Effects of Low Oxygen and 1-Methylcyclopropene on Storage Disorders of ‘Empire’ Apples

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Abstract. The objective of this study was to investigate the effects of low-oxygen storage and 1-methylcyclopropene (1-MCP) on disorders and quality of ‘Empire’ apples. For 2 years, ‘Empire’ apples were obtained from commercial orchards during their harvesting period. After cooling overnight at 3 °C, the apples were treated with or without 1-MCP (1 $\mu\text{L}\cdot\text{L}^{-1}$) for 24 hours and subsequently stored in controlled atmosphere (CA) with 2.5 kPa O₂ (+2 kPa CO₂) or 1.5 kPa O₂ (+1.2 kPa CO₂) for 8 months at 1.5 and 3 °C for the first and second year, respectively. In the second year, a third group of the ‘Empire’ apples was also held in respiratory quotient (RQ)-based dynamic CA storage (SafePod) that reached 0.6 kPa O₂ (+0.5 kPa CO₂), and half of these apples were treated with 1-MCP (1 $\mu\text{L}\cdot\text{L}^{-1}$) for 24 hours at 3 °C upon removal after 8 months. All apples were then evaluated for disorders and quality after 1, 7, or 14 days at room temperature (RT, 23 to 24 °C). Substantial external CO₂ injury, flesh browning, and core browning (up to 38% incidence) developed in ‘Empire’ stored in 2.5 and 1.5 kPa O₂ during both years of study. Storage in 1.5 kPa O₂ reduced flesh browning in the first year and core browning during the second year in apples without 1-MCP, as compared to storage in 2.5 kPa O₂. 1-MCP-treated apples stored in 2.5 or 1.5 kPa O₂ had higher overall incidence of disorders than similar fruit without 1-MCP. In contrast, there was negligible incidence (0% to 1%) of these disorders in ‘Empire’ apples held in 0.6 kPa O₂, regardless of 1-MCP treatment upon removal. Storage in 0.6 kPa O₂ also resulted in the greatest fruit firmness retention while at RT for 14 days. This regime can provide flexibility to postpone 1-MCP treatment until after storage, to prevent increased susceptibility to disorders during storage, without compromising fruit quality. However, results from the RQ-based dynamic CA with 0.6 kPa O₂ were from a single season, and further research is needed to confirm these observations.

‘Empire’ is a major commercial apple cultivar produced in the northeastern United States and Canada, as it appeals to consumers for fresh quality attributes, texture, and flavor profile. Despite its popularity, ‘Empire’ apples are sensitive to elevated CO₂ and chilling conditions, making them susceptible to certain physiological disorders in storage, including external CO₂ injury and flesh browning (DeEll and Ehsani-Moghaddam, 2012; Fawbush et al., 2008; Watkins and Nock, 2012).

External CO₂ injury is characterized by rough bronze lesions that are often partially

sunken on the peel with well-defined edges (Fawbush et al., 2008; Meheriuk et al., 1994; Watkins et al., 1997). Development of external CO₂ injury usually occurs within the early stages of CA storage and it is more prevalent with rapid establishment of elevated CO₂ levels, especially if apples are not sufficiently cooled before storage (Burneister and Dilley, 1995; DeEll et al., 2016; Meheriuk et al., 1994; Watkins and Liu, 2010; Watkins et al., 1997). In most cases, symptoms of external CO₂ injury progress with minimal internal flesh browning or damage.

Flesh browning, also known as internal browning, is characterized by diffuse browning of the flesh tissue, and it is typically not visible from the external surface (DeEll and Ehsani-Moghaddam, 2012; DeEll et al., 2007; Meheriuk et al., 1994; Watkins and Liu, 2010). The onset of flesh browning in apples is postulated to be associated with low temperatures during storage (Jung and Watkins, 2011; Watkins and Liu, 2010).

Postharvest treatment with 1-MCP, an inhibitor of ethylene action, has been shown to improve quality characteristics of apples, including reduced ethylene production and respiration, as well as improved firmness and acidity retention (DeEll et al., 2007; Watkins,

2007). Unfortunately, 1-MCP can also exacerbate the susceptibility of ‘Empire’ apples to external CO₂ injury and flesh browning disorders (DeEll and Ehsani-Moghaddam, 2012; DeEll et al., 2003, 2005; Fawbush et al., 2008).

