Storage Temperature and 1-Methylcyclopropene Treatment Affect Storage Disorders and Physiological Attributes of ‘Royal Gala’ Apples

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Abstract. ‘Royal Gala’ apples [*Malus domestica* (Borkh.) Mansf.] can develop postharvest disorders such as flesh browning, senescent breakdown, peeling, cracking, or shriveling during and after cold storage. The objective of this study was to examine the effects of storage temperature and a range (0, 0.25, 0.5, or 1 $\mu\text{L}\cdot\text{L}^{-1}$) of 1-methylcyclopropene (1-MCP) concentrations on fruit quality attributes and incidence and severity of physiological disorders during and after cold storage. Storage temperature differentially affected internal ethylene concentration (IEC), fruit circumference, and cortex color. 1-MCP treatment resulted in significant effects on fruit quality attributes and severity of physiological disorders, regardless of storage temperature. Incidence and severity of diffuse flesh breakdown (DFB), shriveling, cracking, and peeling were highest in control fruit stored but radial stem-end flesh breakdown (RSFB) only primarily in 1-MCP-treated fruit. Incidence of RSFB was highest following storage at 0.5 °C compared with 3 °C. 1-MCP treatment had the most influence on disorder incidence/severity or quality attributes, while treatment concentration of 1-MCP was not significant. Overall, the results indicate that 1-MCP treatment can reduce the incidence of ‘Royal Gala’ DFB but may enhance sensitivity to RSFB, when fruit are stored at 0.5 or 3 °C. Incidence of DFB and RSFB are influenced differentially by storage temperature or by 1-MCP treatment, respectively, indicating they may be different disorders.

‘Gala’ apple (*Malus domestica*) strains are highly prone to the incidence of stem-end cracking before harvest with symptoms progressing during and after storage (Lee et al., 2013; Opara et al., 1997). The incidence of stem-end cracking increases with advanced fruit maturity and ripening (Byers, 1998; Drake et al., 2006) with increased fruit size (Lee et al., 2013). However, delay of ripening following exposure to 1-MCP treatment reduces cracking development (Lee et al., 2013). ‘Royal Gala’ apples are also susceptible to the development of flesh breakdown during and after cold storage (Lee et al., 2013). However, flesh breakdown

development can be delayed following fruit exposure to 1-MCP (Lee et al., 2013). Nonetheless, 1-MCP-treated fruit might have different types of flesh breakdown symptom appeared at the stem-end region. Typically, this type of flesh breakdown was detected at the later part of cold storage, which was mostly between 3 and 6 months cold storage.

Apple fruit are typically stored at 0–1 °C with exceptions for cultivars that can be chilling sensitive (Watkins et al., 2014). Higher storage temperature can reduce or eliminate chilling injury (CI) for a number of cultivars including ‘Cox’s Orange Pippin’ (Johnson, 2010), ‘Cripps Pink’ (James et al., 2008), ‘Empire’ (Burmeister and Dilley, 1995; Jung et al., 2010; Watkins and Liu, 2010), ‘Fuji’ (Kweon et al., 2013), and ‘Honeycrisp’ (Moran et al., 2010; Tong et al., 2003; Watkins et al., 2004). Higher storage temperature can also result in a change in CI symptom expression (Watkins and Liu, 2010).

The ethylene action inhibitor 1-MCP slows apple fruit ripening (Bai et al., 2005; Mir et al., 2001) but can differentially impact development of physiological disorders (DeEll et al., 2003; DeLong et al., 2004a; Jung et al., 2010; Larrigaudière et al., 2010; Lee et al., 2012b; Moran and McManus, 2005; Zanella, 2003). The differential (increased or decreased incidence) impacts on disorder development following fruit exposure

to 1-MCP may result from differences in how fruit ripening, senescence, and chilling-related metabolism are influenced by ethylene action (Lee et al., 2012b; Leisso et al., 2015).

‘Royal Gala’ flesh breakdown appears to have some similarities to ‘Empire’ flesh browning as symptoms initiate at the fruit stem end and then progress toward the calyx end (Jung and Watkins, 2011; Lee et al., 2012a). ‘Empire’ flesh browning symptom development has a relationship with storage temperature and 1-MCP treatment concentration (Jung and Watkins, 2011; Lee et al., 2012a), factors that have not been evaluated in relation to ‘Royal Gala’ flesh breakdown. As ‘Royal Gala’ and ‘Empire’ are not known to have similar susceptibilities to postharvest disorders, the objectives of this study were to determine if fruit storage temperature and 1-MCP treatment concentration impact the incidence and severity of ‘Royal Gala’ flesh breakdown and other physiological disorders as well as fruit quality attributes.

Materials and Methods

Plant material. ‘Royal Gala’ apple harvested on 26 Aug. 2013 in a commercial orchard near Vantage, WA, were transported to the laboratory in Wenatchee, WA. Fruit absent of external blemishes with weights $\geq$ 240 g/fruit were selected.

Harvest maturity and fruit quality assessment. At harvest and on 0 and 7 d after removal from storage, fruit quality was assessed on 18 fruit, while 36 fruit were evaluated for physiological disorders. Fruit fresh weight and circumference were measured with an analytical balance and a tape measure, respectively. Measurements were conducted using the same fruit at harvest, after cold storage, and 7-d shelf life. IEC was measured by withdrawing a 0.5 mL gas sample from the core cavity using a syringe and analyzing the sample using a Hewlett-Packard 5880A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a 46 cm (length) $\times$ 0.32 cm (diameter) glass column packed with Porapak Q (Supelco Co., Bellefonte, PA). Flow rates for N₂ carrier, H₂, and air were 0.5, 0.17, and 3.3 mL·s⁻¹, respectively. Oven, injector, and detector temperatures were 60, 100, and 200 °C, respectively.

