

1-METHYLCYCLOPROPENE MAINTAINS FIRMNESS AND PEEL COLOR AND REDUCES DECAY AREA OF ARTIFICIALLY WOUNDED FRUITS IN MATURE JAPANESE PEAR (*PYRUS PYRIFOLIA* NAKAI ‘SHIZUKISUI’)

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ABSTRACT

Recently, there has been an increasing need to prolong the quality of matured fruits to promote the distribution of fresh fruits to consumers and processing facilities. Studies have shown that 1-methylcyclopropene (1-MCP), an inhibitor of ethylene, can maintain the firmness and quality of several fruits for a long duration. Therefore, the aim of this study was to examine the effect of 1-MCP treatment on the firmness, rind color, and decay rate of the Japanese pear ‘Shizukisui’. Results showed that 1-MCP treatment alone and 1-MCP treatment after precooling significantly maintained the firmness of mature fruits compared with untreated fruits. However, the presence or absence of ethylene addition did not significantly affect fruit firmness; moreover, 1-MCP treatment after precooling tended to reduce moisture loss in immature fruits. Regarding the peel color of the fruits, 1-MCP treatment alone and 1-MCP after precooling treatment increased the L*, b*, and C* values of mature fruits but reduced the values in immature fruits. Compared with the control group, the 1-MCP treatment caused a decrease in the decay area of wounded ‘Shizukisui’ and ‘Kosui’ fruits and decreased the decay rate of wounded ‘Kosui’. Overall, this study showed that 1-MCP treatment maintained the firmness and peel color of Japanese pear and reduced its decay rate.

Key words: storage, ethylene, ripening, discoloration, precooling, softening

INTRODUCTION

Recently, there has been an increasing need to maintain the freshness of fruits and vegetables for longer durations due to expanding distribution networks, differences in cultivars and harvest times, and diversification of sales and processing. Fruits, such as apples and persimmons, are matured and aged with the participation of ethylene, an important plant hormone. Over the years, ethylene production and activity inhibitors to maintain the freshness of fruits during distribution have received considerable attention. The physiological response of plants to ethylene is triggered by the binding of ethylene to its receptors, which suppress the expression of genes involved in maturation. However, they are inactivated due to binding to ethylene, resulting in the rapid progression of fruit maturation

and aging. Recently, aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) have been identified as potent inhibitors of ethylene production and activities. For instance, AVG prolongs the preservation period of apples (Yuan & Carbaugh 2007), and prevents fruit dropping in apple and satsuma mandarin (Kondo & Takahashi 1989; Kondo & Hayata 1995; Ogata et al. 1997). Recently, post-harvest application of acibenzolar-S-methyl has been shown to modulate the glutathione-ascorbate cycle to delay the senescence of ‘Doctor Jules Guyot’ pears (Huang et al. 2022). Postharvest treatments with the ethylene antagonist 1-MCP have been widely studied as a method of delaying ripening (Watkins 2008), which could be effective in maintaining the quality of fruits such as apple, kiwifruit, avocado, banana, nectarine, peach, tomato, and other climacteric fruits.

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The effectiveness of 1-MCP differs, ranging from almost compromising the ability of the fruit to ripen properly to relatively small and transitory effects. Although 1-MCP treatment has been extensively applied to apple preservation, studies on its effect on Japanese pear are limited. Presently, there is a need to lengthen the preservation time and quality of Japanese pear (*Pyrus pyrifolia* Nakai) by maintaining the texture that was at harvest. Moreover, the cultivar and climatic conditions may affect the response of the pear fruit to 1-MCP treatment. For instance, early-maturing Japanese pear cultivars have low storability and shelf life of approximately 5 days, indicating the need for the fruits to be harvested before maturity to enable the distribution of wholesome fruits to consumers because they may get spoiled before they reach the retail market. However, it is believed that 1-MCP treatment can improve and maintain the quality of pear fruits long after harvest, which will promote the shipment of suitable and high-quality fruits. Several studies have examined the effect of 1-MCP treatment on different *Pyrus communis* pear cultivars (Calvo & Sozzi 2004; Ekman et al. 2004; Bai et al. 2006; Chino et al. 2010; Chiriboga et al. 2013; Huang et al. 2022). Additionally, some studies have reported the effect of 1-MCP on the preservation of Asian pear species, including Japanese cultivars (Li & Wang 2009; Lee et al. 2017). Sakamoto et al. (2021) reported that 1-MCP treatment suppressed the respiration rate and ethanol production rate and preserved the quality of Japanese pear fruit. However, studies on the effect of 1-MCP on rind color and fruit decay, and the effects of pre-cooling temperature at different maturity stages of Japanese pear fruit are limited. Moreover, since the effects of 1-MCP may be affected by the genotype and maturity stage, it is necessary to evaluate different genotypes and maturity stages (Blankenship & Dole 2003; Itai 2004). The Japanese pear ‘Shizukisui’ used in this study was generated by irradiating the ‘Kisui’ cultivar with gamma rays to obtain black spot disease resistance (Sawano et al. 2011).