Previous studies have reported the practice of delaying CA storage to be beneficial for reducing disorders in apples (DeEll and Ehsani-Moghaddam, 2012; Watkins and Nock, 2012). Delayed CA storage involves delaying the establishment of CA by holding fruit in ambient air at low temperatures before exposure to low O₂ and elevated CO₂ (Argenta et al., 2000; de Castro et al., 2007). Delaying CA for 1 or 2 months at 1 °C reduced external CO₂ injury and flesh browning disorders in ‘Empire’ apples compared to fruit placed immediately into CA (DeEll and Ehsani-Moghaddam, 2012). Similarly, delaying CA for 2 or 4 weeks at 0.5 °C reduced CO₂-induced flesh browning in ‘Pink Lady’ apples (de Castro et al., 2007). However, inconsistencies and year-to-year variation in the effects of delayed CA establishment for alleviating certain storage disorders in apples have also been documented (Argenta et al., 2000; DeEll and Ehsani-Moghaddam, 2012; DeEll et al., 2016; Watkins and Nock, 2012).

In recent years, there has been a renewed interest in low O₂ storage of apples due to the advent of dynamic CA in which fruit response to stress is monitored. The application of low O₂ (<2 kPa) during storage has shown advantages for maintaining apple quality characteristics, including reduced ethylene production and respiration, maintained fruit firmness, sugars and acidity levels, and delayed fruit senescence (Both et al., 2016; Thewes et al., 2015). Exposure to low O₂ can also alleviate superficial scald in certain susceptible apple cultivars (Lumpkin et al., 2014; Pesis et al., 2007; Wang and Dilley, 2000; Zanella, 2003). When apples are held in standard CA (2–3 kPa O₂), fruit are generally held in atmospheric conditions greater than their anaerobic compensation point, the point where fruit O₂ consumption and CO₂ production are at minimal rates (Thewes et al., 2015; Yearsley et al., 1996). Consequently, fruit respiration and associated metabolic processes are not at minimum, leading to potential fruit quality loss in standard CA storage. However, further reduction of O₂ levels during storage also raises concerns for increased low O₂-related stress and injury (Wright et al., 2015).

Fruit RQ, defined as the ratio of CO₂ production to O₂ consumption, can be used to assess low O₂-related stress in fruit and as a signal to adjust atmospheric composition within storage systems (Gran and Beaudry, 1993; Yearsley et al., 1996). By monitoring O₂-related stress in real-time with adjustments of O₂ partial pressures throughout the storage period, the respiratory health of apples under low O₂ can be observed autonomously. This is considered dynamic CA storage, which involves the reduction of O₂ partial pressures to the lowest possible tolerance level without inducing excess anaerobic

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metabolism affecting fruit quality and off-flavours associated with fermentation products (Bessemans et al., 2016; Thewes et al., 2015).

Although the benefits of low O₂ storage on apple quality are well-documented in the literature (Bessemans et al., 2016; Köpcke, 2015; Lumpkin et al., 2014; Rebeaud and Gasser, 2015; Veltman et al., 2003; Zanella, 2003), the response of a CO₂ and chilling sensitive apple like 'Empire' to low O₂ levels (<2 kPa) in combination with 1-MCP is not understood. The objective of this study was to investigate the effects of low O₂ and 1-MCP on storage disorders and fruit quality of 'Empire' apples. RQ-based dynamic CA storage with 0.6 kPa O₂ was also evaluated in the second year of this study.

Materials and Methods

Plant material. 'Empire' apples were harvested from two orchards on Sept. 27 in 2013 and a single orchard on Sept. 29 in 2015 located near Simcoe (Norfolk County), Ontario, Canada. There were 24 and 16 boxes of apples (≈13 kg) harvested in 2013 and 2015, respectively, with three box replicates per treatment for each orchard, except for two box replicates per treatment for fruit held in RQ-based dynamic CA storage (SafePod™; Storage Control Systems Inc., Sparta, MI) in the second year. Each box contained fruit from several trees and from various locations within the trees. All apples were transported within 1 h of harvest to the nearby apple storage research facility and cooled overnight at 3 °C.

