HortScience 48(12):1518–1524. 2013.

Fruit Size Affects Physiological Attributes and Storage Disorders in Cold-stored 'Royal Gala' Apples

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Additional index words. 1-MCP, cracking, flesh breakdown, stem-end browning

Abstract. **'Royal Gala' apple [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] fruit can be susceptible to the development of postharvest disorders such as flesh breakdown and cracking (splitting) during and after cold storage. The objective of this research was to investigate fruit size and 1-methylcyclopropene (1-MCP) treatment effects on fruit physiological attributes and incidence and severity of storage disorders in 'Royal Gala' apples held in cold storage. In 2011, fruit segregated at harvest into two groups based on size (120 to 175, 250 to 350 g/fruit) were stored in air at 0.5 °C for 6 months and then at 20 °C for 7 days. In 2012, fruit were sorted into four groups (less than 200, 200 to 240, 241 to 280, and greater than 280 g/fruit), treated with 0 or 1 $\mu L \cdot L^{-1}$ 1-MCP for 12 hours, and then stored in air at 0.5 °C for 3 or 6 months. Storage disorders were only detected at 6 months, regardless of 1-MCP treatment. In both control and 1-MCP-treated fruit, flesh breakdown incidence increased with fruit size, whereas severity was less associated with size. The progression of flesh breakdown developed in overall cortex tissue of control fruit but only detected in the stem-end tissue of 1-MCP-treated fruit. Internal ethylene concentration (IEC) decreased and CO_2 production increased with increased fruit weight; however, 1-MCP-treated fruit had low IEC regardless of weight. Cortex tissue lightness (L^*) increased with fruit size irrespective of tissue localization (stem end, equatorial, calyx end) at harvest. During 6 months' storage, L^* decreased with increased fruit size in controls but not 1-MCP-treated fruit. Fruit fresh weight loss increased with fruit size and storage duration, more so in controls when compared with 1-MCP-treated fruit. Furthermore, fruit circumference increased during storage with fruit size only for control fruit. These physical changes are associated with susceptibility of large fruit to flesh breakdown more so than small fruit. Reduced flesh breakdown incidence, progression of symptoms from the stem end into the cortex, and symptom severity in 1-MCP-treated fruit may indicate flesh breakdown is related to fruit ripening and senescence.**

'Royal Gala' [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] is one of the major apple cultivars produced worldwide. In North America, production is projected to continue to increase (U.S. Apple Association, 2010). The unique flavor and texture at harvest and after storage of 'Gala' apples continues to make this cultivar highly desirable to consumers (Boylston et al., 1994; Cliff et al., 1998).

'Gala' apples are susceptible to development of stem-end cracking (splitting) before and after harvest and disorder risk increases with advanced fruit maturity (Opara et al., 1997) and fruit ripening (Byers, 1998). Inhibition of ethylene production and slower fruit maturation after application of aminoethoxyvinylglycine reduces incidence of stem-end cracking (Byers, 1998). 'Gala' apples can also develop flesh breakdown during cold storage in air or a controlled atmosphere (CA) (Argenta et al., 2006; Johnson, 2000; Stow and Genge, 2000). CA storage CO_2 content (0, 1, or 5 kPa) with 1 kPa O_2 did not impact breakdown incidence (Johnson, 2000; Stow and Genge, 2000). The incidence of senescent breakdown in cold-stored 'Spartan' apple is associated with large fruit diameter (Lidster et al., 1975), and Johnston et al. (2002) reported that large 'Royal Gala' apples harvested at advanced maturity softened faster during cold storage compared with small fruit. Whether the increased rate of softening in large 'Royal Gala' apples reflects faster ripening and an earlier onset of senescent metabolism has not been reported.

The ethylene action inhibitor 1-MCP enhances maintenance of several aspects of 'Gala' fruit quality during and after storage (Mattheis et al., 2005; Moya-Leon et al., 2007). Consumer preference and acceptance of 1-MCP-treated 'Gala' apple is equal to or greater compared with untreated fruit (Marin et al., 2009). 1-MCP treatment helps maintain fruit quality during and after storage by delaying fruit ripening and retarding the loss of flesh firmness, acidity, and soluble solids concentration (Bai et al., 2005; Fan et al., 1999a). 1-MCP prevents apple superficial scald (Fan et al., 1999b; Rupasinghe et al., 2000; Watkins et al., 2000) and can reduce incidence of soft scald of 'Fuji' (Fan et al., 1999b) and 'Honeycrisp' apples (DeEll and Ehsani-Moghaddam, 2010) and senescent flesh browning of 'Gala', 'Imperial Gala', and 'Royal Gala' apple (Argenta et al., 2006). 1-MCP treatment can also exacerbate the development of certain storage disorders such as external CO_2 injury of 'Empire' (Fawbush et al., 2008) and 'McIntosh' apple (DeEll et al., 2003), firm flesh browning of 'Empire' (Lee et al., 2012), and core browning of 'Delicious' (DeEll et al., 2007).