Peel color on an unblushed area of the fruit equator region was measured with a chromameter (Minolta CR-200; Minolta Co., Osaka, Japan). Flesh color was assessed at the stem end (1.5 cm from stem end toward equator cut horizontally), equator (at the fruit equator cut horizontally), and the calyx end (1.5 cm from calyx end toward equator cut horizontally) with six readings per region using the same chromameter. Color measurements were expressed as lightness (*L**), chroma (*C**), and hue angle (*h*°) (McGuire, 1992).

Flesh firmness was assessed using a penetrometer (Mohr Digi-Test; Mohr & Associates,

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Table 1. Internal ethylene concentration (IEC), I_{AD} , and difference in fruit fresh weight and circumference from harvest through storage and shelf life for 'Royal Gala' apples. Fruit were exposed to 0 or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 or 3 °C for 3 or 6 mo. (M) followed by 7 d at 20 °C.

Storage duration	Treatment	IEC ^z ($\mu\text{L}\cdot\text{L}^{-1}$)		I_{AD}^y ($A_{670}-A_{720}$)		$\Delta\text{Fresh wt}^x$ (g/fruit)		$\Delta\text{Fruit circumference}^x$ (mm/fruit)	
		0.5 °C	3 °C	0.5 °C	3 °C	0.5 °C	3 °C	0.5 °C	3 °C
3 M	Control	13 de ^w	28 c	0.11 ab	0.11 b	4.3 g	4.7 g	0.75 cd	1.6 bc
	1-MCP	0.13 f	1.7 f	0.13 ab	0.13 ab	4.4 g	4.1 g	1.6 bc	1.6 bc
3 M + 7 d	Control	130 a	69 b	0.07 cd	0.06 d	10.1 b	11.5 a	1.8 a-c	0.83 cd
	1-MCP	0.67 f	0.30 f	0.13 ab	0.13 ab	9.7 bc	9.7 bc	2.4 ab	2.8 a
6 M	Control	19 cd	7.4 ef	0.10 bc	0.10 bc	7.4 de	6.8 e	0.28 d	-4.2 g
	1-MCP	0.27 f	1.2 f	0.12 ab	0.15 a	7.0 e	5.8 f	-0.75 e	-1.6 ef
6 M + 7 d	Control	9.1 ef	3.4 ef	0.06 d	0.05 d	9.3 c	11.3 a	-4.1 g	-8.1 h
	1-MCP	3.1 ef	19 cd	0.12 ab	0.11 b	8.0 d	7.5 de	-1.4 ef	-2.3 f
Sources		IEC		I_{AD}		$\Delta\text{Fresh wt}$		$\Delta\text{Fruit circumference}$	
Storage temperature (T)		***		NS		NS		***	
1-MCP (P)		****		****		****		****	
Storage duration (D)		****		****		****		****	
T × P		****		NS		****		****	
T × D		****		NS		***		****	
P × D		****		*		****		****	
T × P × D		****		NS		NS		****	

^zValues are means (n = 18).

^yValues are means (n = 18) with the mean of sunny and shade side of measurement per fruit.

^xFruit fresh weight and circumference difference = harvest value – poststorage (or after 7-d shelf life) value (n = 36).

*Means in each category followed by the same letters do not differ significantly, Duncan's multiple range test at $P \leq 0.05$.

NS, *, **, ****Nonsignificant or significant at $P < 0.05$, 0.001, or 0.0001, respectively.

1-MCP = 1-methylcyclopropene.

Table 2. Cortex tissue color of stem-end, equator, and calyx-end tissues from harvest through storage and shelf life for 'Royal Gala' apples. Fruit were exposed to 0 or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 or 3 °C for 3 or 6 mo. (M) followed by 7 d at 20 °C.

Storage duration	Stem end				Equator				Calyx end			
	0.5 °C		3 °C		0.5 °C		3 °C		0.5 °C		3 °C	
	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP
Lightness ^z (L^*)												
0 M	81.9 b-i ^y		81.9 b-i		83.5 a		83.5 a		82.5 a-e		82.5 a-e	
3 M	81.4 d-k	80.7 i-n	81.4 d-k	80.9 g-n	82.7 a-c	82.4 a-e	82.9 ab	82.4 a-e	81.4 d-k	80.5 j-n	81.3 e-m	80.3 j-o
3 M + 7 d	79.9 n-o	80.7 i-n	79.0 m-o	80.8 h-n	82.3 a-g	82.1 b-h	81.4 d-k	82.4 a-e	80.1 k-p	80.0 m-p	80.1 k-p	80.4 j-n
6 M	81.5 c-i	79.9 o-q	80.1 m-o	79.8 n-o	83.1 ab	81.9 b-i	82.8 a-c	82.2 b-g	81.4 e-l	79.9 n-o	81.2 e-n	80.4 j-n
6 M + 7 d	76.3 r	77.9 q	71.7 s	78.6 q	81.1 f-n	81.9 b-i	79.9 n-o	82.3 a-g	79.8 n-o	79.9 n-o	78.9 pq	80.0 m-p
Chroma ^z (C^*)												
0 M	21.0 m-q		21.0 m-q		17.1 x		17.1 x		15.8 y		15.8 y	
3 M	21.1 m-q	21.4 k-o	22.6 g-k	21.3 i-o	18.2 vw	18.0 wx	19.6 r-u	18.7 u-w	19.9 q-t	19.3 s-v	22.4 h-l	20.7 n-r
3 M + 7 d	22.9 f-j	21.4 k-o	23.9 c-f	21.9 j-u	19.7 r-u	18.9 t-w	21.3 l-p	18.9 t-w	24.1 c-f	21.7 k-n	24.7 cd	21.9 j-n
6 M	23.3 e-i	22.2 i-m	24.2 c-e	22.9 f-j	20.4 o-s	18.5 u-w	21.5 k-o	19.9 q-t	23.3 e-i	20.1 p-t	24.9 bc	22.6 g-k
6 M + 7 d	24.7 cd	23.5 e-h	25.9 ab	24.0 c-f	20.9 m-q	19.4 s-u	22.6 g-k	20.7 n-r	24.8 b-d	22.1 j-m	26.1 a	23.7 d-g
Hue angle ^z (h°)												
0 M	104.5 bc		104.5 bc		105.7 ab		105.7 ab		106.7 a		106.7 a	
3 M	101.6 f-n	101.1 j-p	101.1 j-p	101.4 g-o	103.3 c-g	103.1 c-i	102.9 c-j	102.9 c-j	103.9 c-e	104.3 b-d	103.4 c-f	103.6 c-f
3 M + 7 d	98.5 q-s	100.3 l-q	96.5 ut	99.7 r-q	101.2 i-p	102.1 e-l	99.3 p-r	102.3 d-k	101.1 i-p	102.8 c-j	99.5 o-q	102.8 c-j
6 M	99.9 n-q	98.7 q-s	96.4 ut	97.6 r-t	101.9 e-m	102.3 d-k	100.1 m-q	101.3 g-o	102.0 e-m	103.2 c-h	100.3 l-q	101.9 e-m
6 M + 7 d	93.4 v	95.5 u	88.3 w	95.4 u	98.6 q-s	100.9 j-p	95.9 tu	100.6 k-p	98.4 q-s	102.4 d-k	96.9 s-u	101.4 g-o
Sources		Lightness (L^*)				Chroma (C^*)				Hue angle (h°)		
Storage temperature (T)		****				****				****		
1-MCP (P)		****				****				****		
Tissue localization (L)		****				****				****		
Storage duration (D)		****				****				****		
T × P		****				NS				**		
T × L		****				NS				****		
T × D		****				****				****		
P × L		****				**				****		
P × D		****				****				****		
L × D		****				****				****		
T × P × L		****				NS				**		
T × P × D		****				NS				NS		
P × L × D		****				NS				*		
T × P × L × D		****				NS				NS		