Postharvest treatments. In the first year of study, after cooling overnight at 3 °C, half of the apples (12 boxes) were treated with 1-MCP (1 µL·L⁻¹) for 24 h. 1-MCP application was made using SmartFresh™ tablets (AgroFresh Inc., Collegeville, PA) within an air-tight treatment tent (Storage Control Systems Inc., Sparta, MI) at 3 °C. Thereafter, three boxes of 'Empire' apples with and three boxes without 1-MCP treatment from each orchard were placed into CA storage of either 2.5 kPa O₂ + 2 kPa CO₂ (standard) or 1.5 kPa O₂ + 1.2 kPa CO₂ (low O₂) at 1.5 °C for 8 months.

In the second year of study, after cooling overnight at 3 °C, six boxes of apples were treated with 1-MCP (1 µL·L⁻¹) for 24 h. Thereafter, three boxes of 'Empire' apples with and three boxes without 1-MCP treatment were placed into CA storage of either 2.5 kPa O₂ + 2 kPa CO₂ or 1.5 kPa O₂ + 1.2 kPa CO₂ at 3 °C for 8 months. The four remaining boxes of apples without 1-MCP treatment were held in a novel RQ-based dynamic CA storage regime at 3 °C, in which the O₂ was initially pulled down to 1.5 kPa and then gradually decreased to 0.6 kPa O₂ (+0.5 kPa CO₂) within 2 to 3 months. After 8 months of storage, half of the apples (two boxes) from 0.6 kPa O₂ were then treated with 1-MCP (1 µL·L⁻¹) for 24 h at 3 °C.

The CA storage system consisted of small aluminum storage chambers (0.9 m³ volume) fitted with a circulating fan system (Storage Control Systems Inc., Sparta, MI). Atmospheres

were checked hourly and maintained within 0.2 kPa of target values using an ICA 61/CGS 610 CA Control System (International Controlled Atmosphere Ltd., Kent, UK), which was modified with flow controllers for the experimental chambers (Storage Control Systems Inc., Sparta, MI). The RQ-based dynamic CA storage system consisted of SafePod™ technology (Storage Control Systems Inc., Sparta, MI), in which up to 80 kg of fruit could be stored and RQ of the fruit measured. This was connected to the same ICA 61/CGS 610 CA Control System for storage monitoring.

Fruit quality and disorder evaluations. Initial fruit maturity at the time of harvest was evaluated using 10-apple samples from each orchard. Internal ethylene concentration was determined by withdrawing a 3-mL gas sample from the core of each fruit using a syringe and injecting the gas sample into a Varian CP-3380 gas chromatograph (Varian Canada Inc., Mississauga, Ontario, Canada) equipped with a 0.5-mL sample loop, flame ionization detector, and 15 m × 0.32 mm Restek Rt-SPLIT™ capillary column (Chromatographic Specialties Inc., Brockville, Ontario, Canada). The injector, column, and detector temperatures were 120, 35, and 225 °C, respectively. High-grade helium was used as the carrier gas at a flow rate of 0.37 mL·s⁻¹ with a typical run time of 1.5 min.

Fruit firmness was determined on opposite sides of each apple after peel removal, using an electronic texture analyzer fitted with an 11-mm tip (GÜSS, South Africa). Thereafter, juice was extracted and collected from composite samples of segments from all apples used for firmness testing. Titratable acidity (expressed as mg equivalents of malic acid per 100 mL of juice) was determined by titrating a 2-mL juice sample (extracted from composite samples of fragments from all apples used for firmness testing) with 0.1 N NaOH to an endpoint of pH 8.1 (as indicated by phenolphthalein), whereas soluble solids concentration was determined on a juice sample using a digital refractometer [BRX-242 (Erma Inc., Japan) in 2013 and PR-32 (Atago Co. Ltd., Japan) in 2015]. The starch index was determined using a Generic Starch–Iodine Index Chart for Apples (Blanpied and Silsby, 1992). Apples were cut in half at the equator, dipped in potassium-iodine solution and rated on a scale of 1 to 8, where 1 = 100% starch staining and 8 = no starch.