The objective of this study was to investigate fruit size and 1-MCP treatment impacts on physical and physiological changes and the incidence of storage disorders such as senescent breakdown, stem-end browning, and cracking (splitting) in 'Royal Gala' apples stored in air at 0.5 °C.

Materials and Methods

Plant material

Fruit used in these experiments were harvested from 'Royal Gala' [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh) Mansf.] apple trees in a commercial orchard near Vantage, WA.

Expt. 1. Fruit harvested on 6 Sept. 2011 were transported to the laboratory in Wenatchee, WA, and blemish-free fruit (no cracks or mechanical damage) sorted based on fresh weight: 120 to 175 or 250 to 350 g/fruit, 18 fruit for each weight group. The weight ranges were selected to evaluate extremes in fruit weight on development of flesh breakdown and cracking. Fruit was placed on pressed fiber trays (18/tray) and trays packed inside a perforated polyethylene bag in a cardboard box. Fruit were stored in air at 0.5 °C with 90% relative humidity (RH) for 6 months followed by 20 °C for 7 d.

Expt. 2. Fruit harvested on 24 Aug. 2012 were transported to the laboratory and blemish-free fruit sorted based on fruit fresh weight: less than 200, 200 to 240, 241 to 280, or greater than 280 g/fruit, 18 fruit for each group. Additional size categories were added to evaluate a more continuous range of fruit weights. Fruit were treated with 0 or 1 $\mu L \cdot L^{-1}$ 1-MCP (SmartFresh™ powder, 3.8% a.i.; AgroFresh Inc., Spring House, PA) for 12 h on the day of harvest and then packed into cardboard boxes and stored as described for Expt. 1. Fruit were evaluated after storage for 3 or 6 months.

Harvest and quality assessments

Eighteen single fruit replicates were used to assess quality and disorders at harvest and after storage. Assessments of fresh weight, IEC, starch iodine index, flesh firmness, titratable acidity (TA), soluble solids concentration

Received for publication 2 Aug. 2013. Accepted for publication 25 Oct. 2013.
Financial support for this research was received from AgroFresh, Inc.
We thank David Buchanan and Janie Countryman for excellent technical assistance.
Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.
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(SSC), peel and flesh lightness, chroma, and hue angle were performed. For both experiments, fresh weight, fruit circumference, IEC, lightness, chroma, and hue angle were determined at harvest and on the day fruit were removed from storage after 6 months (6M+D0) or after an additional 7 d with fruit held at 20 °C (6M+D7). For Expt. 2, assessments were also conducted at 3 months. CO_2 production rate was determined after 6 months' cold storage and 7 d only for Expt. 1.

Fruit fresh weight and circumference were measured with an analytical balance and a tape measure, respectively. Measurements were conducted before and after storage on the same fruit. The difference calculation was based on values at harvest and those after storage. Internal ethylene concentration in a 0.5-mL gas sample taken from the core cavity was analyzed using a Hewlett-Packard 5880A gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and fitted with a 46 cm (length) × 0.32-cm (diameter) glass column packed with Porapack Q (Supelco Co., Bellefonte, PA). Flow rates for N_2 carrier, H_2, 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 was measured on an unblushed area of the fruit equator region using a chromameter (Minolta CR-200; Minolta Co., Osaka, Japan). Flesh color was assessed at the stem end (1.5 cm from stem end toward the equator cut horizontally), equator (at the fruit equator cut horizontally), and the calyx end (1.5 cm from the calyx end toward the equator cut horizontally) with six readings per region. Color measurements obtained as L*, a*, b* values were expressed as lightness (L^*), chroma (C^*), and hue angle (h^o) color space (McGuire, 1992).

Flesh firmness was assessed using a penetrometer (Mohr Digi-Test; Mohr & Associates, Richland, WA) equipped with a cylindrical plunger 11 mm in diameter (Evans et al., 2010). The measurement was carried out on opposite peeled sides of each equatorial region. The starch index was determined by dipping the cut surface of each fruit into a potassium–iodine (1.5% KI, 0.6% I) solution and assessed by rating hydrolysis of starch based on a scale from 1 (100% starch) to 6 (0% starch) (Brookfield et al., 1997).