The objective of this study was to examine the effect of 1-MCP treatment on the firmness and rind color of the Japanese pear ‘Shizukisui’ at two different maturing stages. Additionally, the suppressive effect of 1-MCP on fruit decay area and decay rate in wounded Japanese pear ‘Shizukisui’ and ‘Kosui’ was examined.

MATERIALS AND METHODS

Effect of 1-MCP on firmness, peel color, moisture loss, and soluble solid content of Japanese pear ‘Shizukisui’ (experiment 1)

The fruits of ‘Shizukisui’ were harvested from an orchard at Shizuoka Prefectural Agriculture and Forestry Research Institute Fruit Tree Research Centre in the Shimizu area, Shizuoka city, in July 2021. The harvested fruits were immediately transported to the laboratory of the Shizuoka Professional University Junior College of Agriculture. Immature (uncolored) fruits (a^* value < 2.0) were separated from mature (colored) fruits. The fruits were randomly assigned to three treatment groups: 1 – 1-MCP treatment (fumigation at 68 mg per m³, 24 h), 2 – 1-MCP treatment after pre-cooling at 5 °C for 48 h, and 3 – nontreated group (control). A total of 32 fruits, including 16 pre-cooling ones, were placed in a container (46 L). The immature and mature fruits were treated in different boxes. Nontreated samples were maintained under same conditions without 24 h 1-MCP treatment. After 1-MCP treatment, the fruits were transferred to a flat plastic container (outer dimensions: 615 mm length × 410 mm width × 190 mm height, inner dimensions: 565 mm × 375 mm × 180 mm), and the container was put in a polypropylene bag (0.025 mm thickness, 1950 mm length, and 1000 mm width). The bags were not sealed throughout the experimental period. The fruits of each treated group were stored under an atmospheric condition with ethylene (approximately 200 ppm in initial concentration) or without ethylene added. The average temperature and relative humidity during the storage period were 26.7 °C and 95.2%, respectively. The ethylene concentration was measured using Gastec detector tube No. 172 (Gastec Corporation, Kanagawa, Japan), and the average temperature and relative humidity were measured using a temperature recorder (RS-13, Espec, Aichi, Japan). The peel color of the pear fruit was measured using a color analyzer (CR-400, Konica Minolta, Tokyo, Japan). The CIELAB (L^* – lightness, a^* – bluish-green/red-purple hue component, b^* – yellow-blue hue component) properties of the peels were measured, and chroma values were calculated [$(C^*) = (a^{*2} + b^{*2})^{1/2}$]. At harvest and 3 and 10 days after starting treatment (DAST), the peel color of the fruits was measured as described above, and changes in L^* , a^* , b^* , and C^* values were calculated according to the following formula: ΔL^* , Δa^* , Δb^* , and $\Delta C^* = (\text{the value of the fruits at 3 or 10 DAST}) - (\text{the value of the fruits before treatment})$.

Fruit firmness was measured at two opposite points on the lateral part of the fruit using a fruit hardness tester (KM-5; Fujiwara Scientific, Tokyo, Japan). The intrusive part of the fruit hardness meter had a conical shape, and the diameter of the base was 12 mm. Moisture loss was calculated using the following formula: Moisture loss (%) = [(fresh weight before treatment) – (fresh weight at 10 DAST)]/(fresh weight before treatment) × 100.

