Biol.* 33:847-855.
- Mitchell, F.G. 1990. Postharvest biology and technology of European pears. In: *Pear Production Manual*. Department of Pomology, University of California Davis, Davis, CA.
- Sisler, E.C., Dupille, E. and Serek, M. 1996. Effect of 1-methylcyclopropene and methylenecyclopropene on ethylene binding and ethylene action on cut carnations. *Plant Growth Regulat.* 18:79-86.
- Trincherro, G.D., Sozzi, G.O., Covatta, F. and Frascina, A.A. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of 'Bartlett' pears. *Postharvest Biol. Technol.* 32:193-204.
- Villalobos Acuna, M.G., Biasi, W.V., Mitcham, E.J. and Holcroft, D. 2011. Fruit temperature and ethylene modulate 1-MCP response in 'Bartlett' pears. *Postharvest Biol. Technol.* 60:17-23.
- Watkins, C.B. 2008. Overview of 1-methylcyclopropene trials and uses for edible horticultural crops. *HortScience* 43:86-94.
- Yoshida, T., Borgic, D.M., Chen, P.M. and Mielke, E.A. 1986. Changes in ethylene, acids and brown-core development of 'Bartlett' pears in low-oxygen storage. *HortScience* 21:472-474.
- Zhang, Z., Huber, D.J., Hurr, B.M. and Rao, J. 2009. Delay of tomato fruit ripening in response to 1-methylcyclopropene is influenced by internal ethylene levels. *Postharvest Biol. Technol.* 54:1-8.
- Zhang, Z., Huber, D.J. and Rao, J. 2010. Short-term hypoxic hypobarica transiently decreases internal ethylene levels and increases sensitivity of tomato fruit to subsequent 1-methylcyclopropene treatments. *Postharvest Biol. Technol.* 56:131-137.

Tables

Table 1. 1-MCP and ethylene co-treatment concentrations.

1-MCP (nl L ⁻¹)	Ethylene (nl L ⁻¹)	1-MCP:ethylene
0	0	-
600	0	-
600	12	50:1
600	30	20:1
600	60	10:1
600	600	1:1

Table 2. Mean ethylene concentrations inside treatment chambers at the beginning and end of 24-hour 1-MCP and ethylene co-treatments at 0°C. Chambers contained fruit obtained at one of three different maturity stages.

Treatment 1-MCP:ethylene	Ethylene concentration (µl L ⁻¹)					
	Early-season		Mid-season		Late-season	
	Beginning	End	Beginning	End	Beginning	End
Control	0.000	0.172	0.006	1.086	0.007	0.258
1-MCP alone	0.000	0.056	0.006	4.200	0.007	0.224
50:1	0.017	0.086	0.015	3.638	0.019	0.480
20:1	0.034	0.108	0.036	8.858	0.036	0.547
10:1	0.072	0.173	0.064	4.540	0.067	0.688
1:1	0.625	1.455	0.690	4.058	0.650	1.573