Fig. 1. Symptoms of flesh breakdown in 'Royal Gala' apples. Fruit were exposed to 0 (control: **A, B**, and **C**) or 1 µL·L^{-1} 1-methylcyclopropene (1-MCP) (**D, E**, and **F**) at harvest and then stored in air at 0.5 °C after 6 months storage.

Table 1. 'Royal Gala' apple harvest indices in 2011 and 2012.[z]

Yr	Fresh wt[y] (g/fruit)	Log IEC (µmol·L^{-1})	Starch index (1–6)	Firmness (N)	TA[x] (%)	SSC (%)	Lightness[w] (L^*)	Chroma (C^*)	Hue angle (h^o)
2011	253	0.08	2.4	75.6	0.36	11.1	69.5	71.7	71.6
2012	252	0.02	1.7	84.8	0.31	9.8	72.8	75.6	91.5
Significance	NS	*	**	****	****	****	NS	***	****

[z]Fruit were obtained from a commercial orchard located near Vantage, WA.
[y]Values for fresh weight, log internal ethylene concentration (IEC), starch index, and flesh firmness are means, n = 18 fruit.
[x]Values for titratable acidity (TA) and soluble solids concentration (SSC) are means, n = 9 replicates of two fruit.
[w]Values for lightenss, chroma, and hue angle measured on unblushed (non-red) fruit skin are means, n = 18 fruit.
NS, *, **, ***, **** Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.

Table 2. Incidence, severity, and progression of flesh breakdown (internal browning) and cracking (splitting) and warming effect (6M+D7–6M+D0) of 'Royal Gala' apple fruit harvested in 2011 and then stored in air at 0.5 °C for 6 months (6M+D0) then 20 °C for 7 d (6M+D7).

	Flesh breakdown						Cracking			
	Incidence[z] (%)		Severity[y] (0–5)		Progression[x] (1–3)		Incidence (%)		Severity[w] (0–5)	
Storage duration	Small[v]	Large[u]	Small	Large	Small	Large	Small	Large	Small	Large
6M+D0	0.0	77.8	0.0 c[t]	2.6 b	1.0 c	2.6 b	0.0	44.4	0.0 d	1.4 b
6M+D7	61.1	88.9	2.6 b	3.8 a	2.2 b	3.0 a	5.6	72.2	1.0 c	2.6 a

	Flesh breakdown			Cracking	
Significance	Incidence	Severity	Progression	Incidence	Severity
Fruit size (S)	****	****	****	****	****
Duration (D)	****	****	****	****	****
S × D	****	****	****	****	****

[z]n = 18 fruit.
[y]Severity of flesh breakdown was subjectively evaluated by slicing fruit into five or six sections parallel to the fruit equator and estimating the percent brown area on the slice with the largest area of browning: 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.
[x]Progression of flesh breakdown was rated as: 1 = no browning, 2 = browning within 1.5 cm of the stem end, 3 = browning extending past 1.5 cm of the stem end.
[w]Severity of cracking was subjectively evaluated by estimating the percent cracked peel: 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.
[v]Fresh weight small fruit 120 to 175 g at harvest.
[u]Fresh weight large fruit 250 to 350 g at harvest.
[t]Means in each category followed by the same letters do not differ significantly, Duncan's multiple range test, $P = 0.05$.
****Significant at $P < 0.0001$.

SSC and TA of freshly prepared juice were determined using an analog refractometer (Atago N1; Atago Co. Ltd., Tokyo, Japan) and autotitrator (TIM850, SAC80; Hach Co., Loveland, CO). TA was determined using juice extracted from composite samples of two segments per two fruit titrated using 0.1 M KOH to pH 8.2.

The CO_2 production rate was determined using three replicates of five fruit placed into 3.79-L glass jars sealed with Teflon lids with two gas ports. Jars were purged with air at 1.7 mL·s^{-1} for 1 h, then 3 mL head space gas collected from the lid outlet port was used for CO_2 analysis by a Hewlett Packard 5890 gas chromatograph (Agilent, Palo Alto, CA) equipped with a 0.5 m, 3.2-mm i.d. stainless steel column packed with Porapak Q (Supelco, Bellefonte, PA), a methanizer (John Booker & Co., Austin, TX), and a flame ionization detector. The N_2 carrier, H_2, and air flows were 0.5, 0.5, and 5 mL·s^{-1}, respectively. Oven and injector temperatures were 35 and 300 °C, respectively. The methanizer temperature was 290 °C controlled by an Instrumentation Temperature Controller (Valco Instruments, Inc., Houston, TX) with a H_2 flow of 0.5 mL·s^{-1}.

