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Controlled-atmosphere Storage of 'Honeycrisp' Apples

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Abstract. **'Honeycrisp' is an apple [*Malus* x*sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] that can be stored in air for several months, but the flavor becomes bland with prolonged storage. Controlled-atmosphere (CA) storage recommendations have not been made in some growing regions, however, because of the susceptibility of fruit to physiological disorders. In the first year of this study, we stored fruit from six orchards in O_2 partial pressures (pO_2) of 1.5, 3.0, and 4.5 kPa with 1.5 and 3.0 kPa pCO_2. In the second year, we stored fruit from three orchards in three storage regimes (2.0/2.0, 3.0/1.5, 3.0/0.5 kPa O_2/kPa CO_2) with and without treatment of fruit with 1-methylcyclopropene (1-MCP) at the beginning and end of the conditioning regime (10 °C for 7 days) that is commercially used for 'Honeycrisp'. CA storage had little effect on flesh firmness, soluble solids concentration (SSC), and titratable acidity (TA) over the range of pO_2 and pCO_2 tested. Greasiness was generally lower in fruit stored in lower pO_2 and higher pCO_2. Susceptibility of fruit to core browning and senescent breakdown varied between years, but a high incidence of internal CO_2 injury in fruit from some orchards occurred in both years. 1-MCP treatment decreased internal ethylene concentration (IEC) and sometimes maintained TA but had little effect on firmness and SSC. Senescent breakdown and core browning incidence were reduced by 1-MCP treatment where orchard susceptibility to these disorders was high. However, 1-MCP treatment sometimes increased internal CO_2 injury, especially if treatment occurred at the beginning of the conditioning period. CA storage cannot be recommended for storage of New York-grown 'Honeycrisp' apples until management of CO_2 injury can be assured.**

'Honeycrisp' [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh) Mansf.] is a popular apple cultivar that commands premium prices in the North American market. The cultivar has a unique crisp, juicy texture that is popular with consumers. Maintenance of crisp texture characteristics for up to 9 months in air storage has been reported (Luby and Bedford, 1992; Tong et al., 1999) associated with high turgor and cell wall integrity (Tong et al., 1999) and low transcript accumulations for some of the genes involved in cell wall disassembly (Harb et al., 2012; Mann et al., 2008). However, industry observations indicate that flavor decreases with prolonged air storage under commercial conditions.

'Honeycrisp' apples are also susceptible to a number of physiological disorders including bitter pit, soft scald, soggy breakdown, low temperature breakdown, and senescent breakdown (DeEll and Ehsani-Moghaddam, 2010; DeLong et al., 2006; Moran et al., 2009; Rosenberger et al., 2004; Tong et al., 2003; Wargo and Watkins, 2004) as well as greasiness (DeLong et al., 2006; Delong et al., 2009; Watkins et al., 2005). Soft scald and soggy breakdown have proven to be serious limitations for air storage of the cultivar but could be greatly alleviated by a conditioning period of 7 d at 10 °C followed by storage at 3 °C (Watkins and Rosenberger, 2000). Subsequent studies confirmed the benefits of conditioning and the requirement of warmer storage temperatures (DeLong et al., 2004, 2006; Delong et al., 2009; Watkins et al., 2004) with some exceptions (Moran et al., 2010), and this protocol is now widely recommended (Tong and Mader, 2009).

Continued plantings of 'Honeycrisp' trees will result in an increasing volume of fruit to be stored in the future and therefore improved methods of maintaining quality are desired. Although the standard practice for apple storage is use of CA regimes, few reports of CA storage for 'Honeycrisp' are available. Nova Scotia-grown 'Honeycrisp' can tolerate pO_2 as low as 0.4 kPa, and treatment of fruit with dynamic low O_2 storage (0.5 to 0.8 kPa O_2/1.5 kPa CO_2) compared with a 1.5 kPa O_2/1.5 kPa CO_2 atmosphere resulted in similar fruit firmness (DeLong et al., 2004). A 2.5 kPa O_2/1 to 1.5 kPa CO_2 atmosphere at 3 °C for 6 months after conditioning resulted in fruit that were slightly firmer, more acidic, less greasy, and with less soft scald than those stored in air (DeLong et al., 2004, 2006), but soft scald was high in Ontario-grown fruit in a 1.7 kPa O_2/2% CO_2 atmosphere without conditioning (DeEll, 2010). In Washington state, a 2 kPa O_2/1 kPa CO_2 atmosphere at 1.7 °C has been commercially successful (Mattheis, personal communication), but no recommendations for CA storage are available in Michigan, New York, or Ontario because of concern about susceptibility of fruit to CO_2 injury (Tong and Mader, 2009).

SmartFresh™ technology, based on the inhibitor of ethylene perception, 1-MCP can help maintain SSC and TA during air storage of 'Honeycrisp' apples (DeEll, 2010; Watkins and Nock, unpublished data). The interaction between 1-MCP and CA storage is not well studied, although DeEll (2010) found that 1-MCP exacerbated CA-related internal storage disorders. Fruit were not conditioned in that experiment, however.

The objective of the current study was to investigate a range of CA regimens for storage of 'Honeycrisp' to develop safe recommendations for the industry. All fruit in this study were subjected to a conditioning treatment of 7 d at 10 °C because it is standard practice for handling of the cultivar in New York. In addition, we compared the effects of the 1-MCP treatment at the beginning and the end of the conditioning period on quality of CA-stored fruit.