After removal from storage, fruit were held at RT (23 to 24 °C) and then evaluated for fruit quality and storage disorders after 1, 7, or 14 d. Ten fruit per box replicate of each treatment were measured for internal ethylene concentration, firmness, soluble solids concentration, and malic acid content. The incidence of storage disorders, namely external CO₂ injury, flesh browning, and core browning, were determined using all apples in each box replicate of each treatment (up to 83 per box). Incidence was calculated as a percentage of fruit displaying the disorder regardless of severity.

Statistical analyses. Data from each year were analyzed using Proc GLM and Proc GLIMMIX of the SAS program (Version 9.2; SAS Institute Inc., Cary, NC), incorporating a split-plot experimental design. All data were subjected to testing of normality and assumptions for analysis of variance and transformed for analysis when appropriate. Mean separations were examined using Duncan's multiple range test and only differences significant at $P \leq 0.05$ are discussed.

Results and Discussion

'Empire' apples were obtained during the commercial harvest period, and fruit maturity at harvest time is presented in Table 1. Internal ethylene concentration and starch index values were not significantly different for the two orchards in 2013, and therefore, these were combined for poststorage analyses. There was a difference in fruit firmness among these two orchards, but firmness is not an appropriate measure of maturity as it tends to be strongly influenced by orchard management and growing conditions (DeEll et al., 2001). Starch index was higher in the 2015 orchard compared with one of the orchards in 2013 (Table 1). There were no significant differences in internal ethylene concentration at harvest time, which is the major fruit maturity indicator. There were also no significant differences in soluble solids concentration and malic acid content. All apples were considered marketable, and no visible symptoms of disorders were present at harvest time.

External CO₂ injury, flesh browning, and core browning developed in the apples during both years of study (Table 2). However, there were no consistent effects of low O₂ or 1-MCP treatment on disorder incidence throughout both years.

During the first year of study, there were no significant effects of low O₂ or 1-MCP on the incidence of external CO₂ injury. In contrast, during the second year, the application of 1-MCP after harvest increased the incidence of external CO₂ injury in 'Empire' apples stored in both 2.5 and 1.5 kPa O₂. Furthermore, 1-MCP-treated apples had less external CO₂ injury when held in 1.5 kPa O₂ than in 2.5 kPa O₂. 'Empire' apples stored in 0.6 kPa O₂ with or without 1-MCP treatment upon removal had very little external CO₂ injury after 8 months of storage.

'Empire' is a CO₂ sensitive apple cultivar, and exposure to elevated CO₂ during the early stages of storage can provoke the development of CO₂ injuries (Fawbush et al., 2008; Watkins and Nock, 2012). Delaying establishment of CA and elevated CO₂ exposure has been reported to reduce the incidence of external CO₂ injury in 'Empire' apples (DeEll and Ehsani-Moghaddam, 2012). Application of the antioxidant diphenylamine (DPA) also controls the development of external CO₂ injury (Argenta et al., 2002; Fawbush et al., 2008; Watkins et al., 1997), but the use of DPA on apples may soon become restricted due to regulatory issues.

Table 1. Maturity indices of 'Empire' apples at harvest.

	Internal ethylene concn ($\mu\text{L}\cdot\text{L}^{-1}$)	Firmness (N)	Soluble solids concn (%)	Malic acid ^z (mg)	Starch index ^y (1–8)
2013					
Orchard 1	0.04 a	72.1 b	11.0 a	788 a	3.8 ab
Orchard 2	0.1 a	77.3 a	11.6 a	885 a	2.8 b
2015	6.5 a	79.2 a	9.8 a	662 a	4.8 a
Significance ^x	NS	***	NS	NS	*

^zmg per 100 mL of juice.^yBased on the generic starch–iodine index chart for apples (Blanpied and Silsby, 1992).^xMeans within the same column with the same letter are not significantly different at $P < 0.05$.*, **Significant at $P < 0.05$ or $P < 0.001$, respectively.

NS = nonsignificant.