Each fruit was assessed for the incidence and severity of peel cracking and then horizontally sliced into five or six sections to evaluate the incidence and severity of internal flesh breakdown (Fig. 1). Disorder incidence is expressed as percent fruit affected (n = 18). For fruit with disorders, symptom severity was scored as 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100% affected area of the peel or the slice with the largest area with browning. Progression of flesh breakdown was rated as: 1 = no browning; 2 = browning within 1.5 cm of the stem end; and 3 = browning extending past 1.5 cm of the stem end.

Statistical analysis

Harvest data were subjected to one-way analysis of variance (ANOVA) and storage data to two-way ANOVA using the general linear model (Proc GLM) to determine main effects and interactions (Version 9.3; SAS Institute Inc., Cary, NC). The Duncan's multiple range test was used to compare means significant at $P = 0.05$. IEC values were transformed to logarithm before statistical analysis, and values in tables are transformed means.

Results

IEC, starch index, and firmness at harvest indicated fruit had reached physiological maturity and had begun to ripen (Table 1). Fruit at harvest in 2012 had lower IEC, starch index, TA, and SSC but higher firmness compared with 2011, whereas fruit size and fruit peel L^* were similar at harvest in both seasons.

Expt. 1. The incidence, severity, and progression of flesh breakdown and cracking were greater for larger fruit (Table 2). Incidence and severity of both storage disorders increased for both large and small fruit during 7 d at 20 °C after fruit were removed from storage. IEC was similar at harvest regardless of fruit size but significantly higher in smaller fruit after storage (Table 3). Although IEC of small fruit did not change during 7 d at 20 °C, large fruit IEC decreased during the same period. Respiration rate increased with fruit weight and post-storage ripening.

Fruit size, storage duration, and tissue localization impacted L^*, C^*, and h^o (Table 4). For L^* and h^o, two-way interactions exist among fruit size, storage duration, and tissue localization. L^* and h^o of stem-end cortex tissue of large fruit after post-storage ripening was lower compared with small fruit. Only L^* had a significant three-way interaction.

Circumference of small fruit held 7 d after removal from cold storage and all large fruit increased relative to values at harvest (Table 5). The increase was greater for large compared with small fruit during both storage and ripening. The loss of fruit fresh weight was greater in larger fruit, regardless

Table 3. Log internal ethylene concentration (IEC) and CO_2 production of 'Royal Gala' apple fruit at harvest or after storage in air at 0.5 °C for 6 months (6M+D0) and then 20 °C for 7 d (6M+D7).[z]

	Log IEC (µmol·L^{-1})		CO_2 production (µmol CO_2/kg·h^{-1})	
Storage duration	Small[y]	Large[x]	Small	Large
0M	0.02[w] c[v]	0.03 c	—	—
6M+D0	2.15 a	0.76 b	150[u] d	200 c
6M+D7	2.34 a	0.09 c	393 b	494 a

Significance	Log IEC	CO_2 production
Fruit size (S)	****	****
Duration (D)	****	****
S × D	****	*

[z]Apples harvested in 2011.
[y]Fresh weight small fruit 120 to 175 g at harvest.
[x]Fresh weight large fruit 250 to 350 g at harvest.
[w]Values are means of 18 fruit (n = 18).
[v]Means in each category followed by the same letters do not differ significantly, Duncan's multiple range test, $P = 0.05$.
[u]Values are means of 3 replicates (n = 3) of five fruit.
*, ****Significant at $P < 0.05$ or 0.0001, respectively.

Table 4. Lightness (L^*), chroma (C^*), and hue angle (h^o) of stem-end, equator, and calyx-end tissue[z] of 'Royal Gala' apple at harvest after storage in air at 0.5 °C for 6 months (6M+D0) and after 7 d at 20 °C (6M+D7).[y]

	Small[x]			Large[w]		
Storage duration	Stem end	Equator	Calyx end	Stem end	Equator	Calyx end
			Lightness (L^*)			
0M	83.2[v]	83.6	82.0	82.6	83.6	82.8
6M+D0	82.2	82.9	81.6	81.2	82.0	81.7
6M+D7	79.9	82.5	80.7	75.0	80.7	80.8
			Chroma (C^*)			
0M	19.3	15.7	17.0	21.6	18.3	18.7
6M+D0	19.9	17.3	19.7	22.8	20.6	23.0
6M+D7	21.2	19.3	21.8	25.5	22.3	25.3
			Hue angle (h^o)			
0M	106.2	107.3	106.7	104.6	105.9	106.3
6M+D0	103.6	105.0	104.3	98.4	101.9	102.1
6M+D7	97.9	101.9	101.0	90.1	96.8	97.6