Materials and Methods

Plant material. Fruit used in these experiments were harvested from 'Honeycrisp' apple trees grown in commercial orchards in western New York. In Expt. 1 (2009), fruit were obtained from six orchard blocks that had been harvested to commercial quality criteria of red coloration (greater than 50%) and delivered to two major storage operations. Three blocks (1, 2, and 3) were harvested on 24 Sept. and kept at ambient conditions overnight, and the other three (4, 5, and 6) were harvested on 25 Sept. Fruit from the six blocks were transported to Ithaca on 25 Sept. In Expt. 2 (2010), fruit were harvested from three orchard blocks to commercial red color standards on 17 Sept. and transported on the day of harvest to Ithaca. Approximately 500 fruit were obtained for each block. The picking dates each year were approximately mid-harvest for the cultivar.

On arrival of fruit at the laboratory, fruit were sorted for uniform size, freedom from blemishes including bitter pit, and randomized to provide experimental units of 30 to 40 fruit for each orchard. In Expts. 1 and 2, one sample of 10 fruit and three samples of 10 fruit, respectively, were taken randomly from each orchard lot for measurement of harvest indices as described subsequently. The remaining fruit as experimental units (replicates) were conditioned at 10 °C, 96% relative humidity, for 7 d. Fruit were then transferred to a 3 °C room for 24 h.

Storage treatment—Expt. 1. Four replicates per orchard lot were placed into each of six CA chambers with a volume of 0.9 m^3 fitted with a circulating fan system (Storage

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Control Systems, Sparta, MI) and the following atmospheres established within 48 h: 1.5, 3.0 and 4.5 kPa O_2, each with 1.5 or 3.0 kPa CO_2. Atmospheres were checked hourly and maintained within 0.2 kPa of target values with a ICA 61/CGS 610 CA Control System (International Controlled Atmosphere Ltd., Kent, U.K.) modified with flow controllers for the experimental chambers (Storage Control Systems, Sparta, MI). Fruit were stored for 6 months and evaluated after 4 d at 20 °C.

Storage treatment—Expt. 2. Three replicates per orchard lot were treated with 1 $\mu L \cdot L^{-1}$ 1-MCP for 24 h on either Day 1 or 6 of the preconditioning treatment. Fruit were treated in 4000-L plastic tents using SmartFresh tablets and a release and fan system supplied by the manufacturers (AgroFresh Inc., Rohm & Haas Company, Philadelphia, PA). Treated and untreated fruit were placed in each of three CA chambers and the following atmospheres established within 48 h: 2.0 kPa O_2/2.0 kPa CO_2, 3.0 kPa O_2/0.5 kPa CO_2, and 3.0 kPa O_2/1.5 kPa CO_2. Atmospheres were maintained as in Expt. 1.

Harvest and quality assessments. Ten fruit replicates were used for all storage analyses. In both experiments, IECs, flesh firmness, SSC, and TA were measured at harvest and after storage, except in Expt. 1 in which IEC was not measured after storage. Starch pattern indices were measured at harvest. Acetaldehyde and ethanol concentrations of the fruit were measured in Expt. 2.

The IEC of each fruit was measured on 1-mL samples of internal gas from the core cavity (Watkins et al., 2000). Ethylene was measured using a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Wilmington, DE) equipped with a flame ionization detector and fitted with a stainless steel column packed with 60/80 mesh alumina F-1 (2 m × 2 mm, i.d.). Analyses were run isothermally with an oven temperature of 200 °C and injector and detector temperatures of 220 and 250 °C, respectively. The flow rates for nitrogen, hydrogen, and compressed air were 30, 30, and 230 $mL \cdot min^{-1}$, respectively. Samples were injected directly into the gas chromatograph. Ethylene was quantified by peak area, and an external standard of 10 $\mu L \cdot L^{-1}$ was used for calibration.

Firmness was measured on opposite peeled sides of each fruit using an electronic pressure tester fitted with an 11.1-mm diameter probe [Guss Fruit Texture Analyzer; Guss Manufacturing (Pty) Ltd., Strand, South Africa] and the expressed juice used for SSC measurement with a refractometer (Atago PR-100; Atago Co. Ltd., Tokyo, Japan). Titratable acidity was measured on juice extracted from composite samples of segments using 0.1 M NaOH to an end point of pH 8.1 with an autotitrator (Mettler DL12, Hightstown, NJ).

For measurement of acetaldehyde and ethanol concentrations, one wedge (no core tissue) from each of the 10 apples per replicate were juiced with an Acme 6001 Supreme Juicerator (Waring Products Division of Conair Corp., Windsor, NJ). A saturated NaCl solution (2.5 g) and distilled water (2.5 g) were added to duplicate samples of 5.0 g of juice in 20 mL VWR TraceClean open screw-capped vials with 3.2-mm fluoropolymer resin/silicone septa. Samples were frozen at –20 °C. For gas chromatography (GC) analysis, sample vials were individually removed from the freezer and incubated at 60 °C for 20 min in a heating block (dry bath incubator; Fisher Scientific, Waltham, MA) before 0.5 mL of the headspace was manually injected (Fernandez-Trujillo et al., 2001). The analysis was carried out using a Hewlett-Packard Model 5890 GC equipped with a flame ionization detector and a 0.53-mm × 15-m Stabilwax capillary column with 1.0-μm film thickness (Restek Corp, Bellefonte, PA). The oven temperature was held at 40 °C for 4 min and then raised to 240 °C at a rate of 20 $°C \cdot min^{-1}$ to clear the column after each injection. The sample volatiles were identified by comparison of their retention times with those of standards. The standard curve for each volatile was established with up to 10 concentrations from 0 to 205 $mg \cdot kg^{-1}$.