^zValues are means (n = 18) with three readings per replicate.

^yMeans in each color parameter followed by the same letters do not differ significantly, Duncan's multiple range test at $P \leq 0.05$.

NS, *, **, ****Nonsignificant or significant at $P < 0.05$, 0.01, or 0.0001, respectively.

1-MCP = 1-methylcyclopropene.

Fig. 1. Symptoms of diffuse flesh breakdown (A), radial, stem-end flesh breakdown (B), cracking (C), and peeling (D) in cold-stored 'Royal Gala' apples.

Richland, WA), equipped with a cylindrical plunger 11 mm in diameter (Evans et al., 2010). The measurement was performed on two pared surfaces on opposite sides of the fruit equator region. Starch pattern index was estimated by cutting each fruit horizontally through the equator then staining the cut surface using a potassium-iodine (1.5% KI, 0.6% I) solution. Starch pattern index from 1 (100% starch) to 6 (0% starch) was determined using the scale described by Brookfield et al. (1997).

Soluble solids concentration (SSC) and titratable acidity (TA) in freshly prepared juice extracted from composite samples of two segments per two fruit were determined using a refractometer (Atago N1; Atago Co. Ltd., Tokyo, Japan) and autotitrator (TIM850, SAC80; Hach Co., Loveland, CO), respectively. TA was determined by titrating juice with 0.1 M KOH to pH 8.2.

Fruit index of absorbance difference (I_{AD}) was determined using a DA meter (53500 DA meter; T.R. Turonosrl, Forli, Italy). I_{AD} on the fruit blush and shade side was used to calculate overall mean for each fruit (Costamagna et al., 2013).

Incidence and severity of peel shriveling, cracking, and peeling were recorded and then fruit were horizontally sliced into five or six sections. Incidence and severity of internal disorders were assessed. Disorder incidence is expressed as percent fruit affected ($n = 36$). The severity of external and internal physiological disorders was subjectively scored as 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100% area of the peel or the slice with the largest area with breakdown (Lee et al., 2013).

Expt. 1. Fruit on pressed fiber trays (18 fruit/tray) were exposed to 0 or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (SmartFresh™ powder, 3.8% a.i.; Agro-Fresh Inc., Spring House, PA) at ambient temperature for 16 h on the day of harvest. Following removal from the treatment chambers, trays, four per box, were placed into cardboard boxes lined with a perforated polyethylene bag. Fruit were stored in air at 0.5 or 3 °C with 90% relative

humidity (RH) for up to 6 months followed by 20 °C for 7 d.

Expt. 2. Fruit on pressed fiber trays (18 fruit/tray) were exposed to 0, 0.25, 0.5, or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (SmartFresh™ powder, 3.8% a.i.; Agro-Fresh Inc.) for 16 h on the day of harvest. The fruit were packed as described for Expt. 1 and then stored in air at 0.5 °C with 90% RH for up to 6 months followed by 7 d at 20 °C.

Experimental design and statistical analyses. Expt. 1 was conducted with two factors, 1-MCP treatment and storage temperature. Expt. 2 consisted of one factor, four levels of 1-MCP concentration. Fruit quality attributes and incidence and severity of physiological disorders were analyzed according to a completely randomized experimental design. For the assessment of fruit quality attributes, 18 fruit were used with 6 fruit per replication, and for the evaluation of fruit physiological disorders, 36 fruit were assessed. All statistical analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC). Fruit quality attributes and storage disorder results were subjected to analysis of variance using the general linear model (Proc GLM) to determine main effects and interactions and means compared using Duncan's multiple range test, $P \leq 0.05$. Pearson correlation coefficient analysis was performed to identify relationships between response variables and fruit quality and storage disorders.

Results

Mean values for fruit maturity and quality attributes at harvest were fresh weight: 281.4 g; circumference: 265 mm; starch pattern index: 2.1; flesh firmness: 78.9 N; SSC: 11.2%; TA: 0.375 g/100 g; IEC: 1.03 $\mu\text{L}\cdot\text{L}^{-1}$; peel L^* : 75.3; peel C^* : 28.1; peel h° : 84.7.