Significance	Lightness (L^*)	Chroma (C^*)	Hue angle (h^o)
Fruit size (S)	****	****	****
Duration (D)	****	****	****
Localization (L)	****	****	****
S × L	****	NS	****
S × D	****	NS	***
D × L	****	**	****
S × D × L	**	NS	NS

[z]Stem end: cortex tissue 1.5 cm from the stem end toward the equator cut horizontally; Equator: cortex tissues at the fruit equator cut horizontally; Calyx end: cortex tissue 1.5 cm from the calyx end toward the equator cut horizontally.
[y]Apples harvested in 2011.
[x]Fresh weight small fruit 120 to 175 g at harvest.
[w]Fresh weight large fruit 250 to 350 g at harvest.
[v]Values are the mean of 18 fruit (n = 18), six measurements per fruit.
NS, **, ***, **** Nonsignificant or significant at $P < 0.01$, 0.001, or 0.0001, respectively.

of cold storage and post-storage ripening with most fresh weight loss during post-storage ripening.

Expt. 2. No disorders were observed at 3 months in storage (data not presented) but flesh breakdown was present after 6 months in control fruit and incidence, severity, and progression of flesh breakdown were higher in control fruit compared with 1-MCP-treated fruit (Table 6). Incidence of flesh breakdown was higher in larger fruit, regardless of 1-MCP treatment. Fruit treated with 1-MCP had flesh breakdown only in the stem-end region and only in fruit larger than 241 g at harvest. Severity of flesh breakdown in 1-MCP-treated fruit was slight (Fig. 1).

IEC was significantly affected by 1-MCP, storage duration, and fruit size (Table 7). Although IEC was unaffected by fruit size at harvest, IEC was lowest in larger control fruit after storage. Fruit treated with 1-MCP had lower IEC compared with controls regardless of size or storage duration. Control fruit IEC was lower at 6 months than at 3 months, although it was higher for 1-MCP-treated fruit after 6 months. Two-way and three-way interactions were found among 1-MCP treatment, storage duration, and fruit size. After 3 months, fruit circumference decreased regardless of 1-MCP treatment in all but the largest control fruit (data not presented). By contrast, fruit circumference increased with fruit size after 6 months' storage for control fruit; however, 1-MCP-treated fruit were smaller after storage compared with harvest. Change in fruit circumference in 1-MCP-treated fruit decreased with fruit size. Fresh weight decreased with storage duration and fruit size, irrespective of 1-MCP treatment. The change in fruit fresh weight was less for 1-MCP-treated fruit compared with controls.

L^* was significantly affected by 1-MCP treatment and tissue localization and two-way interactions such as treatment × storage duration, treatment × tissue localization, storage duration × fruit size, and storage duration × tissue localization were also significant (Table 8). L^* of stem-end cortex tissue after 6 months for 241 to 280 g/fruit was lowest. The main effects and many two-way interactions were significant for C^* and h^o. Non-significant two-way interactions included 1-MCP treatment and tissue localization for h^o and fruit size and tissue localization for C^* and h^o. Hue angle (h^o) was lowest in control fruit flesh at 6 months. However, h^o of 1-MCP-treated flesh after 6 months segregated into two groups where h^o was higher in fruit less than 240 g.

Table 5. Difference in fruit circumference and fresh weight between 'Royal Gala' apples at harvest and after storage in air at 0.5 °C for 6 months (6M+D0) and then at 20 °C for 7 d (6M+D7).[z]

	ΔFruit circumference[y] (mm/fruit)		ΔFresh wt (g/fruit)	
Storage duration	Small[x]	Large[w]	Small	Large
H-(6M+D0)	0.7 b[v]	– 5.2 a	5.2 b	9.1 b
H-(6M+D7)	– 0.3 b	– 4.9 a	8.6 b	31.6 a

Significance	ΔFruit circumference	ΔFresh weight
Size (S)	****	****
Duration (D)	****	NS
S × D	****	NS

[z]Apples harvested in 2011.
[y]Circumference and fresh weight difference = harvest value – post-storage value (n = 18).
[x]Fresh weight small fruit 120 to 175 g at harvest.
[w]Fresh weight large fruit 250 to 350 g at harvest.
[v]Means in each category followed by the same letters do not differ significantly, Duncan's multiple range test, $P = 0.05$.
NS, **** Nonsignificant or significant at $P < 0.0001$.