Three 10-fruit replicates from each orchard were used for mineral analyses at harvest. A 1.5-cm disc was equatorially cut to include the core in each fruit. The skin was removed with a single knife cut on opposite sides of the disc. Then a further cut was made parallel to the initial cut but 1.5 cm into the flesh on both sides of the fruit. This was trimmed to a 1.5-cm cube, making sure to avoid all core material and skin. Only sound flesh was used. Samples were put in paper bags with foil lining the bottom. Fruit samples were dried in a forced-air oven to constant dry weight and then ground to pass through a 1-mm screen. Tissue nitrogen concentration was determined with a C/N analyzer (LECO Corporation, St. Joseph, MI) via combustion, and phosphorus, potassium, calcium, magnesium, sulfur, boron, zinc, copper, manganese, and iron concentrations were measured through an inductively coupled plasma emission spectrometry (Fison Instrument, Dearborn, MI). Analyses were carried out by Agri Analysis, Inc., PA.

Each fruit, including those used for quality assessment, were assessed for presence or absence of greasiness, determined subjectively by touch, and any external disorders and then sliced at least three times to assess internal disorders.

Statistical analyses. Harvest data were subjected to one-way analysis of variance (ANOVA) and storage data to two-way ANOVA using the general linear model to determine main effects and interactions (Release 15; Minitab, State College, PA). SEs of the mean are provided for highest-order interaction. Pearson correlations were used to investigate relationships among harvest indices and mineral concentrations with disorder incidences and volatile concentrations.

Results

Expt. 1. The harvest indices of fruit were assessed within 2 d after (Orchards 1 to 3) or on the day (Orchards 4 to 6) of picking. The IECs of fruit from Orchard 1 were all less than 1 $\mu L \cdot L^{-1}$ except for one fruit, but IECs of fruit from the other orchards ranged from 3.4 to 28.1 $\mu L \cdot L^{-1}$ (Table 1). The starch indices indicated that starch hydrolysis was close to complete at the time of harvest. Flesh firmness ranged from 58.1 N in Orchard 6 to 67.6 N in Orchard 2 (Table 1). Although the absence of biological replication for TA and SSC does not permit definitive statements about orchard-to-orchard variation, informal tasting confirmed that orchards varied greatly and that higher acidity in the fruit was preferred.

Calcium, magnesium, potassium, and nitrogen concentrations in the fruit varied among orchard, differences for phosphorus being barely not significant at $P = 0.05$ (Table 1). The most pronounced differences were for calcium, in which concentrations were more than twice as high in Orchards 1 to 3 than in 4 to 6.

Table 1. Harvest indices and mineral concentrations (dry weight basis) in 'Honeycrisp' fruit from the six orchard blocks used in Expt. 1.

Orchard No.	IEC[z] ($\mu L \cdot L^{-1}$)	Starch index (1–8)	Flesh firmness (N)	Titratable acidity[y] (% malic acid)	Soluble solids concn (%)	Calcium[x] ($\mu g \cdot g^{-1}$)	Magnesium ($\mu g \cdot g^{-1}$)	Potassium ($\mu g \cdot g^{-1}$)	Nitrogen ($\mu g \cdot g^{-1}$)	Phosphorus ($\mu g \cdot g^{-1}$)
1	0.4[w]	6.9	62.5	0.216	11.1	43.3	30.0	613.3	353.3	63.0
2	3.4	7.1	67.6	0.303	11.4	46.7	32.2	766.7	260.0	76.7
3	28.1	7.0	60.9	0.280	11.5	40.0	36.7	643.3	313.3	60.0
4	19.6	6.9	66.2	0.361	10.8	20.3	30.0	806.7	310.0	70.0
5	14.4	7.4	67.5	0.386	12.6	21.7	40.0	846.7	300.3	76.7
6	11.1	7.0	58.1	0.272	10.8	20.0	29.5	763.3	310.0	80.0
Pooled SD	11.02	0.53	6.90	—	—	0.03	0.01	54.92	23.41	8.17
Significance	<0.001	0.727	<0.001	—	—	<0.001	<0.001	0.001	0.011	0.059

[z]Internal ethylene concentration (IEC), starch index, flesh firmness represent means of 10 individual fruit samples.
[y]Titratable acidity and soluble solids concentration are based on bulked samples of 10 fruit.
[x]Minerals are based on three 10 fruit replicates per orchard.
[w]Without one of 10 apples, which had an IEC of 40.9 $\mu L \cdot L^{-1}$.

After storage, firmness varied by orchard block (Table 2), averaging 64.0, 65.1, 62.7, 62.0, 63.4, and 62.0 nitrogen in Orchards 1 to 6, respectively. Firmness was unaffected by storage atmosphere. In contrast, highly significant effects of both orchard and storage atmosphere were detected for TA and SSC (Table 2). The lowest TA occurred at 4.5 kPa O_2 compared with 1.5 and 3.0 kPa O_2, and concentrations were slightly higher in 1.5 kPa CO_2 than at 3.0 kPa CO_2. Overall, the highest TA (0.224%) was found in 3.0 kPa O_2 and was unaffected by CO_2, whereas at 1.5 kPa O_2, the TAs averaged 0.233% in 1.5 kPa CO_2 and 0.207% in 3.0 kPa CO_2. For SSC, values among pO_2 and between pCO_2 were significant, but no interaction between the gases was detected. Differences were small and not commercially significant, however, being 11.2%, 11.0%, 11.1% for 1.5, 3.0, and 4.5 kPa O_2, respectively, and 11.0% and 11.2% for 1.5 and 3.0 kPa CO_2, respectively.