Freshly extracted juices were collected for the determination of the soluble solid content (SSC), using a digital refractometer (DBX-55A; Atago, Tokyo, Japan). Each treatment was repeated four times (replications), and one fruit was used per replication. In this experiment, we chose fruits of the same quality from one orchard. The treatments combined the three factors to verify the main effect.

Suppressive effect of 1-MCP on fruit decay area and decay rate in wounded Japanese pear ‘Shizukisui’ and ‘Kosui’ (experiment 2)

The harvested ‘Shizukisui’ fruits mentioned above, and the Japanese pear ‘Kosui’, which were harvested from an orchard as above in August 2021, were used for this analysis. The fruits were immediately transported to the laboratory of the Shizuoka Professional University Junior College of Agriculture. 1-MCP treatment and control treatments were performed as mentioned above (Table 1). After 1-MCP treatment, a diameter incision (of 10 mm depth × 4 mm diameter) was made between the pedicel and lateral part of one fruit using a needle equipped with a stopper to ensure wound uniformity. Exactly at 10 DAST, the diameter of the decay area on the fruit surface due to natural fungal infection was measured using a ruler. The infection was identified by water-soaked soft rot spots and brown coloration of the surface. The measured diameter was the average of the long and short lengths. There were twelve replications in each treatment, using one fruit per replication in both cultivars. Additionally, the number of decayed fruits was counted. The total decay rate was calculated using the following formula: decay rate = (number of fruits with soft rot area/total number of examined fruits) × 100.

Statistical analysis

Statistical analyses were performed using R v. 3.6.2 software. Fruit quality data, including firmness and peel color, after storage were analyzed using a two-way analysis of variance (ANOVA). Data on changes in peel color were analyzed using Tukey’s multiple range test, with the significance level set at $p < 0.05$.

Data on decay area diameter in wounded fruit were analyzed using the Mann–Whitney U test. Fruit decay data were analyzed using Fisher’s exact test.

RESULTS

Effect of 1-MCP on moisture loss, peel color, firmness, and soluble solid content in Japanese pear ‘Shizukisui’ (experiment 1)

1-MCP treatment alone and 1-MCP after precooling treatment significantly improved the maintenance of the firmness of both matured and immature fruits compared with untreated fruits (Table 2). The presence or absence of ethylene did not significantly affect fruit firmness; moreover, 1-MCP treatment after precooling tended to reduce moisture loss in immature fruits. Additionally, the interaction between 1-MCP and ethylene treatments caused a significant increase in the SSC of both mature and immature fruits, with 1-MCP and ethylene-treated pears having the highest SSC. Regarding the peel color of the fruits, 1-MCP treatment alone and 1-MCP after precooling treatment increased the L^* , b^* , and C^* values of the mature fruits, whereas 1-MCP treatment after precooling decreased the a^* value compared with that of the untreated group (Table 2 & Fig. 1). Regarding the peel color of immature fruits, 1-MCP treatment alone and 1-MCP treatment after precooling decreased the L^* , a^* , b^* , and C^* values of the fruits 10 DAST compared with the untreated group. Three-way ANOVA of the interactive effect of 1-MCP, ethylene treatment, and maturity stage showed that the interaction between 1-MCP and maturity stage significantly influenced the change in peel color (ΔL^* , Δa^* , Δb^* , and ΔC^*) as shown in Table 3.

Suppressive effect of 1-MCP on the diameter of decay area and fruit decay rate in wounded Japanese pear ‘Shizukisui’ and ‘Kosui’ (experiment 2)

The suppressive effects of 1-MCP on the diameter of the decay area and the rate of fruit decay of wounded ‘Shizukisui’ and ‘Kosui’ fruits are illustrated in Figures 2A and B, respectively. In addition, the visual appearance of fruits after 10 days post wounding in ‘Shizukisui’ and ‘Kosui’ are shown in Figures 2C and D, respectively. The diameter of the decay area of ‘Shizukisui’ and ‘Kosui’ fruits with 1-MCP was 53% and 43% smaller, respectively, than that of the control group. Furthermore, 1-MCP significantly reduced the decay rate of wounded ‘Kosui’, but not that of wounded ‘Shizukisui’ 10 DAST compared with that of the control group.