Expt. 1. Fruit IEC after storage and shelf life was higher for controls compared with 1-MCP-treated fruit except for 6 months at 3 °C and 6 months plus 7 d at 0.5 °C (Table 1). Poststorage IEC for control fruit was not consistently related to storage temperature, and IEC for all 1-MCP-treated fruit was similar except after 6 months plus 7 d when IEC was higher for fruit stored at 3 °C compared with 0.5 °C. Fruit I_{AD} was similar following 3 and 6 months at both temperatures, however, values were higher for 1-MCP-treated fruit after 6 months at 3 °C and after 7 d at 20 °C. Fresh weight loss was highest after 3 months storage plus 7 d compared with 6 months plus 7 d for fruit stored at 3 °C compared with 0.5 °C. Storage temperature did not significantly impact fresh weight loss for 1-MCP-treated fruit with the exception that 1-MCP-treated fruit lost more fresh weight at 0.5 °C than at 3 °C during 6 months storage, and weight loss for 1-MCP-treated fruit was relatively less compared with controls regardless of previous cold-storage temperature. Fruit circumference decreased through 3 months plus 7 d with no significant effects from storage temperature or 1-MCP treatment. After 6 months, circumference

decreased for controls stored at 0.5 °C but increased for controls stored at 3 °C and for 1-MCP-treated fruit regardless of storage temperature. At 6 months plus 7 d at 20 °C, circumference increased regardless of storage temperature or 1-MCP treatment. The increase in circumference was greater for controls stored at 3 °C compared with 0.5 °C and less in 1-MCP-treated fruit compared with controls.

Fruit cortex appearance generally was lighter (higher L^*) at the equator compared with the stem ends and calyx ends (Table 2). 1-MCP treatment resulted in lower L^* value for both storage temperatures and all cortex tissue locations compared with control. However, L^* generally decreased with increased storage duration and values were typically higher in 1-MCP-treated fruit compared with control fruit after 7 d at 20 °C. The lowest L^* values were for control fruit stored at 3 °C for 6 months plus 7-d shelf life. Fruit cortex C^* increased with increased storage duration, storage temperature (controls only), and during 7-d shelf life. Less change generally occurred in 1-MCP-treated fruit. Hue angle (h°) value generally decreased with increased storage temperature, storage duration, and during 7-d shelf life. However, the reduction in h° was typically less for 1-MCP-treated fruit compared with controls. The lowest h° values were for control fruit stored at 3 °C for 6 months plus 7-d shelf life.

DFB (Fig. 1A) developed only in control fruit (Table 3). DFB severity was highest in fruit stored at 3 °C and incidence and severity increased during 7 d at 20 °C. RSFB was detected in two control fruit stored at 0.5 °C for 3 months and in 1-MCP-treated fruit regardless of storage temperature and duration. RSFB incidence and severity in 1-MCP-treated fruit increased with increased storage duration and during 7 d at 20 °C after cold storage. RSFB incidence and severity were typically less for fruit stored at 3 °C compared with 0.5 °C. Shriveling, cracking, and peeling in control fruit increased with storage duration, storage temperature, and during 7-d shelf life. Incidence and severity of shriveling, cracking, and peeling were lower in 1-MCP-treated fruit relative to controls.

The Pearson correlation coefficient matrices (Fig. 2) present correlations among all response variables and class variables, storage temperature, and 1-MCP treatment to evaluate overall responses of all the variables to storage temperature and 1-MCP treatment. Flesh color variables were more significant in 1-MCP-treated fruit compared with controls. Furthermore, the correlation among flesh color variables was much greater for 1-MCP-treated fruit stored at 3 °C than at 0.5 °C. The incidence and severity of storage disorders were more highly correlated at 3 °C than at 0.5 °C for control fruit and 1-MCP-treated fruit. Fresh weight loss, fruit circumference change, and IEC of controls had higher correlations at 3 °C than at 0.5 °C but the opposite pattern was observed for

Table 3. Incidence and severity of diffuse flesh breakdown, radial stem-end flesh breakdown, shriveling, cracking, and peeling disorders in 'Royal Gala' apple exposed to 0 or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 or 3 °C for 3 or 6 mo. (M) followed by 7 d at 20 °C.

	Disorder incidence ^z (%)				Disorder severity rate ^y (0–5)					
	0.5 °C		3 °C		0.5 °C		3 °C			
	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP		
Storage duration	Diffuse flesh breakdown									
3 M	0	0	0	0	0.0 g ^x	0.0 g	0.0 g	0.0 g		
3 M + 7 d	44	0	81	0	1.1 e	0.0 g	2.2 c	0.0 g		
6 M	25	0	89	0	0.4 f	0.0 g	1.7 d	0.0 g		
6 M + 7 d	97	0	100	0	3.2 b	0.0 g	4.3 a	0.0 g		
	Radial stem-end flesh breakdown									
3 M	6	14	0	6	0.1 f	0.2 f	0.0 f	0.1 f		
3 M + 7 d	0	78	0	53	0.0 f	1.2 d	0.0 f	0.7 e		
6 M	0	97	0	53	0.0 f	2.1 b	0.0 f	1.6 c		
6 M + 7 d	0	92	0	92	0.0 f	2.4 a	0.0 f	2.0 b		
	Shriveling									
3 M	0	0	0	0	0.0 c	0.0 c	0.0 c	0.0 c		
3 M + 7 d	0	0	0	0	0.0 c	0.0 c	0.0 c	0.0 c		
6 M	0	0	78	3	0.0 c	0.0 c	1.1 b	0.0 c		
6 M + 7 d	64	0	97	0	1.0 b	0.0 c	2.0 a	0.0 c		
	Cracking									
3 M	0	0	0	0	0.0 e	0.0 e	0.0 e	0.0 e		
3 M + 7 d	0	0	0	0	0.0 e	0.0 e	0.0 e	0.0 e		
6 M	3	0	89	14	0.1 e	0.0 e	1.5 b	0.2 e		
6 M + 7 d	69	11	100	34	1.2 c	0.1 e	2.5 a	0.5 d		
	Peeling									
3 M	0	0	0	0	0.0 d	0.0 d	0.0 d	0.0 d		
3 M + 7 d	0	0	0	0	0.0 d	0.0 d	0.0 d	0.0 d		
6 M	0	0	72	3	0.0 d	0.0 d	0.9 b	0.0 d		
6 M + 7 d	28	0	78	3	0.3 c	0.0 d	1.8 a	0.0 d		
Sources	Diffuse breakdown		Radial breakdown		Shriveling		Cracking		Peeling	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Temperature (T)	****	****	***	****	****	****	****	****	****	****
1-MCP (P)	****	****	****	****	****	****	****	****	****	****
Duration (D)	****	****	****	****	****	****	****	****	****	****
T × P	****	****	***	****	****	****	****	****	****	****
T × D	**	**	NS	NS	****	****	****	****	****	****
P × D	****	****	****	****	****	****	****	****	****	****
T × P × D	**	**	NS	NS	****	****	****	****	****	****

^zThe incidence of physiological disorders was expressed as percent fruit affected (n = 36).