Internal CO_2 injury, characterized by flesh browning, and often accompanied by cavities, was detected in fruit from all orchards but was almost absent in fruit from Orchard 1 and highest in fruit from Orchard 5 (Table 3). No effect of pO_2 was detected, but overall, 10% injury occurred in fruit stored in 3.0 kPa CO_2 compared with 5% in 1.5 kPa CO_2. However, the effects of pCO_2 interacted with orchard.

Incidence of greasiness was affected by an interaction among orchard, pCO_2, and pO_2. Greasiness was negligible in fruit from Orchard 2 (1%) but much higher incidences in other orchards, especially 3 and 4 (Table 3). Overall, the incidence of greasiness was lower (11%) in 1.5 kPa O_2 than in 3.0 and 4.5 kPa O_2 (20% and 18%, respectively) and lower (13%) in 3.0 kPa CO_2 than in 1.5% kPa CO_2 (20%).

The incidence of core browning was very low (data not shown) but overall higher (0.3%) in 3.0 kPa CO_2 and 0% in 1.5 kPa CO_2 ($P = 0.039$). Bitter pit, lenticel breakdown, and decay incidences varied by orchard but did not exceed 5% overall and were unaffected by atmosphere (data not shown). Soft scald incidence was negligible (less than 2%) and unaffected by any factor. Another disorder, in which the skin had a wrinkled appearance, was observed at low levels and was unaffected by any factor. Senescent breakdown was detected in all except Orchard 2, but at less than 2% incidence (data not shown). Overall, incidence of senescent breakdown averaged 1.2% and 0.6% in 1.5 and 3.0 kPa CO_2, respectively ($P = 0.051$).

Expt. 2. The range of IECs and flesh firmness at harvest varied across orchards, but no significant differences were detected for the starch indices, TA, or SSC (Table 4). Maturity indices were similar to those in the first experiment. Of the minerals, only potassium and nitrogen concentrations varied significantly ($P = 0.05$) in fruit among the orchards.

Fruit were either untreated during the conditioning period of 10 °C or treated with 1-MCP after 1 or 6 d of conditioning. The effect of orchard on IECs, firmness, TA, and SSC was highly significant and therefore results are shown separately for each orchard (Table 5). An effect of atmosphere was detected in two of three orchards, but 1-MCP treatment always resulted in much lower IECs than without treatment. Flesh firmness was affected by atmosphere in Orchard 1 only with no other main effects or interactions being detected, whereas TA was affected by treatment only in Orchard 2. Differences were small, however. The SSC was unaffected by atmosphere or treatment.

Internal CO_2 injury was essentially absent in Orchard 1 compared with Orchards 2 and 3 (Table 6). Injury was typically higher in the 2.0 kPa O_2/2.0 kPa CO_2 treatment than in the two 3.0 kPa O_2 treatments. Also, injury was consistently worse in fruit treated with 1-MCP after 1 d than after 6 d. Core browning was negligible in fruit from Orchards 1 and 3 but higher incidences in Orchard 2 were decreased by treatment with 1-MCP at either timing (Table 6). Senescent breakdown was absent in fruit from Orchard 1 but generally lower in 1-MCP-treated fruit from Orchards 2 and 3 (Table 6).

Greasiness incidence in fruit from Orchard 1 was unaffected by atmosphere or treatment (Table 6), but in Orchard 2, fruit from the 3.0 kPa O_2/0.5 kPa CO_2 atmosphere averaged 29% compared with 26% and 21% in the 2.0 kPa O_2/2.0 kPa CO_2 and 3.0 kPa O_2/1.5 kPa CO_2 atmospheres, respectively. In fruit from Orchard 3, greasiness was lower in fruit treated with 1-MCP on Day 1 compared with no treatment or treatment on Day 6 but only at the 3.0 kPa O_2/0.5 kPa CO_2 atmosphere.

Table 2. Firmness, titratable acidity and soluble solids concentration of 'Honeycrisp' apples stored in six CA storage regimes for 6 months at 3 °C plus 4 d at 20 °C.[z]

Orchard no.		CA storage regime (kPa O_2/kPa CO_2) 1.5/1.5	3.0/1.5	4.5/1.5	1.5/3.0	3.0/3.0	4.5/3.0
		Firmness (N)					
1		63.7	64.0	63.6	64.4	64.3	64.2
2		65.3	66.0	64.3	65.2	65.0	64.7
3		62.4	63.2	63.2	62.7	62.8	62.2
4		62.3	62.7	61.7	61.1	62.6	61.5
5		62.9	65.2	64.9	63.0	61.9	62.7
6		60.7	61.7	63.5	61.3	62.2	62.8
	SEM[y]	0.29					
	Orch	<0.001					
	O_2	0.186					
	CO_2	0.097					
	Orch × O_2	0.288					
	Orch × CO_2	0.092					
	O_2 × CO_2	0.427					
	Orch × O_2 × CO_2	0.661					
		Titratable acidity (% malic acid)					
1		0.155	0.147	0.135	0.142	0.146	0.133
2		0.240	0.254	0.233	0.225	0.222	0.219
3		0.212	0.191	0.148	0.178	0.183	0.171
4		0.267	0.259	0.217	0.246	0.267	0.240
5		0.299	0.288	0.249	0.247	0.276	0.257
6		0.226	0.216	0.204	0.207	0.245	0.212
	SEM	0.0066					
	Orch	<0.001					
	O_2	<0.001					
	CO_2	0.002					
	Orch × O_2	0.029					
	Orch × CO_2	0.003					
	O_2 × CO_2	<0.001					
	Orch × O_2 × CO_2	0.004					
		Soluble solids concentration (%)					
1		10.2	9.3	9.6	9.7	9.5	9.7
2		11.7	11.5	11.3	11.7	11.3	11.0
3		10.7	10.7	10.8	11.4	11.3	10.9
4		11.5	11.3	11.1	12.0	11.6	11.9
5		12.1	12.0	12.2	12.8	11.5	12.9
6		10.5	11.1	10.5	11.0	10.8	11.3
	SEM	0.17					
	Orch	<0.001					
	O_2	0.004					
	CO_2	0.001					
	Orch × O_2	0.001					
	Orch × CO_2	<0.001					
	O_2 × CO_2	0.102					
	Orch × O_2 × CO_2	0.006					