Table 1. Schedule of experiments

Experiment 1	Days after starting treatment			
	0	1	2	3–10
1-MCP	1-MCP		storage (26.7°C)	
1-MCP (precooling)	precooling (5°C)	1-MCP	storage (26.7°C)	
control	storage (26.7°C)			
Experiment 2	Days after starting treatment			
	0	1	2	3–10
1-MCP	1-MCP		storage (26.7°C)	
control	storage (26.7°C)			

Table 2. Effect of 1-MCP on moisture loss, peel color, firmness, and soluble solid content in Japanese pear ‘Shizukisui’

Mature fruits		Moisture loss		Peel color			Firmness	SSC
		(%)	L*	a*	b*	C*	(N)	Brix
Ethylene (+)	1-MCP	4.1 ± 0.1	58.1 ± 0.8	7.8 ± 1.0	39.2 ± 1.2	40.0 ± 1.2	20.3 ± 1.0	12.2 ± 0.02
	1-MCP (Precooling)	3.0 ± 0.1	58.6 ± 0.5	6.3 ± 0.8	37.0 ± 0.5	37.6 ± 0.6	19.6 ± 0.6	11.5 ± 0.1
	Control	4.7 ± 0.8	50.5 ± 2.0	6.4 ± 0.8	27.5 ± 2.5	28.3 ± 2.6	15.8 ± 0.9	11.3 ± 0.3
Ethylene (-)	1-MCP	3.6 ± 0.3	58.3 ± 0.8	8.7 ± 0.9	37.1 ± 0.8	38.2 ± 0.8	21.5 ± 1.3	11.5 ± 0.2
	1-MCP (Precooling)	3.5 ± 0.5	61.5 ± 0.5	5.5 ± 1.1	38.8 ± 0.5	39.2 ± 0.5	20.6 ± 0.4	12.1 ± 0.05
	Control	3.8 ± 0.3	48.8 ± 1.9	8.3 ± 0.6	27.2 ± 1.5	28.4 ± 1.4	16.9 ± 0.8	11.0 ± 0.2
Significance ^z	1-MCP (A)	ns	**	**	**	**	**	**
	Ethylene (B)	ns	ns	ns	ns	ns	ns	ns
	(A)×(B)	ns	ns	ns	ns	ns	ns	*
Immature fruits		Moisture loss		Peel color			Firmness	SSC
		(%)	L*	a*	b*	C*	(N)	Brix
Ethylene (+)	1-MCP	3.3 ± 0.2	54.1 ± 1.7	1.8 ± 0.8	33.6 ± 0.9	33.7 ± 0.9	25.4 ± 2.3	10.8 ± 0.2
	1-MCP (Precooling)	2.9 ± 0.2	54.4 ± 0.5	-1.1 ± 0.5	33.1 ± 0.1	33.1 ± 0.1	25.4 ± 0.4	10.3 ± 0.4
	Control	3.3 ± 0.2	60.5 ± 0.6	5.6 ± 0.4	38.1 ± 0.3	38.5 ± 0.3	23.6 ± 0.6	9.1 ± 0.1
Ethylene (-)	1-MCP	3.6 ± 0.3	54.6 ± 0.7	1.8 ± 0.9	34.0 ± 0.8	34.1 ± 0.8	22.1 ± 0.8	9.9 ± 0.2
	1-MCP (Precooling)	1.9 ± 0.2	55.4 ± 0.6	1.6 ± 1.2	34.5 ± 0.8	34.6 ± 0.9	25.4 ± 0.7	9.9 ± 0.2
	Control	3.6 ± 0.2	58.4 ± 0.9	5.4 ± 0.8	37.1 ± 0.6	37.5 ± 0.6	24.0 ± 0.8	9.5 ± 0.2
Significance ^z	1-MCP (A)	**	**	**	**	**	ns	**
	Ethylene (B)	ns	ns	ns	ns	ns	ns	ns
	(A)×(B)	*	ns	ns	ns	ns	ns	*