Figures

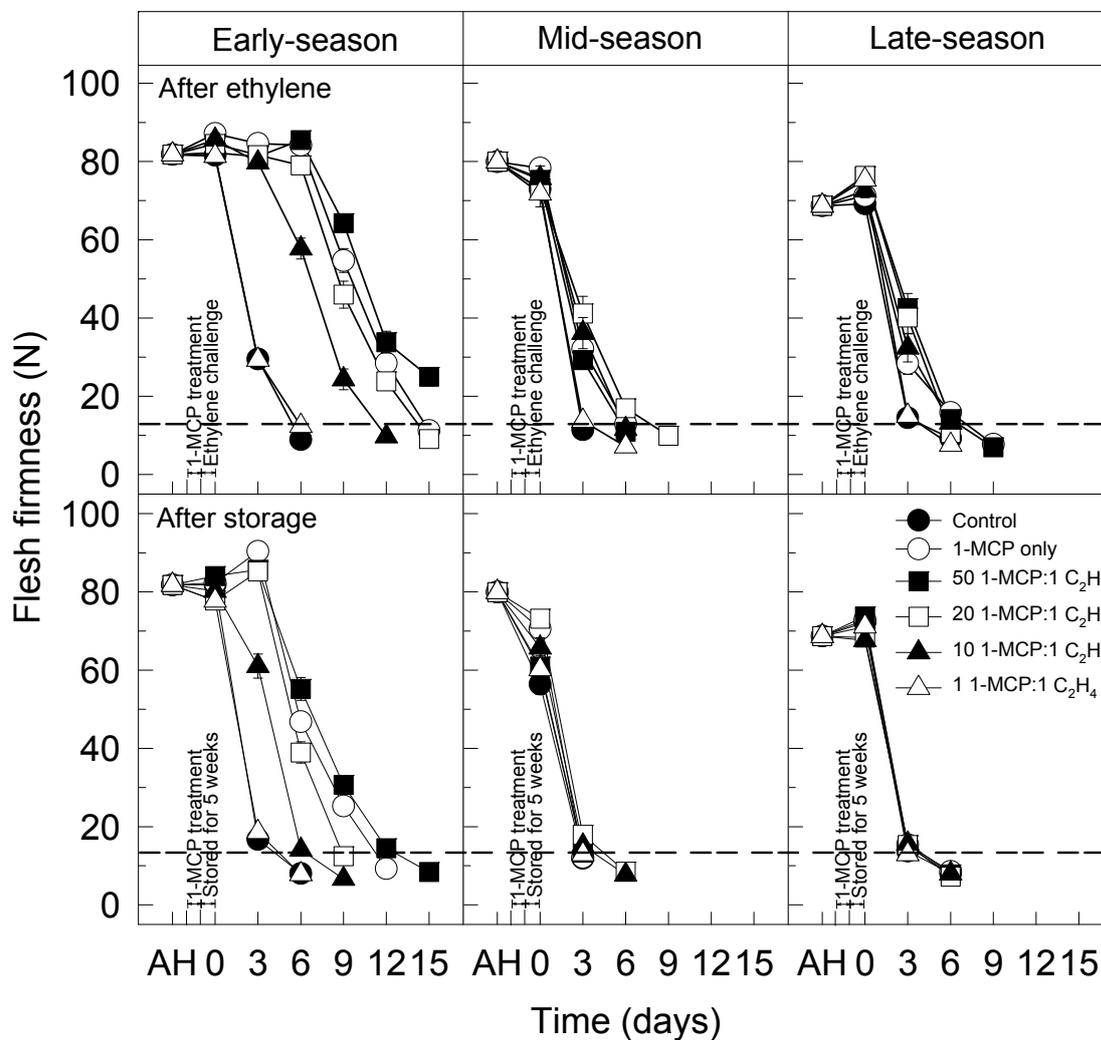


Fig. 1. Fruit firmness at harvest (AH) and during ripening at 20°C for ‘Bartlett’ pears obtained at three stages of maturity from a packinghouse. Fruit were treated with 600 nl L⁻¹ 1-MCP alone or in combination with 12, 30, 60 and 600 nl L⁻¹ ethylene (C₂H₄) for 24 hours at 0°C. Fruit were then challenged with 100 µl L⁻¹ ethylene for 24 hours at 20°C, or stored for 5 weeks at 1°C prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 13.4 N.