^ySeverity rate of physiological disorders was subjectively evaluated by estimating the percentage of each incidence: 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.

^xMeans in each category followed by the same letters do not differ significantly, Duncan's multiple range test at $P \leq 0.05$.

NS, **, ***, ****Nonsignificant or significant at $P < 0.01$, 0.001, or 0.0001, respectively.

1-MCP = 1-methylcyclopropene.

1-MCP-treated fruit. Fruit circumference change had much higher correlations with the incidence and severity of storage disorders at 0.5 °C than at 3 °C, irrespective of 1-MCP treatment. By contrast, fresh weight loss correlated positively with DFB, shrivel and cracking and negatively with RSFB for control fruit but not 1-MCP-treated fruit, regardless of storage temperature. The incidence and severity of DFB were positively correlated with the other disorders except for RSFB at both storage temperatures.

Expt. 2. IEC for 1-MCP-treated fruit was lower compared with controls except after 6 months plus 7 d (Table 4). IEC levels were not statistically different among 1-MCP-treated fruit except for fruit exposed to 0.25 $\mu\text{L}\cdot\text{L}^{-1}$ then stored for 6 months plus 7 d.

The incidence and severity of DFB not only increased with increased storage duration in control fruit, compared with 1-MCP-treated

fruit but was also much worse during shelf life than during cold storage (Table 6). Although 1-MCP-treated fruit had the symptoms of DFB during cold storage, these were negligible. In contrast, the symptoms of RSFB were only detected in 1-MCP-treated fruit, regardless of 1-MCP concentration levels. The incidence and severity of RSFB increased with increased storage duration. The symptoms of shriveling and peeling were only detected in control fruit after 6 months plus 7 d but were not observed in 1-MCP-treated fruit. The incidence and severity of fruit cracking were highest in control fruit after 6 months plus 7 d. Although 1-MCP treatment was highly significant for the incidence and severity of fruit cracking disorder, the severity of fruit cracking incidence in 1-MCP-treated fruit was negligibly detected within 0.1 ratio of severity.

The correlation matrices indicated that fruit quality attributes were more correlated with the increase in 1-MCP-treated concentration but the correlation of fruit physiological disorders was more positively responded to control fruit than 1-MCP-treated fruit (Fig. 3). As 1-MCP-treated concentration increased, flesh L^* variables were more significantly correlated with flesh C^* and h^o variables. Postharvest storage disorder variables were positively correlated with fruit fresh weight loss in control fruit. However, in 1-MCP-treated fruit, RSFB disorder was positively correlated with IEC but negatively with fruit circumference increase, regardless of 1-MCP-treated concentration.

Discussion

Incidence and severity of fruit cracking and flesh breakdown in cold-stored 'Royal

Fig. 2. Pearson correlation coefficient (r) matrices among the responses of fruit quality attributes and incidence and severity of physiological disorders of 'Royal Gala' apples exposed to 0 or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest and stored in air at 0.5 or 3 °C for 3 or 6 months followed by 20 °C for 7 d. Red and blue colors indicate positive and negative correlation coefficients among variables, respectively. The lower or upper mirrored halves on plots are control or 1-MCP treated fruit, respectively at each storage temperature. 1-MCP = 1-methylcyclopropene.

Table 4. Internal ethylene concentration (IEC), I_{AD} , and difference in fruit fresh weight and circumference from harvest through storage and shelf life for 'Royal Gala' apples. Fruit were exposed to 0, 0.25, 0.5, or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 °C for 3 or 6 mo. (M) followed by 7 d at 20 °C.

	1-MCP ($\mu\text{L}\cdot\text{L}^{-1}$)				1-MCP ($\mu\text{L}\cdot\text{L}^{-1}$)			
	0.0	0.25	0.5	1.0	0.0	0.25	0.5	1.0
Storage duration	IEC ^z ($\mu\text{mol}\cdot\text{L}^{-1}$)				I_{AD} ^y ($A_{670}-A_{720}$)			
3 M	13 bc ^w	0.10 d	0.08 d	0.13 d	0.11 ^v b-d	0.15 ab	0.16 a	0.13 a-d
3 M + 7 d	130 a	0.76 d	0.72 d	0.67 d	0.09 d-f	0.14 a-c	0.12 a-d	0.15 ab
6 M	19 b	0.30 d	0.37 d	0.27 d	0.07 ef	0.10 c-e	0.13 a-d	0.13 a-d
6 M + 7 d	9.2 cd	13 bc	6.7 cd	3.1 d	0.06 f	0.10 c-e	0.10 c-f	0.13 a-d
	$\Delta\text{Fresh wt}^x$ (g/fruit)				$\Delta\text{Fruit circumference}^x$ (mm/fruit)			
3 M	4.3 ef	3.1 g	3.6 fg	4.4 ef	0.75 e-g	2.2 b-d	1.1 d-f	1.3 c-f
3 M + 7 d	10.1 a	10.0 a	9.9 ab	9.7 ab	1.8 b-e	4.0 a	2.7 b	2.4 bc
6 M	7.4 d	5.1 e	7.0 d	7.0 d	0.28 f-h	-0.22 g-i	-0.42 g-i	-0.75 hi
6 M + 7 d	9.4 ab	8.9 bc	7.3 d	8.0 cd	-4.1 k	-0.83 hi	-1.2 ij	-2.3 j
Sources	IEC				I_{AD}			
1-MCP (P)	****				****			
Duration (D)	****				****			
P × D	****				NS			

^zValues are means (n = 18).