Data are expressed as the means ± standard error (SE; n = 4)

^z ns, *, ** indicate nonsignificant and significant differences at p < 0.05 and p < 0.01, respectively, by two-way ANOVA

Table 3. Three-way ANOVA of the effects of 1-MCP and ethylene treatment on peel color at different maturation stages

Source	p value			
	ΔL*	Δa*	Δb*	ΔC*
1-MCP (A)	< 0.05	< 0.001	< 0.001	< 0.01
Ethylene (B)	0.074	0.211	0.285	0.674
Maturation stage (c)	< 0.001	< 0.001	< 0.001	< 0.001
(a)*(b)	0.549	0.956	0.749	0.895
(a)*(c)	< 0.001	< 0.001	< 0.001	< 0.001
(b)*(c)	0.192	0.895	0.729	0.506
(a)*(b)*(c)	0.977	0.113	0.774	0.900

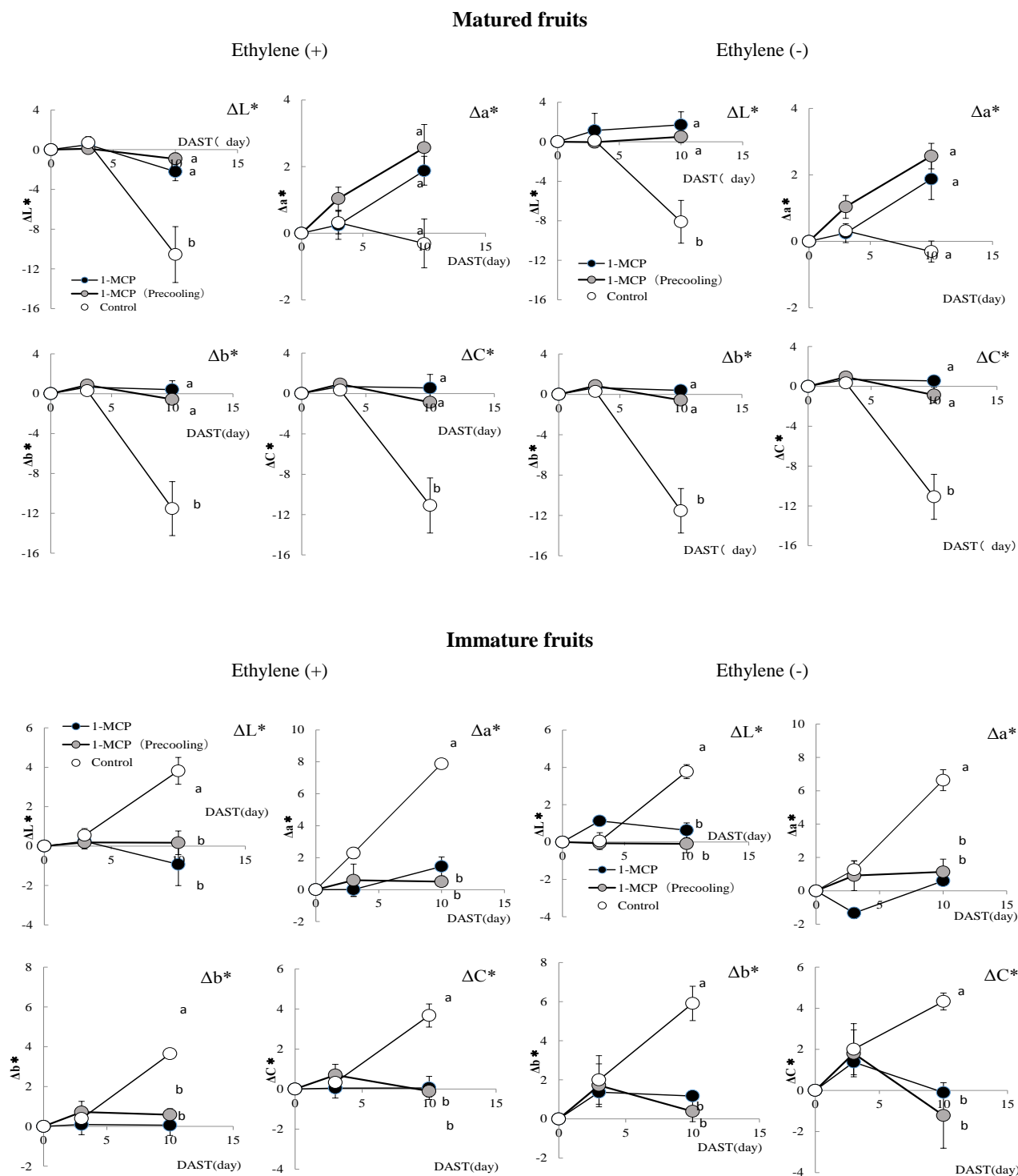


Figure 1. The effect of 1-MCP treatment on L*, a*, b*, and C* values of Japanese pear ‘Shizukisui’ fruits. Vertical bars indicate standard error (SE) (n = 4); different letters indicate significant differences between the treatments; p < 0.05 by Tukey’s multiple range test