[z]Fruit were conditioned for 7 d at 10 °C before cooling for 24 h and atmospheres imposed.
[y]SEM for the highest significant interaction, where df in parentheses are: orchard (5); O_2 (2); CO_2 (1); orchard × O_2 (10); orchard × CO_2 (5); O_2 × CO_2 (2), and orchard × O_2 × CO_2 (10).
CA = controlled atmosphere.

Decay incidence was unaffected by atmosphere or treatment (data not shown). Bitter pit incidence was less than 5% in Orchards 1 and 3. In Orchard 2, less pit (9%) occurred in 3.0 kPa O_2/1.5 kPa CO_2 than in 2.0 kPa O_2/2.0 kPa CO_2 (14%) and 3.0 kPa O_2/0.5 kPa CO_2 (17%) ($P = 0.034$). Pit was also lower in 1-MCP-treated fruit (9% and 12% for 1 and 6 d, respectively) than without 1-MCP (18%) ($P = 0.018$). Skin wrinkling occurred at low levels (less than 2%), was unaffected by treatment, but was absent in two of three orchards in 3.0 kPa O_2/1.5 kPa CO_2 (data not shown).

Acetaldehyde and ethanol concentrations in fruit at harvest did not differ significantly among orchards, averaging 0.153 and 0.994 $\mu g \cdot g^{-1}$. After CA storage, acetaldehyde concentrations were variable and unaffected consistently (data not shown), but ethanol concentrations were affected by orchard ($P = 0.005$) and, within each orchard by atmosphere, 1-MCP treatment and an interaction between them (Table 7). In general, ethanol concentrations were lower in 1-MCP-treated fruit than untreated fruit, higher in fruit kept at 3 kPa O_2 than 2 kPa O_2. When stored with 3 kPa O_2, there was usually higher ethanol accumulation in 0.5 kPa CO_2 than at 1.5 kPa CO_2.

Discussion

This study reveals several features of 'Honeycrisp' apples under CA storage conditions. Despite storage of fruit in a wide range of pCO_2 and pO_2, with or without 1-MCP application, little effect of storage treatments was detected for firmness (Tables 2 and 5). The range of partial pressures used in this study and/or the use of 1-MCP would typically result in markedly different softening in other cultivars (Fan et al., 1999; Johnson and Ertan, 1983; Stow, 1989; Stow and Genge, 2000; Watkins et al., 2000). However, 'Honeycrisp' apples maintain crispness for extended periods in storage (Tong et al., 1999; Watkins et al., 2005) and can sometimes increase above harvest levels as occurred in orchards in both years (Tables 2 and 5). 'Honeycrisp' is subjected to a conditioning period that causes weight loss in the fruit and which can physically affect firmness readings. Moreover, firmness as measured by standard pressure tester techniques does not necessarily relate to eating quality (Wargo and Watkins, 2004). Therefore, for 'Honeycrisp', firmness is not a useful indicator of storage potential in terms of responses of fruit to different atmospheres.

After firmness, SSC and TA are two criteria that appear to relate to eating quality of apples (Harker et al., 2008). Differences in SSC and TA in 'Honeycrisp' were mainly associated with levels in the fruit of different orchards at the time of harvest (Tables 1 and 4) rather than the effects of different CA regimes (Tables 2 and 5). Informal analyses suggest that high SSC and high TA levels are associated with best eating quality of 'Honeycrisp' apples, and DeEll et al. (2011) describe sensory panel results that indicate that 1-MCP maintained acidity and reduced incidence of unfavorable off-flavors in air-stored fruit after various conditioning periods. Because the flavor of the fruit at harvest appears to be the primary determinant of 'Honeycrisp' eating quality, further research on the interaction between preharvest factors and quality is needed. In this study, fruit were obtained from commercial packing sheds and little is known about preharvest treatment of these fruit. However, fruit from different orchards varied greatly in flavor. Negative impacts of high crop load on size, color, and flavor of 'Honeycrisp' has been identified (Baugher and Schupp, 2010; Robinson and Watkins, 2003).