^yValues are means (n = 18) with the mean of sunny and shade side of measurement per fruit.

^xFruit fresh weight and circumference difference = harvest value – poststorage (or after 7-d shelf life) value (n = 36).

^wMeans in each category followed by the same letters do not differ significantly, Duncan's multiple range test at $P \leq 0.05$.

NS, **, ***, ****Nonsignificant or significant at $P < 0.01$, 0.001, or 0.0001, respectively.

1-MCP = 1-methylcyclopropene.

Gala' apples have a positive relationship with fruit size (Lee et al., 2013). Disorder development is reduced by 1-MCP treatment before cold storage, indicating that fruit ripening and senescence may be factors

influencing disorder development. Development of several apple fruit internal physiological disorders is associated with storage temperature (DeEll and Prange, 1998; DeLong et al., 2004b; James et al., 2008;

Johnson and Ertan, 1983; Jung et al., 2010; Kweon et al., 2013; Watkins et al., 2004). Disorder development that increases with decreased apple fruit storage temperature includes low-temperature breakdown and

Table 5. Cortex tissue color of stem-end, equator, and calyx-end tissues from harvest through storage and shelf life for 'Royal Gala' apples. Fruit were exposed to 0, 0.25, 0.5, or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 °C for 3 or 6 mo. (M) followed by 7 d at 20 °C.

Storage duration	1-MCP ($\mu\text{L}\cdot\text{L}^{-1}$)				1-MCP ($\mu\text{L}\cdot\text{L}^{-1}$)				1-MCP ($\mu\text{L}\cdot\text{L}^{-1}$)			
	0.0	0.25	0.5	1.0	0.0	0.25	0.5	1.0	0.0	0.25	0.5	1.0
	Stem-end				Equator				Calyx-end			
					Lightness ^a (L^*)							
0 M	82.1 c-e ^v				83.6 a				82.6 bc			
3 M	81.4 d-i	80.7 h-m	81.1 e-j	80.7 h-m	82.7 bc	82.0 c-f	82.6 bc	82.4 b-d	81.4 d-i	80.1 l-n	80.7 h-m	80.5 i-m
3 M + 7 d	79.9 l-o	80.9 g-l	80.6 h-m	80.7 h-m	82.3 b-d	82.0 c-f	82.4 b-d	82.1 c-e	80.1 l-n	79.8 l-o	80.4 j-m	80.0 l-o
6 M	81.5 d-h	80.2 j-n	79.9 l-o	79.9 l-o	83.1 ab	81.9 c-f	82.2 b-d	81.9 c-f	81.4 d-h	80.7 h-m	80.6 h-m	79.9 l-o
6 M + 7 d	76.3 q	79.1 o	79.4 no	77.9 p	81.1 f-k	81.8 c-g	82.4 b-d	81.9 c-f	79.8 m-o	79.8 k-n	80.1 k-n	79.9 l-o
					Chroma ^a (C^*)							
0 M	20.9 i-o				16.7 y				15.6 z			
3 M	21.1 i-n	20.8 j-p	21.6 h-l	21.4 h-m	18.2 v-x	17.8 x	18.0 wx	18.0 wx	19.9 n-t	19.5 p-v	19.5 p-v	19.3 r-w
3 M + 7 d	22.9 c-q	20.9 i-o	21.7 g-k	21.4 h-m	19.7 o-u	18.4 u-x	18.3 v-x	18.9 s-x	24.0 a-c	20.5 k-r	20.6 k-q	21.7 h-l
6 M	23.3 c-f	22.6 d-h	22.7 d-h	22.2 e-i	20.4 l-r	18.7 t-x	18.9 s-x	18.5 u-x	23.3 c-f	20.3 m-r	20.0 n-t	20.1 m-s
6 M + 7 d	24.7 ab	23.0 c-f	23.6 b-d	23.5 c-e	20.9 i-o	19.2 r-w	19.5 q-v	19.4 q-v	24.8 a	21.6 h-l	22.6 d-h	22.1 f-j
					Hue angle ^a (h°)							
0 M	104.5 b				105.9 a				106.8 a			
3 M	101.6 i-l	101.2 j-m	101.1 j-m	101.1 j-m	103.3 b-i	103.1 b-i	103.4 b-h	103.1 b-i	103.9 b-e	103.8 b-f	104.1 b-d	104.3 bc
3 M + 7 d	98.5 o-q	101.1 j-m	99.9 m-o	100.3 l-n	101.2 j-m	103.6 b-g	102.6 c-j	102.1 e-k	101.1 j-m	103.8 b-f	103.5 b-g	102.8 b-j
6 M	99.9 m-o	98.9 n-q	98.9 n-q	98.7 n-q	101.9 h-l	102.6 c-k	102.7 c-k	102.3 d-k	102.0 f-k	103.3 b-i	103.5 b-g	103.2 b-i
6 M + 7 d	93.4 s	97.6 pq	97.3 q	95.5 r	98.6 o-q	101.1 j-m	101.7 h-l	100.9 k-m	98.4 o-q	102.6 c-k	102.8 b-j	102.4 d-k
Sources	Lightness (L^*)				Chroma (C^*)				Hue angle (h°)			
1-MCP (P)	*				****				****			
Duration (D)	****				****				****			
Localization (L)	****				****				****			
P × D	****				****				****			
P × L	*				****				NS			
D × L	****				****				****			
P × D × L	**				NS				NS			

^aValues are means (n = 18) with mean of three readings per replicate.

^vMeans in each color parameter followed by the same letters do not differ significantly, Duncan's multiple range test at $P \leq 0.05$.

NS, *, **, ****Nonsignificant or significant at $P < 0.05$, 0.01, or 0.0001, respectively.