Table 6. The incidence, severity, and progression of flesh breakdown in 'Royal Gala' apples exposed to 0 or 1μL·L^{-1} 1-MCP at harvest and stored in air at 0.5 °C for 6 months.[z]

	Less than 200 g/fruit		200 to 240 g/fruit		241 to 280 g/fruit		Greater than 280 g/fruit	
Flesh breakdown	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP
Incidence (%)	16.7	0.0	16.7	0.0	50.0	38.9	61.1	44.4
Severity[y] (0–5)	2.0 b[w]	0.0 d	4.0 a	0.0 d	2.4 b	1.4 c	2.3 b	1.1 c
Progression[x] (1–3)	1.3 cd	1.0 d	1.3 cd	1.0 d	1.9 b	1.4 c	2.2 a	1.4 c

Significance	Incidence	Severity	Progression
Treatment (T)	****	****	****
Storage duration (D)	****	****	****
Fruit size (S)	NS	NS	***
T × D	****	****	****
T × S	NS	*	NS
D × S	NS	*	****
T × D × S	NS	*	NS

[z]Apples harvested in 2012.
[y]Severity of flesh breakdown was subjectively evaluated by slicing fruit into five or six sections parallel to the fruit equator and estimating the % brown area on the slice with the largest area of browning: 0 = 0%, 1 = 1% to 10%, 2 = 11% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100%.
[x]Progression of flesh breakdown was rated as: 1 = no browning, 2 = browning within 1.5 cm of the stem end, 3 = browning extending past 1.5 cm of the stem end.
[w]Means in each category followed by the same letters do not differ significantly, Duncan's multiple range test, $P = 0.05$.
NS, *, ***, **** Nonsignificant or significant at $P < 0.05$, 0.001, or 0.0001, respectively.
1-MCP = 1-methylcyclopropene.

Discussion

Susceptibility to flesh breakdown and cracking in 'Royal Gala' apple is shown to be associated with fruit size, storage duration, 1-MCP treatment at harvest, and ripening at 20 °C after cold storage. Disorder incidence and severity are likely to be associated with physical fruit attributes. Breakdown symptoms are initially visible at the fruit stem end and progress over time in storage toward the fruit equator. The increased fruit area with browning symptoms is consistent with injury resulting from the progression of fruit ripening and senescence (Argenta et al., 2006) because flesh breakdown symptoms were only detected in the stem-end region of 1-MCP-treated fruit where ripening and senescence are delayed.

Large fruit had the highest incidence of flesh breakdown and cracking in both experiments but severity was higher in Expt. 1. Johnston et al. (2002) reported that large fruit softened more rapidly compared with small fruit when harvested at advanced maturity, and earlier onset of maturation and ripening in larger fruit has also been observed (Harker et al., 1997). Based on these previous results, larger fruit typically soften and ripen sooner after harvest compared with small fruit and, therefore, may be at a higher risk for developing flesh breakdown and cracking related to ripening and senescence. The results reported here indicate fruit size did not impact at-harvest IEC, firmness, or starch pattern index within the same year (data not shown), although fruit IEC and quality attributes were different between the 2 years with fruit more mature in Expt. 1. A starch pattern index of 2.5 to 4 (1 to 6 scale, 1 = no starch clearing, 6 = all starch cleared) assures good eating quality after various periods of storage (Plotto et al., 1995). The difference in harvest maturity could be a factor contributing to the lack of cracking and lower incidence of flesh breakdown in Expt. 2. In 'Honeycrisp' apple, development of soft scald and soggy breakdown is more likely in fruit with advanced maturity at harvest (Watkins et al., 2005). Larger fruit can be comprised of larger cells (Malladi and Hirst, 2010), and larger cell size is associated with reduced cell number per unit area (Mann et al., 2005). Large cell size may reduce the amount of cell wall matrix components and the overall surface area of cell-to-cell attachments (Harker et al., 1997); therefore, larger fruit may soften more rapidly. Consequently, accelerated ripening could

Table 7. Log internal ethylene concentration (IEC) and difference in fruit circumference (ΔFruit circumference) and fresh weight (ΔFruit fresh weight) between 'Royal Gala' apples at harvest exposed to 0 or 1 μL·L^{-1} 1-MCP and after storage in air at 0.5 °C for 6 months.[z]

	Less than 200 g/fruit		200 to 240 g/fruit		241 to 280 g/fruit		Greater than 280 g/fruit	
	Control	1-MCP	Control	1-MCP	Control	1-MCP	Control	1-MCP
Log IEC (μmol·L^{-1})	1.06[y] a[v]	0.17 e	0.79 b	0.25 e	0.62 c	0.24 e	0.44 d	0.19 e
ΔFruit circumference[x]	– 0.5 c	1.1 e	– 1.3 bc	1.4 e	– 1.8 b	0.9 e	– 3.1 a	0.3 d
ΔFruit fresh weight[w]	5.0 d	4.5 de	6.3 c	4.8 d	7.5 b	7.3 b	8.5 a	7.1 b