'Honeycrisp' apples are susceptible to a range of physiological disorders. The disorders that are most associated with the cultivar in air storage, that is, soft scald, soggy breakdown, and low temperature breakdown (DeEll and Ehsani-Moghaddam, 2010; DeLong et al., 2006; Moran et al., 2009; Tong et al., 2003; Wargo and Watkins, 2004; Watkins et al., 2004, 2005), were essentially absent in our study. In part, the absence of soft scald and soggy breakdown results from the conditioning treatment of 7 d at 10 °C and subsequent storage at 3 °C (Watkins and Rosenberger, 2000; Watkins et al., 2004), but CA storage

Table 3. Storage disorders of 'Honeycrisp' apples stored in six CA storage regimes for 6 months at 3 °C plus 4 d at 20 °C.[z]

Orchard no.		CA storage regime (kPa O_2/kPa CO_2) 1.5/1.5	3.0/1.5	4.5/1.5	1.5/3.0	3.0/3.0	4.5/3.0
		Internal CO_2 injury (%)					
1		2	0	0	2	1	1
2		3	3	6	13	9	17
3		5	1	1	4	4	7
4		5	9	4	10	9	8
5		18	14	8	34	22	25
6		2	1	2	1	4	7
	SEM[y]	1.6					
	Orch	<0.001					
	O_2	0.262					
	CO_2	<0.001					
	Orch × O_2	0.086					
	Orch × CO_2	0.001					
	O_2 × CO_2	0.246					
	Orch × O_2 × CO_2	0.884					
		Greasiness (%)					
1		5	13	2	2	8	2
2		1	1	0	1	2	0
3		16	40	57	19	26	22
4		33	51	47	17	28	36
5		5	21	16	9	14	18
6		8	31	10	19	9	9
	SEM	3.8					
	Orch	<0.001					
	O_2	<0.001					
	CO_2	<0.001					
	Orch × O_2	<0.001					
	Orch × CO_2	<0.001					
	O_2 × CO_2	0.001					
	Orch × O_2 × CO_2	0.001					

[z]Fruit were conditioned for 7 d at 10 °C before cooling for 24 h and atmospheres imposed.
[y]SEM for the highest significant interaction, where df in parentheses are: orchard (5); O_2 (2); CO_2 (1); orchard × O_2 (10); orchard × CO_2 (5); O_2 × CO_2 (2), and orchard × O_2 × CO_2 (10).
CA = controlled atmosphere.

Table 4. Harvest indices and mineral concentrations (dry weight basis)[z] in 'Honeycrisp' fruit from the three orchard blocks used in Expt. 2.

Orchard no.	IEC[y] ($\mu L \cdot L^{-1}$)	Starch index (1–8)	Flesh firmness (N)	Titratable acidity (% malic acid)	Soluble solids concn (%)	Calcium ($\mu g \cdot g^{-1}$)	Magnesium ($\mu g \cdot g^{-1}$)	Potassium ($\mu g \cdot g^{-1}$)	Nitrogen ($\mu g \cdot g^{-1}$)	Phosphorus ($\mu g \cdot g^{-1}$)
1	31.9	7.8	55.7	0.299	10.7	41.0	30.0	726	300.0	76.7
2	14.4	7.9	63.5	0.347	11.8	39.2	36.7	1017	343.3	90.0
3	14.2	7.9	56.2	0.322	11.3	40.0	30.0	860	253.3	90.0
Pooled SD	6.20	0.07	1.64	0.020	0.46	0.02	0.03	25.6	24.33	6.71
P value	0.020	0.147	0.002	0.070	0.089	0.474	0.079	<0.001	0.011	0.079

[z]Means of three replicates of 10 fruit.
[y]Internal ethylene concentration.

Table 5. Internal ethylene concentration (IEC), firmness, titratable acidity, and soluble solids concentration (SSC) of 'Honeycrisp' apples stored in three CA storage regimes for 6 months at 3 °C plus 4 d at 20 °C.[z]

		CA storage regime (kPa O_2/kPa CO_2)								
		2/2	3/0.5	3/1.5	2/2	3/0.5	3/1.5	2/2	3/0.5	3/1.5
1-MCP		Orch. 1			Orch. 2			Orch. 3		
treatment		*IEC (μL· L^{-1})*								
None		160.3	192.4	177.9	46.5	81.0	82.9	82.9	96.7	92.5
Day 1		3.3	7.7	4.9	2.0	4.7	2.3	3.0	7.7	6.3
Day 6		5.5	16.3	14.1	1.9	4.6	2.8	3.3	8.8	9.0
	SEM[y]		3.91			3.11			3.12	
	P atm (A)		0.034			<0.001			0.195	
	Treatment (T)		<0.001			<0.001			<0.001	
	A × T		0.355			<0.001			0.918	
		Firmness (N)								
None		58.7	58.2	59.2	59.5	59.4	60.9	55.7	54.4	52.6
Day 1		58.0	57.4	58.7	61.4	61.2	61.9	55.4	57.3	56.1
Day 6		57.2	58.9	59.4	57.6	59.5	58.0	55.7	55.9	53.9
	SEM		0.29			0.99			1.15	
	P atm (A)		0.028			0.847			0.565	
	Treatment (T)		0.270			0.117			0.479	
	A × T		0.156			0.917			0.890	
		Titratable acidity (%)								
None		0.240	0.233	0.253	0.246	0.290	0.284	0.195	0.223	0.219
Day 1		0.236	0.241	0.265	0.309	0.319	0.296	0.232	0.218	0.240
Day 6		0.248	0.246	0.237	0.278	0.291	0.298	0.210	0.239	0.204
	SEM		0.0128			0.0076			0.0135	
	P atm (A)		0.503			0.147			0.446	
	Treatment (T)		0.884			0.019			0.282	
	A × T		0.563			0.356			0.258	
		SSC (%)								
None		10.6	10.2	11.6	11.8	11.7	12.0	10.0	10.7	10.5
Day 1		10.9	10.9	11.5	11.9	12.0	12.0	10.9	10.8	11.2
Day 6		11.2	11.1	11.1	11.5	12.4	12.0	10.7	10.9	10.8
	SEM		0.189			0.140			0.33	
	P atm (A)		0.081			0.299			0.529	
	Treatment (T)		0.352			0.775			0.122	
	A × T		0.273			0.336			0.718	