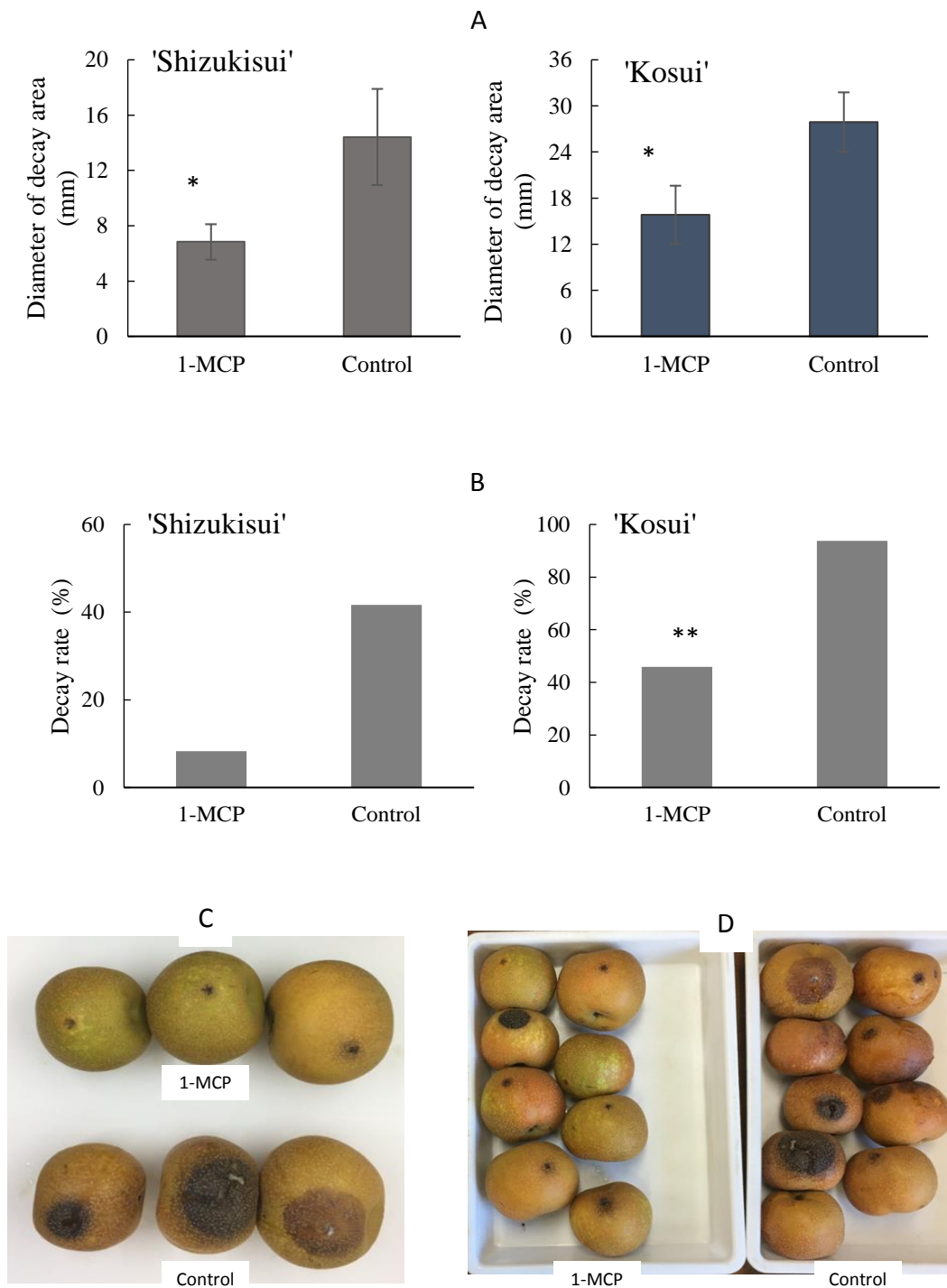


Figure 2. The effect of 1-MCP treatment on the diameter of decay area [A] and decay rate [B] in wounded fruits, and visual appearance after 10 days post wounding 'Shizukisui' [C] and 'Kosui' [D] fruits. [A]: vertical bars indicate standard error (SE) ($n = 12$); *significant difference at $p < 0.05$ by Mann–Whitney U test; [B]: **significant difference at $p < 0.01$ by Fisher's exact test

DISCUSSION

In the present study, we examined the responses of immature and mature fruits of Japanese pear to

1-MCP treatment. The results of the study showed that 1-MCP treatment for 24 h before storage suppressed peel color change and maintained the firmness of 'Shizukisui' fruit, which was consistent with

previous findings in pear (Hiwasa et al. 2003; Kubo et al. 2003). Particularly, it is believed that 1-MCP treatment can extend the freshness, firmness, and peel color of mature fruits, as well as inhibit senescence in several fruits by competing with ethylene for attachment to the enzyme receptor (Kashimura 2005). The findings of the present study show that 1-MCP treatment extended/maintained fruit firmness and peel color in the presence or absence of ethylene treatment, indicating that its effect might be either through inhibition of ethylene or stimulation of some metabolic systems. Presently, both climacteric and nonclimacteric Japanese pear (*P. pyrifolia* Nakai) cultivars have been identified (Kitamura et al. 1981). Itai (2004) reported that there are significant variations in the ethylene production of Japanese pear cultivars, ranging from low to high. Generally, early-maturing cultivars tend to produce more ethylene than late-maturing cultivars; however, 'Kosui' has an ethylene production rate of approximately $1\text{--}2\ \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. Thus, it seems that 'Shizukisui' and 'Kosui' are close to nonclimacteric fruits; therefore, it is possible that the nonsignificant effect of exogenous ethylene on peel color and firmness could be due to the characteristics of the fruits used in this study. Moreover, previous findings suggest that nonclimacteric fruits do not require ethylene for ripening. In contrast, some studies indicated that nonclimacteric fruits may also produce ethylene to promote fruit ripening postharvest (Tatsuki 2007). Perkins-Veazie et al. (1996) reported that ethylene production by strawberry, which is a nonclimacteric fruit, is extremely low compared to that of apples, and the exposure of detached green strawberry fruit to $5,000\ \mu\text{L}\cdot\text{L}^{-1}$ propylene failed to stimulate respiration or ethylene production, but advanced pigmentation change and fresh weight. Additionally, it is believed that the ethylene receptors in Japanese pear may differ from those of other fruits, such as apple, pear, mango, and persimmon.

1-MCP delayed the softening of kiwifruit when treated at harvest (Cantin et al. 2011). However, 1-MCP had no effect on kiwifruit during cold storage, even at high concentrations (Kim et al. 2001). By contrast, Murakami et al. (2016) reported that precooling at temperatures below $10\ ^\circ\text{C}$ for 2 days prior to 1-MCP treatment maintained the hardness of kiwifruit 'Rainbow Red' up to 4 months after harvest. However, the present study's findings showed that precooling did not increase

the effects of 1-MCP treatment. The differing results can be attributed to differences in the ethylene content in Japanese pear and kiwifruit. Further studies are necessary to elucidate the reasons for these differences.