Table 2. Storage disorders of 'Empire' apples treated with or without 1-MCP ($1 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h and then held in 2.5 kPa O₂ + 2 kPa CO₂ or 1.5 kPa O₂ + 1.2 kPa CO₂ at 1.5 °C (Year 1) or 3 °C (Year 2) for 8 months, plus 7 d (Year 1) or 14 d (Year 2) at room temperature (23 to 24 °C). Year 2 also included storage in 0.6 kPa O₂ + 0.5 kPa CO₂ for 8 months, followed by treatment with or without 1-MCP ($1 \mu\text{L}\cdot\text{L}^{-1}$) for 24 h.

	External CO ₂ injury (%)	Flesh browning (%)	Core browning (%)
Year 1			
2.5 kPa O ₂			
No 1-MCP	12.7 a	19.0 a	23.2 c
+1-MCP at harvest	20.5 a	11.2 ab	32.3 ab
1.5 kPa O ₂			
No 1-MCP	24.6 a	8.9 b	24.6 bc
+1-MCP at harvest	22.5 a	5.8 b	38.3 a
Significance ^z	NS	**	**
Year 2			
2.5 kPa O ₂			
No 1-MCP	3.6 c	1.0 b	19.5 a
+1-MCP at harvest	24.8 a	8.8 a	21.4 a
1.5 kPa O ₂			
No 1-MCP	2.2 c	1.5 b	5.2 b
+1-MCP at harvest	12.5 b	12.2 a	17.6 a
0.6 kPa O ₂			
No 1-MCP	0 c	0.5 b	0.5 b
+1-MCP at opening	1.1 c	0.6 b	0 b
Significance ^z	****	****	****

^zMeans within the same column with the same letter are not significantly different at $P < 0.05$.**, ****Significant at $P < 0.01$ or $P < 0.0001$, respectively.Each value represents the average of 204 and 249 apples in Year 1 and 2, respectively, except each value from 0.6 kPa O₂ represents the average of 148 apples. 1-MCP = 1-Methylcyclopropene; NS = nonsignificant.

In the current study, neither delayed CA nor DPA were used to increase the likelihood of developing storage disorders.

Postharvest 1-MCP treatment has been shown to increase the susceptibility of 'Empire' apples to external CO₂ injury (DeEll et al., 2003, 2005; Fawbush et al., 2008), and this was observed in the second year of the current study. Previous studies have shown that the effects of 1-MCP on external CO₂ injury can become less prominent when there is a high incidence of external CO₂ injury, as well as be influenced by year-to-year and orchard-to-orchard variations (DeEll and Ehsani-Moghaddam, 2012; Deyman et al., 2014).

Treatment with 1-MCP had negligible effects on flesh browning in the first year, whereas apples treated with 1-MCP after harvest and then held in 2.5 or 1.5 kPa O₂ displayed higher incidence of flesh browning than similar non-1-MCP-treated fruit in the second year (Table 2). It has been previously shown that 1-MCP treatment can exacerbate flesh browning in 'Empire' apples and more so at warmer storage temperatures (Jung and Watkins, 2011; Lee et al., 2012). In the current study, apples were stored at 1.5 and 3 °C in the first and second year, respectively. Therefore, the warmer temperature may have been a factor in 1-MCP-treated apples having

higher incidence of fleshing browning compared with those not treated only in the second year.

In the first year of study, non-1-MCP-treated 'Empire' had less flesh browning when held in 1.5 kPa O₂ than in 2.5 kPa O₂ (Table 2). However, this effect was not present in the second year. 'Empire' apples stored in 0.6 kPa O₂ with or without 1-MCP treatment upon removal had very little flesh browning after 8 months of storage, and the incidence was not significantly different from fruit without 1-MCP and stored in 2.5 or 1.5 kPa O₂. Similarly, Thewes et al. (2015) found no difference in the incidence of flesh breakdown (browning) in non-1-MCP-treated 'Royal Gala' and 'Galaxy' apples stored in 1.2 or 0.4 kPa O₂. The onset of flesh browning in 'Empire' apples can also be influenced by fruit maturity at harvest (Ehsani-Moghaddam and DeEll, 2009), as well as demonstrate year-to-year and orchard-to-orchard variation (DeEll and Ehsani-Moghaddam, 2012; Deyman et al., 2014; Jung and Watkins, 2011).