[z]Fruit were preconditioned for 7 d at 10 °C, either untreated or treated with 1-MCP on Days 1 or 6 before cooling for 24 h and atmospheres imposed.
[y]SEM for the highest significant interaction, where df in parentheses are: atmosphere (2); 1-MCP treatment (2); atmosphere × 1-MCP treatment (4).
CA = controlled atmosphere; 1-MCP = 1-methylcyclopropene.

itself can decrease soft scald if combined with conditioning (DeLong et al., 2006). However, in the current study, fruit were susceptible to internal CO_2 injury in both years and high incidences of core browning and senescent breakdown in 2010 (Tables 3 and 5). The difference in predominant storage disorders between the 2 years is likely to be an effect of two very different growing seasons, a colder 2009 followed by a warmer 2010 with earlier than normal harvest dates. Warmer years, in which harvest can be later for adequate commercial red color to develop, are more likely to be associated with occurrence of senescent breakdown in apple fruit (Marmo et al., 1985; Smock, 1977). Core browning can occur as a result of low-temperature storage, but senile forms are recognized (Smock, 1977). The effect of orchard on susceptibility of 'Honeycrisp' to physiological disorders was often significant (Tables 3 and 5). Orchard-to-orchard variation in susceptibility of fruit is common for many disorders, e.g., bitter pit (Ferguson and Watkins, 1989), senescent breakdown (Marmo et al., 1985), and external and internal CO_2 injury (Watkins et al., 1997; Watkins and Liu, 2010).

Although at least one orchard was relatively free of internal CO_2 injury in each year, incidence of the disorder was significant in the fruit from most orchards (Tables 3 and 6). Cultivars vary in susceptibility to external or internal CO_2 injury (Fernandez-Trujillo et al., 2001). In 'Honeycrisp', the internal form is predominant. As would be expected for a CO_2-related injury, incidence was generally higher with higher pCO_2 (Tables 3 and 6) as shown for both external and internal CO_2 injury (Burmeister and Dilley, 1995; de Castro et al., 2007; Elgar et al., 1998; Fawbush et al., 2008; Watkins et al., 1997). 1-MCP is known to increase the susceptibility of fruit to CO_2 injury (Fawbush et al., 2008), but treatment increased CO_2 injury incidence only when applied on Day 1 followed by storage at 2.0 kPa O_2/2.0 kPa CO_2 (Table 6). The reason for this effect is not known, but interestingly, greater susceptibility to CO_2 injury has been found in 'McIntosh' and 'Empire' apples that have been treated with 1-MCP 1 d after harvest and kept at warmer temperatures before CA storage (Watkins and Nock, unpublished data).

In other apple cultivars, CO_2 injury can be managed by maintaining low pCO_2, delaying exposure of fruit to CA storage, and by treatment of fruit with the antioxidant diphenylamine (DPA) applied to fruit to inhibit development of superficial scald (Burmeister and Dilley, 1995; de Castro et al., 2007; Fawbush et al., 2008; Mattheis and Rudell, 2008; Watkins et al., 1997). Further investigation into the effects of delayed CA storage as well as possible benefits of DPA treatment is warranted.

No significant correlations between mineral concentrations (Tables 1 and 4) and incidence of CO_2 injury (Tables 3 and 6) were detected (data not shown). Watkins and Liu (2010) also found no such relationships for external CO_2 injury in 'Empire' apples. That study also showed the confounding effect of storage atmosphere and storage temperature on relationships between physiological disorders and minerals, and the dramatic effect of different pO_2 and pCO_2 on internal CO_2 injury is likely to provide the same difficulties in developing meaningful relationships. To our knowledge, there have been no meaningful relationships between CO_2 injuries and mineral composition, similar to those for bitter pit and other calcium-related disorders (Ferguson and Watkins, 1989), described in the literature. However, the range of mineral concentrations found in the current study is limited, and further work may identify meaningful relationships with CO_2 injuries.

Also, although there were significant differences in ethanol concentrations in fruit from the three orchards used in Expt. 2 after CA storage (Table 7), no correlations with injury were detected (data not shown). Indeed, 1-MCP treatment on Day 1, which increased injury (Table 6), was not associated with higher ethanol accumulation. Ethanol accumulation in fruit may be related to soft scald development in 'Honeycrisp' apples, although associations are not always strong (Watkins et al., 2004). It is unclear if relationships between acetaldehyde and ethanol accumulations in flesh and core browning disorders in apples and pears are cause or effect (Argenta et al., 2002; Fernandez-Trujillo et al., 2001; Franck et al., 2007; Smagula and Bramlage, 1977).