1-MCP = 1-methylcyclopropene.

core flush for 'Idared' apples (Johnson and Ertan, 1983), corky flesh browning, low-temperature breakdown, vascular breakdown, and core browning of 'McIntosh' (Lau et al., 1987) and 'Cortland' apples (DeEll and Prange, 1998), and diffuse flesh browning in 'Cripps Pink' (James et al., 2008). Incidence of senescent breakdown and core browning was highly associated with higher storage temperature in 'Empire' apple (Watkins and Liu, 2010). Disorders enhanced by higher storage temperatures include core browning in 'Braeburn' (Lau, 1998), senescent breakdown and core browning in 'Empire' (Watkins and Liu, 2010), and the results herein for DFB and shriveling in 'Royal Gala'. The differential responses of physiological disorders to diverse storage temperatures may result from storage conditions, cultivar specificity, or other factors. In general, long-term stored apple fruit is more ripened after cold storage in air compared with a controlled atmosphere (CA) where low O_2 and high CO_2 inhibit ethylene responses in stored fruit, thereby suppressing fruit respiration and ripening (Bai et al., 2005). However, CA conditions can enhance apple fruit sensitivity to low temperature and enhance incidence and severity of CI (Jung et al., 2010; Watkins and Liu, 2010). 'Honeycrisp' apple fruit are highly sensitive to CI at lower storage temperature during the early stage of

storage (Watkins et al., 2004, 2005). To control physiological disorders including soft scald, CO_2 injury, and superficial scald, preconditioning by which fruit after harvest are held at a lower than ambient temperature and higher than storage temperature for 7–10 d can reduce subsequent injury development (Argenta et al., 2000; Bai et al., 2006; Moran et al., 2010).

The ethylene action inhibitor 1-MCP can enhance the development of several apple fruit physiological disorders induced by chilling including firm flesh browning, flesh breakdown, and diffuse skin browning in 'Empire', 'Royal Gala', and 'Golden Delicious' (Jung et al., 2010; Larrigaudière et al., 2010; Lee et al., 2013, 2014; Watkins, 2008). These adverse physiological responses to low temperature following 1-MCP treatment may occur due to the lack of ethylene action required to metabolically respond to chilling temperatures (Lee et al., 2013). The increase in RSFB in 'Royal Gala' reported here and firm flesh browning in 'Empire' (Jung et al., 2010; Watkins, 2008) fruit previously exposed to 1-MCP then stored at low temperature suggests a unique fruit response to low temperature in the absence of ethylene action can lead to injury that results in development of symptoms unlikely to occur in fruit with a fully functional ethylene response system. The change in predominant

symptomology in 'Royal Gala' from DFB to RSFB in 1-MCP-treated fruit and a reversal of the pattern of temperature sensitivity (increased DFB with increased storage temperature in controls, decreased RSFB with increased storage temperature in 1-MCP-treated fruit) suggests an inherent low-temperature sensitivity for this cultivar that exists regardless of the state of fruit ethylene action. The negative and positive relationships between 1-MCP treatment concentration and DFB and RSFB, respectively, are consistent with a 1-MCP dose effect on inhibition of ethylene action. DFB has been considered to be a senescent disorder as its incidence is reduced in fruit previously treated with 1-MCP (Jung et al., 2010; Lee et al., 2012a, 2013) and 1-MCP treatment results in slowed apple fruit ripening and delayed senescence based on fruit quality attributes as well as reduced fruit ethylene production and lower respiration rate (Fan et al., 1999; Jung and Watkins, 2011; Watkins and Nock, 2012; Watkins et al., 2000). However, the occurrence of both disorders in the same fruit lot may suggest flesh breakdown is a physiological disorder with browning arising during a developmental period coincident with senescence rather than associated with senescent metabolism. This hypothesis is consistent with another apple fruit CI superficial scald

Table 6. Incidence and severity of diffuse flesh breakdown, radial stem-end flesh breakdown, shriveling, cracking, and peeling disorders in ‘Royal Gala’ apple exposed to 0, 0.25, 0.5, or 1 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 °C for 3 or 6 mo. (M) followed by 7 d at 20 °C.

Storage duration	1-MCP (μL·L ⁻¹)				1-MCP (μL·L ⁻¹)					
	0.0	0.25	0.5	1.0	0.0	0.25	0.5	1.0		
	Disorder incidence ^z (%)				Disorder severity ^y (0–5)					
Diffuse flesh breakdown										
3 M	0	0	0	0	0.0 d ^x	0.0 d	0.0 d	0.0 d		
3 M + 7 d	44	0	0	0	1.1 b	0.0 d	0.0 d	0.0 d		
6 M	25	8	6	0	0.4 c	0.2 cd	0.1 d	0.0 d		
6 M + 7 d	97	0	0	0	3.2 a	0.0 d	0.0 d	0.0 d		
Radial stem-end flesh breakdown										
3 M	0	8	8	0	0.0 f	0.1 f	0.1 f	0.0 f		
3 M + 7 d	0	44	64	78	0.0 f	0.6 e	1.0 d	1.2 d		
6 M	0	89	92	97	0.0 f	1.6 c	2.0 b	2.1 b		
6 M + 7 d	0	100	94	92	0.0 f	2.0 b	2.0 b	2.4 a		
Shriveling										
3 M	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
3 M + 7 d	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
6 M	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
6 M + 7 d	64	0	0	0	1.0 a	0.0 b	0.0 b	0.0 b		
Cracking										
3 M	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
3 M + 7d	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
6 M	3	14	3	0	0.1 b	0.1 b	0.1 b	0.0 b		
6 M + 7 d	69	6	6	11	1.2 a	0.1 b	0.1 b	0.1 b		
Peeling										
3 M	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
3 M + 7 d	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
6 M	0	0	0	0	0.0 b	0.0 b	0.0 b	0.0 b		
6 M + 7 d	28	0	0	0	0.3 a	0.0 b	0.0 b	0.0 b		
Sources	Diffuse breakdown		Radial breakdown		Shriveling		Cracking		Peeling	
	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
1-MCP (P)	****	****	****	****	****	****	****	****	****	****
Duration (D)	****	****	****	****	****	****	****	****	****	****
P × D	****	****	****	****	****	****	****	****	****	****

^zThe incidence of physiological disorders was expressed as percent fruit affected (n = 36).