Core browning was more prevalent in 'Empire' apples with 1-MCP treatment after harvest than nontreated fruit in the first year, regardless of O₂ level (Table 2). In the second year of study, 1-MCP-treated 'Empire' apples had higher incidence of core browning than nontreated fruit held in 1.5 kPa O₂, while

there was no significant difference in apples with or without 1-MCP stored in 2.5 kPa O₂. Previous work showed that 1-MCP treatment at harvest resulted in less core browning in 'Empire' apples held in ambient air at 0 to 1 °C for 6 months, whereas there was no effect on core browning when stored in CA (2.5 kPa O₂ + 2.5 kPa CO₂) at 2 °C for 9 or 12 months (DeEll et al., 2007). The efficacy of 1-MCP on reducing core browning in apples typically declines with apples that are of advanced maturity at harvest (DeEll et al., 2007; Watkins et al., 2000), which may have influenced the above results.

'Empire' apples stored in 0.6 kPa O₂ with or without 1-MCP treatment upon removal had very little core browning after 8 months of storage and the incidence was not significantly different from fruit not treated with 1-MCP and stored in 1.5 kPa O₂.

Overall, low O₂ reduced flesh browning in the first year and core browning during the second year in 'Empire' apples without 1-MCP (Table 2). Otherwise low O₂ had no significant effects on storage disorders and it never increased the incidence. Storage disorders in non-1-MCP-treated fruit had similar low incidence in all O₂ regimes utilized in the second year, except for core browning in 2.5 kPa O₂. There was minimal incidence of disorders (0% to 1.1%) in apples

held in 0.6 kPa O₂, regardless of 1-MCP treatment upon removal. It is an important difference that apples stored in 0.6 kPa O₂ had 1-MCP applied after storage rather than at harvest time like for the fruit held in 2.5 or 1.5 kPa O₂. Therefore, 1-MCP was unable to influence disorder development in 'Empire' apples during storage in 0.6 kPa O₂. 1-MCP treatment at harvest resulted in higher incidence of external CO₂ injury and flesh browning in apples held in 2.5 or 1.5 kPa O₂ for at least one year of study and increased core browning in fruit stored in 1.5 kPa O₂ for both years (Table 2). It has been previously postulated that 1-MCP delays ripening and maintains apples in the "at harvest" state, which would consequently extend the period of fruit sensitivity to stress during the early stages of storage and ultimately result in more storage disorders (Fawbush et al., 2008; Jung and Watkins, 2011). The exact biochemical events for 1-MCP to increase fruit susceptibility to certain disorders are not well understood.

The effect of low O₂ on internal ethylene concentration was noticeable 1 d after removal from storage, as 'Empire' apples without 1-MCP had lower internal ethylene with lower O₂ levels (Tables 3 and 4). However, this effect was no longer present after 7 and 14 d at RT. There was also no significant effect of low O₂ on internal ethylene concentration in fruit treated with 1-MCP at harvest time.

As expected, 1-MCP reduced internal ethylene in 'Empire' apples, often regardless of O₂ level and shelf life duration at RT (Tables 3 and 4). Lower ethylene production in apples treated with 1-MCP treatment is in agreement with previous reports (Argenta et al., 2000; DeEll et al., 2005; Fawbush et al., 2008; Jung and Watkins, 2011; Watkins and Nock, 2012).

It is of particular interest that apples treated with 1-MCP upon removal from storage in 0.6 kPa O₂ had lower internal ethylene concentration than those not treated only 1 d later at RT (Table 4). This effect was

also observed after 7 d at RT, but was then lost after 14 d.

Ethylene production in apples has been previously shown to decrease with lower O₂ levels in storage, as 'Royal Gala' and 'Galaxy' apples held in 0.4 kPa O₂ at 1 °C had a lower rate of ethylene production than similar fruit in 1.2 kPa O₂ (Thewes et al., 2015). Similarly, 'Scarlett Spur Red Delicious' apples in 0.3 kPa O₂ had lower internal ethylene content than fruit held in 1.6 kPa O₂ after 8 months of storage plus 7 d at 20 °C (Lumpkin et al., 2014).