Furthermore, the results of the present study showed that a combination of 1-MCP and exogenous ethylene treatment significantly increased the SSC of fruits, which was similar to the findings of Itai and Tanahashi (2008) in Japanese pears 'Gold Nijisseiki' and 'Hosui' (late-maturing cultivars). However, a previous study showed that 1-MCP treatment did not significantly affect the SSC and titratable acidity of climacteric fruits (Egea et al. 2010), whereas Lurie (2007) reported inconsistent titratable acidity levels of 1-MCP-treated fruits.

Additionally, the effect of 1-MCP treatment on the decay rate of Japanese pear was examined. Fruit decay is discussed in terms of cell wall digestion and fungal infection resistance. In pears, not all maturation-related events are necessarily ethylene dependent; however, some important cell wall-modifying enzymes for the softening process are regulated by ethylene. Hiwasa et al. (2003) indicated that 1-MCP treatment decreased the expression of the pear polygalacturonase genes. However, the expression pattern of pear endo-1,4- β -D-glucanase gene was not affected by propylene and 1-MCP treatments. The findings of the present study showed that 1-MCP treatment probably had some effect on cell wall-modifying enzymes, as evidenced by the decrease in the decay rate of 1-MCP-treated 'Kosui' fruits. However, it is necessary to examine the expression of cell wall modifying genes and enzymes in response to 1-MCP treatment in future studies. Although pathogen infection has been shown to facilitate fruit decay, the fruits were not inoculated with specific pathogens in this study, thus the involvement of the pathogen and the disease resistance could not be clarified. 1-MCP treatment may play a role in disease control for postharvest fruit; however, this is yet to be examined. Generally, an increase in fruit decay rate is highly correlated with storage duration. Ergun et al. (2005) reported that electrolyte leakage from 1-MCP-treated melon fruit was maintained at 20% during the first 5 days of storage, and it never exceeded 27% thereafter, whereas electrolyte leakage from fruits in the control group increased up to 35% during the storage period.

To the best of our knowledge, the present study is the first to report the effect of 1-MCP on firmness, peel color, moisture loss, SSC, and decay rate of wounded fruits of ‘Shizukisui’ pear. Overall, it is more economical to use 1-MCP in mature fruits rather than in immature ones to prolong fruit firmness and peel color. Additionally, the ability of 1-MCP to maintain fruit firmness may vary depending on the maturity stage during processing. For instance, apricot softening can be suppressed by 1-MCP treatment when the fruit is mature (Botondi et al. 2003), whereas 1-MCP treatment could not delay the ripening of early maturing tomato fruit (Hurr et al. 2005). This study revealed that the effect of 1-MCP on Japanese pear fruits at different maturation stages was similar to that of other crops (apricot and tomato). 1-MCP may affect other metabolites in addition to ethylene receptors since 1-MCP maintained fruit quality regardless of ethylene treatment. The effects of 1-MCP on antioxidant activity and other secondary metabolites need to be investigated.

CONCLUSION

In conclusion, the findings of this study showed that 1-MCP treatment maintained the firmness and peel color of Japanese pears and reduced the decay rate of wounded fruit. Additionally, 1-MCP treatment increased the SSC of the fruits. However, it is possible that 1-MCP treatment may affect other properties of Japanese pears, such as titratable acidity. Therefore, further studies are necessary to comprehensively examine the effect of 1-MCP treatment on the chemical properties of the Japanese pear and its mechanisms. Additionally, it is necessary to determine the optimal 1-MCP concentration and fruit storage conditions, such as storage temperature, to prolong fruit freshness and quality. Although the findings of the present study showed that 1-MCP treatment, among other positive effects, reduced the decay rate of wounded Japanese pears, the mechanisms of action are poorly understood. Therefore, further studies should examine the expression profiles of cell wall-modifying and ethylene synthesis genes in response to 1-MCP treatment to understand the mechanisms for preventing fruit senescence.

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