Greasiness is a feature of 'Honeycrisp' apples that can be aggravated by conditioning treatments before storage (DeLong et al., 2004, 2006; Delong et al., 2009; Watkins et al., 2005). Greasiness is most often associated with later harvest and longer storage periods of susceptible cultivars (Curry, 2008; Ehsani-Moghaddam and DeEll, 2009; Leake et al., 1989a, 1989b; Veraverbeke et al., 2001; Wargo and Watkins, 2004), and its development results from changes in the wax composition of the fruit (Curry, 2008; Morice and Shorland, 1973; Veraverbeke et al., 2001). Although we consider greasiness is a physiological disorder, Ehsani-Moghaddam and DeEll (2009) suggest that greasiness is more appropriately a ripening index because of its close association with higher IEC. In our study, greasiness incidence was not detected at harvest but, after storage, varied greatly by orchard. Interestingly, Orchards 1 and 2, which had the

Table 6. Storage disorders of 'Honeycrisp' apples stored in three CA storage regimes for 6 months at 3 °C plus 4 d at 20 °C.[z]

1-MCP treatment		CA storage regime (kPa O_2/kPa CO_2) 2/2	3/0.5	3/1.5	2/2	3/0.5	3/1.5	2/2	3/0.5	3/1.5
		Orch. 1			Orch. 2			Orch. 3		
		Internal CO_2 injury (%)								
None		0	0	0	2	0	4	6	1	2
Day 1		2	0	0	23	3	7	20	2	7
Day 6		0	0	0	9	0	5	5	9	5
	SEM[zy]		0.4			2.5			2.8	
	P atm (A)		0.041			<0.001			0.036	
	Treatment (T)		0.041			0.001			0.031	
	A × T		0.020			0.012			0.018	
		Core browning (%)								
None		0	0	0	15	8	12	3	0	1
Day 1		0	0	0	2	3	2	3	1	0
Day 6		0	0	0	0	5	1	0	0	1
	SEM		0			1.6			0.7	
	P atm (A)		—			0.896			0.286	
	Treatment (T)		—			<0.001			0.519	
	A × T		—			0.329			0.481	
		Senescent breakdown (%)								
None		0	0	0	36	36	29	11	24	22
Day 1		0	0	0	12	24	17	2	12	2
Day 6		0	0	0	20	26	20	13	12	8
	SEM		0							
	P atm (A)		—			0.209			0.189	
	Treatment (T)		—			0.002			0.013	
	A × T		—			0.695			0.452	
		Greasiness (%)								
None		7	10	10	13	37	27	23	40	20
Day 1		3	10	10	17	27	10	10	10	20
Day 6		0	13	3	20	23	20	23	53	10
	SEM		3.0			2.9			6.6	
	P atm (A)		0.214			0.020			0.014	
	Treatment (T)		0.731			0.203			0.020	
	A × T		0.816			0.278			0.028	

[z]Fruit were preconditioned for 7 d at 10 °C, either untreated or treated with 1-MCP on Days 1 or 6 before cooling for 24 h and atmospheres imposed.
[y]SEM for the highest significant interaction, where df in parentheses are: atmosphere (2); 1-MCP treatment (2); atmosphere × 1-MCP treatment (4).
CA = controlled atmosphere; 1-MCP = 1-methylcyclopropene.

Table 7. Ethanol concentrations (μg·kg^{-1}) of 'Honeycrisp' apples stored in three CA storage regimes for 6 months at 3 °C plus 4 d at 20 °C.[z]

1-MCP treatment		CA storage regime (kPa O_2/kPa CO_2) 2/2	3/0.5	3/1.5	2/2	3/0.5	3/1.5	2/2	3/0.5	3/1.5
		Orch. 1			Orch. 2			Orch. 3		
None		631	2360	1721	401	1467	625	999	2688	905
Day 1		281	288	421	494	323	280	280	281	468
Day 6		281	281	362	281	280	303	350	413	358
	SEM[y]		123.6			89.8			265.7	
	P atm (A)		<0.001			0.001			0.025	
	Treatment (T)		<0.001			<0.001			<0.001	
	A × T		<0.001			<0.001			0.007	

[z]Fruit were preconditioned for 7 d at 10 °C, either untreated or treated with 1-MCP on Days 1 or 6 before cooling for 24 h and atmospheres imposed.
[y]SEM for the highest significant interaction, where df in parentheses are: atmosphere (2); 1-MCP treatment (2); atmosphere × 1-MCP treatment (4).
CA = controlled atmosphere; 1-MCP = 1-methylcyclopropene.

lowest greasiness development after storage (Table 3), had the lowest IECs at harvest (Table 1). We have not located any studies on the effects of different CAs on greasiness, but in the current study, less incidence was associated with lower pO_2 (Table 3) and higher pCO_2 (Tables 3 and 6). 1-MCP is also known to inhibit greasiness development on apple fruit (Curry, 2008; Fan et al., 1999), but effects of 1-MCP on greasiness of 'Honeycrisp' were significant only in fruit from one orchard at the 3.0 kPa O_2/0.5 kPa CO_2 atmosphere treated with 1-MCP on Day 1 (Table 6).

In summary, firmness of 'Honeycrisp' apples is unaffected over a wide range of pO_2 and pCO_2 and, therefore, is not a useful determinant of responses of fruit to different partial pressures. Effects of partial pressures on SSC appear small, whereas highest TA was found at 3.0 kPa O_2, irrespective of pCO_2. The flavor at harvest, therefore, appears to be the primary determinant of 'Honeycrisp' quality, and further research on the interaction between preharvest factors and quality is needed. The susceptibility of 'Honeycrisp' fruit to physiological disorders, and specifically internal CO_2 injury, is a major limitation to the application of CA storage for this cultivar. It is likely that CO_2 injury will be manageable by methods such as delaying the application of CA storage regimes and/or the use of DPA. Until these methods of control have been evaluated, however, we do not yet have a CA recommendation for 'Honeycrisp' apples for New York.

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