After removal from storage in 2.5 kPa O₂ plus 1, 7, and 14 d at RT, fruit firmness was greater in 'Empire' apples treated with 1-MCP than those not treated (Tables 3 and 4). Similar effects of higher firmness in 1-MCP-treated fruit stored in 1.5 or 0.6 kPa O₂ were not found until 7 or 14 d at RT, respectively. In the first year of study, apples without 1-MCP and stored in 1.5 kPa O₂ had comparable firmness after 7 d at RT to those stored in 2.5 kPa O₂ plus only 1 d at RT. In the second year, apples without 1-MCP and stored in 0.6 kPa O₂ had comparable firmness after 14 d at RT to those treated with 1-MCP and stored in 1.5 or 2.5 kPa O₂. Furthermore, 'Empire' apples from 0.6 kPa O₂ and treated with 1-MCP were firmer than all other treatments after 14 d at RT.

The enhanced firmness retention found in this study with lowering O₂ in the atmosphere during storage is consistent with previous reports (Köpcke, 2015; Rebeaud and Gasser, 2015; Thewes et al., 2015; Veltman et al., 2003). For example, 'Royal Gala' and 'Galaxy' apples from 0.4 kPa O₂ had greater flesh firmness than fruit from 1.2 kPa O₂ after 9 months of storage plus 7 d shelf life (Thewes et al., 2015). These apples in 0.4 kPa O₂ also showed reduced activity of 1-aminocyclopropane-1-carboxylate (ACC) oxidase, an ethylene biosynthesis enzyme. Ethylene reduction can affect ethylene-regulated genes and encoding enzymes associated with cell wall degradation and modifications (Bennett and Labavitch, 2008), thus leading

to enhanced firmness retention. The effect of 1-MCP for retaining firmness in apples has also been well documented (DeEll and Ehsani-Moghaddam, 2012; DeEll et al., 2007; Jung and Watkins, 2011; Watkins et al., 2000).

There were no significant effects of low O₂ or 1-MCP on soluble solids concentration in 'Empire' apples during both years of study (Tables 3 and 4). 'Empire' apples with 1-MCP had slightly higher malic acid content than untreated fruit in the second year, specifically in those from 2.5 or 1.5 kPa O₂ plus 14 d at RT (Table 4). Previous reports also showed inconsistent or no effects of 1-MCP on soluble solids concentration in 'Empire' apples (DeEll et al., 2007; DeEll and Ehsani-Moghaddam, 2012). The marginal changes in soluble solids and malic acid in this study suggest that sugar and organic acid metabolism were not readily altered by the low O₂ levels during storage.

In summary, the results from this study suggest that lowering the O₂ levels in storage rooms can have varying beneficial effects on storage disorders and fruit quality in 'Empire' apples. 1-MCP treatment at harvest provided additional fruit firmness retention and reduced internal ethylene but it also tended to increase storage disorders. The use of RQ-based dynamic CA storage with 0.6 kPa O₂ resulted in negligible storage disorders and greater fruit firmness retention. This could provide flexibility to postpone 1-MCP treatment until after storage, to prevent increased susceptibility to disorders during storage without compromising fruit quality, which would in turn allow producers to decide the marketing avenue (i.e., conventional vs. organic) throughout the storage season instead of at harvest time. However, results from RQ-based dynamic CA with 0.6 kPa O₂ and 1-MCP treatment after storage were from a single season, and further research is needed to confirm these observations.

Table 3. Quality of 'Empire' apples treated with or without 1-MCP (1 µL·L⁻¹) for 24 h and then held in 2.5 kPa O₂ + 2 kPa CO₂ or 1.5 kPa O₂ + 1.2 kPa CO₂ at 1.5 °C for 8 months, plus 1 or 7 d at room temperature (RT, 23 to 24 °C), in Year 1.