^ySeverity rate of physiological disorders was subjectively evaluated by estimating the percentage of each incidence: 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.

^xMeans in each category followed by the same letters do not differ significantly, Duncan’s multiple range test at $P \leq 0.05$.

ns, ****Nonsignificant or significant at $P < 0.0001$.

1-MCP = 1-methylcyclopropene.

for which the initial events causing injury occur soon after harvest but symptom development follows months later after an extended cold-storage period (Lee et al., 2012b; Rudell et al., 2009).

RSFB is an uncommon pattern of apple CI. Symptoms in ‘Royal Gala’ and ‘Cripps Pink’ appear to be similar (James and Jobling, 2009). Diffuse and radial flesh browning in cold stored ‘Cripps Pink’ apples are influenced by tissue location with diffuse flesh browning occurring in cortex tissue and radial flesh browning in vascular tissue as well as by orchard location (James and Jobling, 2009).

Considering various applied 1-MCP levels, incidence and severity of RSFB increased with longer storage duration and increased 1-MCP treatment concentration at harvest. In contrast, apple fruit superficial scald severity increased with decreased 1-MCP treatment concentration (Argenta et al., 2007; Rupasinghe et al., 2000). The different symptomatic responses to a wide range of 1-MCP levels would be driven from the

different etiology of storage disorders in which can be mainly categorized into low temperature-associated CI and ripening and senescence-associated storage disorders (Watkins, 2007).

Flesh color darkening and yellowing were greatest in the stem-end region of untreated fruit stored at 3 °C, coincident with high disorder severity in the stem-end region similar to Lee et al. (2013). In contrast, color change in 1-MCP-treated fruit (Table 5) was much less consistent with a delay in fruit ripening and senescence. The same pattern was not observed in 1-MCP-treated ‘Empire’ apples (Lee et al., 2012a). This difference in cortex color change may reflect disorder etiology in which firm flesh browning in ‘Empire’ apples results from CI in long-term CA (Lee et al., 2012a), but fruit senescence and ripening contribute to provoking flesh breakdown in cold-stored ‘Royal Gala’ apples (Lee et al., 2013) as previously mentioned by Watkins (2007).

Of fruit physiological attributes, fruit weight loss and circumference change were

higher at higher storage temperature than at lower storage temperature but were suppressed by 1-MCP treatment (Table 1), regardless of 1-MCP dosage levels (Table 4). The increase in storage temperature was linked to the increase in fruit respiration during storage (Fidler and North, 1971), consequently leading to fruit weight loss. 1-MCP treatment reduced fresh weight loss during shelf life in ‘Tommy Atkins’ mangoes, regardless of 1-MCP dosage (Alves et al., 2004). The result also provides that 1-MCP application could contribute to reducing fruit physiological changes in terms of fruit weight loss and circumference change, irrespective of 1-MCP applied concentrations. Previously, the bigger in fruit size, the greater in fruit weight loss and circumference change in cold-stored ‘Royal Gala’ apples (Lee et al., 2013). In result, it is considered that storage temperature could be another crucial factor to be considered for controlling fruit cracking and flesh breakdown disorders. Additionally, fruit weight loss and fruit circumference change were

Fig. 3. Pearson correlation coefficient (r) matrices among the responses of fruit quality attributes and incidence and severity rate of physiological storage disorders in ‘Royal Gala’ apples exposed to 0, 0.25, 0.5, or 1 $\mu\text{L L}^{-1}$ 1-MCP at harvest then stored in air at 0.5 °C for 3 or 6 months followed by 20 °C for 7 d. Red and blue colors indicate positive and negative correlation coefficients between variables, respectively. 1-MCP = 1-methylcyclopropane.

highly correlated with the incidence of storage disorders (Figs. 2 and 3). The correlation between these two parameters and storage disorders was highly appeared in control fruit, especially in higher storage temperature. It is reported that higher storage temperature was involved in proceeding fruit senescence and ripening during storage in terms of the firmness loss (Mir et al., 2001). In turn, senescence- and ripening-associated storage disorders including core browning and internal browning were negatively

associated with firmness in cold- or CA-stored ‘Ambrosia’ and ‘Empire’ apples (Ehsani-Moghaddam and DeEll, 2009).

I_{AD} has been applied as a nondestructive approach for identifying fruit maturity and ripeness by indirectly measuring chlorophyll a content in fruit mesophyll cells (Ziosi et al., 2008) where increased chlorophyll a content was strongly associated with increased I_{AD} in outer mesocarp of ‘Stark Red Gold’ nectarines. The result suggests I_{AD} can be applied for the characterization

of fruit maturity and ripeness because there is a positive correlation between I_{AD} values and peel chlorophyll a content (Ziosi et al., 2008). Nevertheless, consistent responses of I_{AD} to internal apple fruit quality attributes were not evident (Toivonen and Hampson, 2014). In this study, I_{AD} was highest in 1-MCP-treated fruit during shelf life rather than during cold storage, regardless of storage temperature. These results indicate the 1-MCP delay in fruit chlorophyll loss is detectable using I_{AD} , and that there is no

apparent relationship between I_{AD} and internal disorder development.

In conclusion, senescence- and fruit ripening-associated disorders, DFB, shriveling, cracking, and peeling, were enhanced by a higher storage temperature. By contrast, 1-MCP treatment led to the development of CI-associated storage disorder in which incidence was radial pattern of flesh breakdown, mainly detected in stem-end tissue. Interestingly, the CI-associated incidence was only influenced by 1-MCP application rather than 1-MCP concentration within the range of concentrations applied. The physiological responses of fruit quality attributes during storage mirrored the development of different types of physiological disorders depending on storage temperature and 1-MCP application.

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