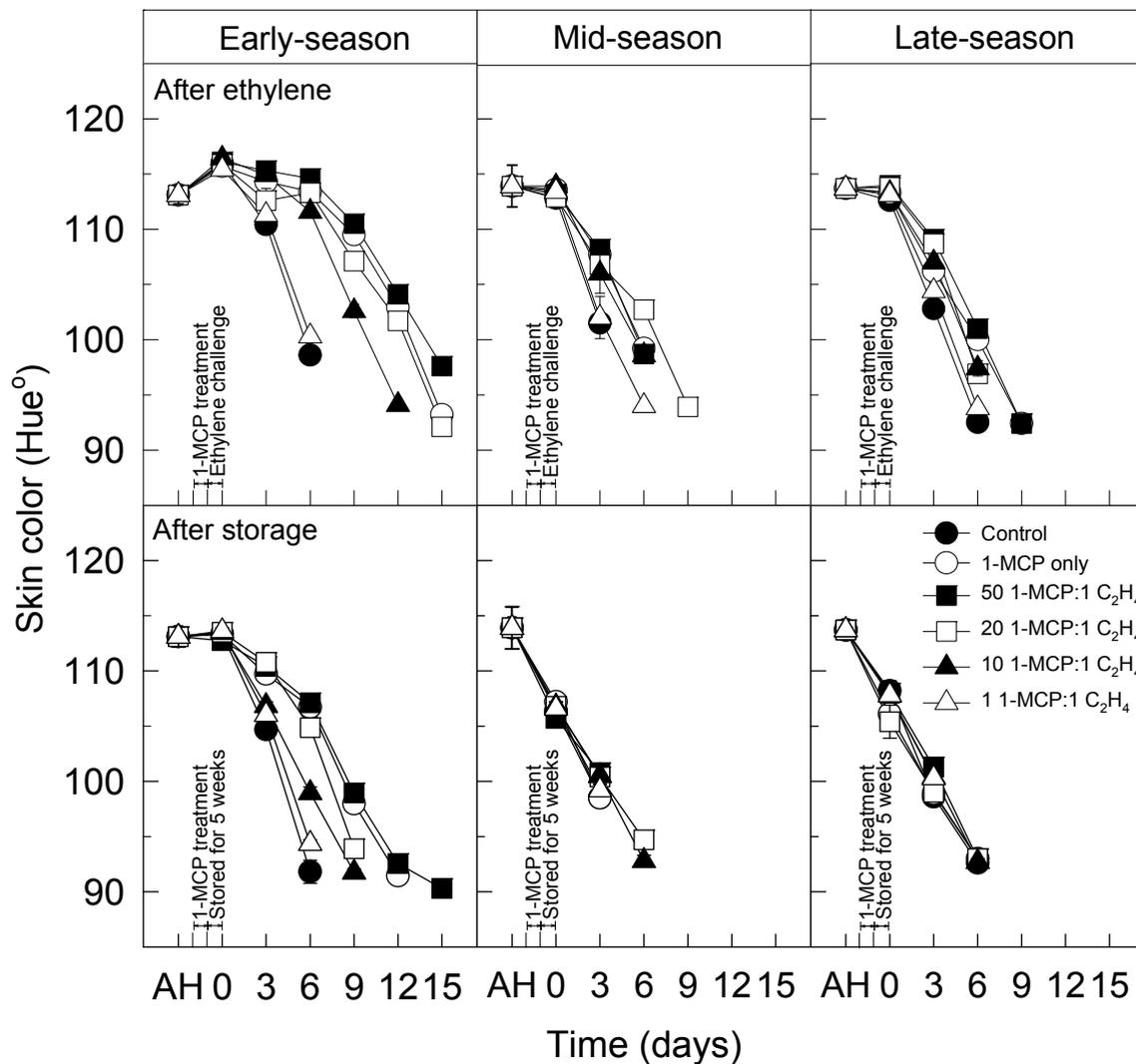


Fig. 2. Fruit skin color (hue angle decreases as the fruit change from green to yellow) at harvest (AH) and during ripening at 20°C for ‘Bartlett’ pears obtained at three stages of maturity from a packinghouse. Fruit were treated with 600 nl L⁻¹ 1-MCP alone or in combination with 12, 30, 60 and 600 nl L⁻¹ ethylene (C₂H₄) for 24 hours at 0°C. Fruit were then challenged with 100 µl L⁻¹ ethylene for 24 hours at 20°C, or stored for 5 weeks at 1°C prior to shelf life evaluation.