	Internal ethylene concn (µL·L ⁻¹)	Firmness (N)	Soluble solids concn (%)	Malic acid ^z (mg)
1 d at RT				
2.5 kPa O ₂				
No 1-MCP	28.9 c	65.3 d	11.6 a	420 ab
+1-MCP at harvest	0.3 d	74.3 a	11.4 a	458 a
1.5 kPa O ₂				
No 1-MCP	3.9 d	72.0 bc	11.8 a	407 ab
+1-MCP at harvest	0.1 d	72.9 ab	11.8 a	421 ab
7 d at RT				
2.5 kPa O ₂				
No 1-MCP	291.6 b	57.2 e	11.8 a	365 bc
+1-MCP at harvest	28.3 c	70.2 c	11.5 a	407 ab
1.5 kPa O ₂				
No 1-MCP	315.8 a	65.7 d	11.5 a	333 c
+1-MCP at harvest	22.4 cd	73.4 ab	11.5 a	393 abc
Significance ^y	****	****	NS	**

^zmg per 100 mL of juice.

^yMeans within the same column with the same letter are not significantly different at $P < 0.05$.

, **Significant at $P < 0.01$, or $P < 0.0001$, respectively.

Each value represents the average of 30 apples.

1-MCP = 1-Methylcyclopropene; NS = nonsignificant.

Table 4. Quality of ‘Empire’ apples treated with or without 1-MCP (1 $\mu\text{L}\cdot\text{L}^{-1}$) for 24 h and then held in 2.5 kPa O_2 + 2 kPa CO_2 or 1.5 kPa O_2 + 1.2 kPa CO_2 at 3 °C for 8 months, or stored in 0.6 kPa O_2 + 0.5 kPa CO_2 for 8 months followed by treatment with or without 1-MCP (1 $\mu\text{L}\cdot\text{L}^{-1}$) for 24 h, plus 1, 7, or 14 d at room temperature (RT, 23 to 24 °C), in Year 2.

	Internal ethylene concn ($\mu\text{L}\cdot\text{L}^{-1}$)	Firmness (N)	Soluble solids concn (%)	Malic acid ^z (mg)
1 d at RT				
2.5 kPa O_2				
No 1-MCP	163.3 a	68.0 b	10.2 a	466 c
+1-MCP at harvest	7.1 cd	75.6 a	10.1 a	543 a
1.5 kPa O_2				
No 1-MCP	74.1 b	75.6 a	10.2 a	502 abc
+1-MCP at harvest	2.3 d	73.8 a	10.3 a	531 ab
0.6 kPa O_2				
No 1-MCP	21.3 c	75.2 a	10.2 a	482 c
+1-MCP at opening	3.1 d	74.7 a	10.1 a	486 bc
Significance ^y	****	****	ns	**
7 d at RT				
2.5 kPa O_2				
No 1-MCP	453.1 ab	54.0 d	10.2 a	432 b
+1-MCP at harvest	389.7 b	68.9 b	10.2 a	495 a
1.5 kPa O_2				
No 1-MCP	493.0 a	62.1 c	10.1 a	455 ab
+1-MCP at harvest	248.3 c	73.8 a	10.0 a	515 a
0.6 kPa O_2				
No 1-MCP	485.6 a	72.0 a	10.3 a	495 a
+1-MCP at opening	132.5 d	73.4 a	10.3 a	517 a
Significance ^y	****	****	NS	*
14 d at RT				
2.5 kPa O_2				
No 1-MCP	719.5 a	46.4 c	9.9 a	338 d
+1-MCP at harvest	468.9 b	50.9 b	10.0 a	427 a
1.5 kPa O_2				
No 1-MCP	669.3 a	47.3 c	10.2 a	371 cd
+1-MCP at harvest	464.7 b	49.5 b	10.2 a	426 a
0.6 kPa O_2				
No 1-MCP	733.3 a	51.3 b	10.3 a	378 bc
+1-MCP at opening	684.3 a	59.4 a	10.0 a	411 ab
Significance ^y	****	****	NS	****

^zmg per 100 mL of juice.

^yMeans within the same column at a given time with the same letter are not significantly different at $P < 0.05$.

*, **, ****Significant at $P < 0.05$, $P < 0.01$, or $P < 0.0001$, respectively.

Each value represents the average of 30 apples for 2.5 and 1.5 kPa O_2 or 20 apples for 0.6 kPa O_2 .

1-MCP = 1-Methylcyclopropene; NS = nonsignificant.

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