Significance	Log IEC	Fruit circumference	Fruit fresh weight
Treatment (T)	****	****	****
Storage duration (D)	****	****	****
Fruit size (S)	****	****	****
T × D	****	**	****
T × S	****	*	**
D × S	****	**	*
T × D × S	**	NS	NS

[z]Apples harvested in 2012.
[y]Values are means of 18 fruit (n = 18).
[x]Fruit circumference difference (mm/fruit) = harvest value – post-storage value (n = 18).
[w]Fresh weight difference (g/fruit) = harvest value – post-storage value (n = 18).
[v]Means in each category followed by the same letters do not differ significantly, Duncan's multiple range test, $P = 0.05$.
NS, *, **, **** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.0001, respectively.
1-MCP = 1-methylcyclopropene.

Table 8. Lightness (L^*), chroma (C^*), and hue angle (h^o) of the stem-end (stem), equator, and calyx-end (calyx) cortex tissue[z] in 'Royal Gala' apples exposed to 0 or 1 μL·L^{-1} 1-MCP at harvest (0M) and then stored in air at 0.5 °C for 3 (3M) or 6 months (6M).[y]

		Less than 200 g/fruit			200 to 240 g/fruit			241 to 280 g/fruit			Greater than 80 g/fruit		
Storage duration	Treatment	Stem	Equator	Calyx	Stem	Equator	Calyx	Stem	Equator	Calyx	Stem	Equator	Calyx
							Lightness (L^*)						
0M	Control	81.7	82.9	81.7	82.0	83.6	82.1	82.3	83.7	82.6	82.9	84.1	82.8
3M	Control	82.1	84.0	81.9	81.9	83.8	81.5	82.3	83.7	81.6	81.9	83.2	81.3
	1-MCP	80.7	82.0	79.5	81.0	82.3	79.6	81.1	82.1	80.1	81.4	82.5	80.3
6M	Control	82.2	84.6	82.6	81.9	84.3	82.2	77.3	83.8	81.7	81.1	83.1	81.3
	1-MCP	80.9	82.3	80.2	81.1	82.8	80.9	81.7	82.5	80.6	81.0	82.4	80.7
							Chroma (C^*)						
0M	Control	15.1	11.8	12.2	17.5	13.3	13.5	19.1	14.8	14.2	19.5	15.4	15.5
3M	Control	21.4	19.1	22.1	21.0	18.8	22.3	21.7	19.9	23.0	22.2	20.2	24.0
	1-MCP	19.4	18.0	19.5	19.8	17.9	19.5	21.2	19.6	21.7	21.5	19.6	21.5
6M	Control	22.0	18.9	22.0	22.5	19.2	22.6	23.2	19.8	23.0	23.7	20.8	23.9
	1-MCP	21.1	18.9	20.9	21.5	19.4	21.4	23.0	20.6	23.5	23.3	20.1	22.4
							Hue angle (h^o)						
0M	Control	108.1	108.7	108.7	107.0	108.3	108.2	105.7	107.0	107.1	106.0	107.0	107.1
3M	Control	104.6	105.3	104.4	104.6	105.3	104.4	103.8	104.6	104.0	102.7	103.4	102.5
	1-MCP	105.6	106.4	105.6	104.9	105.9	105.1	103.1	103.9	103.3	103.7	104.6	104.3
6M	Control	102.6	104.7	104.1	101.1	103.5	102.6	99.6	102.2	101.6	98.4	101.0	100.3
	1-MCP	104.1	104.9	104.5	103.4	104.5	104.1	100.6	102.0	101.4	101.4	103.2	102.9

Significance	Lightness (L^*)	Chroma (C^*)	Hue angle (h^o)
Treatment (T)	****	****	****
Storage duration (D)	NS	****	****
Fruit size (S)	NS	****	****
Tissue localization (L)	****	****	****
T × D	NS	***	**
T × S	**	**	****
T × L	**	***	NS
D × S	****	****	****
D × L	***	****	***
S × L	NS	NS	NS
T × D × S	NS	NS	NS
T × D × L	NS	NS	NS
T × S × L	NS	NS	NS
D × S × L	NS	NS	NS
T × D × S × L	NS	NS	NS

[z]Apples harvested in 2012.
[y]Stem end: cortex tissue 1.5 cm from the stem end toward the equator cut horizontally; Equator: cortex tissue at the fruit equator cut horizontally; Calyx end: cortex tissue 1.5 cm from the calyx end toward the equator cut horizontally. Values are means of 18 fruit, six measurements per fruit.
NS, *, **, ***, **** Nonsignificant or significant at $P < 0.05$, 0.01, 0.001, or 0.0001, respectively.
1-MCP = 1-methylcyclopropene.

contribute to a higher risk of flesh breakdown and cracking during and after storage.

Increased incidence and severity of physiological disorders during storage is also associated with physical and physiological changes in flesh color, fresh weight loss, and fruit circumference change. Flesh breakdown development increased as flesh tissue L^* and h^o decreased. These results are consistent with those for 'Empire' apples where 1-MCP treatment reduced the change in fruit L^* and h^o during CA storage (Lee et al., 2012). The

change in flesh tissue color during storage coincides with development of internal browning disorders such as firm flesh browning (Lee et al., 2012), senescent breakdown, and stem-end browning. Flesh tissue browning results from the enzymatic oxidation of phenolic compounds by polyphenol oxidase after cell death during storage (Toivonen and Brummell, 2008). Cell death in apple fruit flesh tissue develops into numerous distinct browning disorders during storage associated with different disorder characteristics dependent on cultivar, storage conditions, pre-storage treatment, and tissue location. The current results support fruit size as an additional factor contributing to disorder susceptibility.

Fruit cracking was only detected in Expt. 1. Because all fruit in both experiments were placed inside perforated polyethylene box liners before storage to minimize moisture loss and maintain a consistent RH during cold storage, season and maturity at harvest rather than storage conditions may have contributed to cracking susceptibility. Apple fruit typically lose weight and shrink during storage; however, cracks in 'Royal Gala' apples developing before or after harvest or during storage can result in increased fruit circumference and weight loss. The incidence and severity of cracking in this study were highly associated with fruit size. Cracking at harvest can result from suboptimum fruit mineral content, particularly for calcium and potassium (Perring, 1984). Knoche and Grimm (2008) reported microcracking of 'Golden Delicious' cuticle increased with storage duration but differed among apple cultivars with the number of microcracks 2-fold higher in 'Braeburn' compared with 'Idared'. The number of microcracks might be dependent on cultivar and/or total fruit surface area. Because large fruit has greater surface area compared with small fruit, large fruit may be more likely to crack. Under equivalent conditions of RH, larger fruit may be more likely to develop cracks. The lack of cracking in the second year of this study may indicate the importance of harvest maturity as a factor influencing cracking because fruit in Year 2 were less mature at harvest. Furthermore, the increase in fruit circumference during storage was coincident with increased fresh weight loss possibly resulting from increased fruit surface area resulting from crack development. A greater microcracking area of the cuticle proportionally enhances water vapor permeance of apples (Maguire et al., 1999).

IEC and CO_2 production mirrored the severity of flesh breakdown. IEC at harvest was similar in both experiments, but other maturity indices, including starch, firmness, SSC, TA, and h^o, indicated fruit used for Expt. 2 were less mature compared with Expt. 1 fruit. The difference in maturity could have contributed to lower IEC after storage and ripening in Expt. 2 compared with Expt. 1, regardless of fruit size. CO_2 production reflected the incidence and severity of physiological disorders in Expt. 1 and the higher respiration rate coincided with greater weight loss. The increase in CO_2 production could hasten the loss of fruit fresh weight through loss of carbohydrate as well as by the physical disruption of peel tissue resulting from cracking and the increased surface area over which water can be lost to diffusion through the peel. The increase in CO_2 production also is indicative of the progression of ripening associated with increased incidence and severity of flesh breakdown.

Fruit treated with 1-MCP had less flesh breakdown and the symptoms were only detected in the stem-end tissue compared with untreated controls. These results are in contrast to a stem-end localized flesh breakdown disorder induced by 1-MCP treatment in 'Empire' apple (Lee et al., 2012). This difference might be driven from a different storage condition, in which 'Royal Gala' apples were stored in air at 0.5 °C, whereas 'Empire' apples were held at 2 kPa CO_2 and O_2 at 0.5 °C. 1-MCP treatment delays fruit ripening and softening during storage in terms of the responses of flesh firmness, ethylene, and CO_2 production (Fan et al., 1999a).

In conclusion, fruit size contributed to differential development of flesh breakdown and cracking. Inhibition of ethylene action by 1-MCP treatment reduced the incidence of flesh breakdown and cracking, and 1-MCP-treated fruit developed flesh breakdown only in the stem-end localized tissue. Furthermore, ripening of larger fruit was more rapid based on IEC and CO_2 production, L^*, and h^o. Cracking incidence may play a pivotal role in enhancing weight loss. Flesh color changed alongside symptom development in cortex tissue with flesh breakdown disorder.

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