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Prestorage Conditioning and Diphenylamine Improve Resistance to Controlled-atmosphere-related Injury in 'Honeycrisp' Apples

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Abstract*. 'Honeycrisp' apples were found to be sensitive to injury from O_2 and CO_2 partial pressures typical of those in controlled-atmosphere (CA) storage. A preliminary study was conducted in 2008 to investigate the effect of the following O_2/CO_2 partial pressure (kPa) combinations: 1/0, 3/0, 1/3, 3/3, 21/3, 21/0 (air), and 21/0 with 1-methylcyclopropene (1-MCP; 1 $\mu L \cdot L^{-1}$) on CA-related injuries of 'Honeycrisp' during storage for 6 months at 3 °C. 'Honeycrisp' apples were found to be sensitive to an injury comprised of irregular-edged brown lesions in the cortex occasionally accompanied by the formation of lens-shaped voids. The symptoms are similar to CA-related injuries described for other apple cultivars and often characterized as a "CO_2 injury." Injury severity increased as O_2 declined and as CO_2 increased and was evident within the first month of storage. During 2009, 2010, and 2011, a study was conducted to evaluate options for avoiding injury during CA storage for this cultivar. Fruit were conditioned at 3, 10, and 20 °C for 5 days and then exposed to the following O_2/CO_2 partial pressure combinations: 3/0, 3/3, 21/0 (regular air); 3/3 with diphenylamine (DPA) drench (1 $g \cdot L^{-1}$); and 21/0 with 1-MCP (1 $\mu L \cdot L^{-1}$). Injury severity declined as the temperature of the prestorage conditioning period increased; holding fruit for 5 days at 20 °C almost completely eliminated the disorder. The antioxidant DPA also provided nearly complete control of CA injury. 1-MCP, although not studied in conjunction with a modified atmosphere, was found to cause no injury in air storage and may provide an alternative to CA storage and avoid the risk of CA injury for 'Honeycrisp'. The relationship between disorder development and growing degree-days, rainfall, and maturity indexes was studied. Ethylene was the only factor with a significant linkage to the development of CA injury (R^2 = 0.35; P = 0.0043). Suggestions for handling of 'Honeycrisp' for extended storage are presented.

The Minnesota Agricultural Experimental Station released 'Honeycrisp' in 1991 (Luby and Bedford, 1992). Since then, its unusual texture attributes and flavor has made 'Honeycrisp' very popular among consumers (The Packer, 2013; Yue and Tong, 2011). 'Honeycrisp' has been described as a cultivar with excellent storage characteristics with the potential for low-temperature storage of 6 months without atmosphere modification (Luby and Bedford, 1992; Tong et al., 1999). Significant production can now be found in Michigan, New York, and Washington (NASS 2011a, 2011b, 2011c).

As the growing area dedicated to 'Honeycrisp' has grown, the need for continued improvement of storage performance has taken on a greater significance. Unfortunately, long-term storage has been a challenge for 'Honeycrisp', which has proven to be very sensitive to low temperatures (Watkins et al., 2004, 2005; Watkins and Rosenberger, 2000) and can be damaged by CA conditions (Beaudry and Contreras, 2009). The low-temperature disorders [i.e., chilling injuries (CIs)] described for 'Honeycrisp' have been diagnosed as soggy breakdown and soft scald (also known as ribbon scald or deep scald) as described by Plagge (1925, 1929) and Ramsey et al. (1917). Tong et al. (2003) reported 'Honeycrisp' susceptibility to soft scald is orchard-specific. Sensitivity to this disorder is enhanced by greater fruit maturity at harvest (Brooks and Harley, 1934; Watkins et al., 2004) but is not necessarily related to ethylene production rate (Tong et al., 2003). In some apple cultivars, soft scald and soggy breakdown may or may not appear simultaneously (Plagge, 1929; Watkins et al., 2005). Delayed cooling or prestorage conditioning of 'Honeycrisp' appears to be effective in controlling soft scald and soggy breakdown (DeLong et al., 2004; Watkins et al., 2004, 2005; Watkins and Rosenberger, 2000). Successful prestorage conditioning temperatures range from 10 to 20 °C and 4 to 7 d in duration.

Many fruit cultivars develop physiological disorders in response to exposure to the low O_2 and elevated CO_2 partial pressures of CA storage. CA storage injury (CA injury) can be caused by low O_2, elevated CO_2, or a combination of both (Pierson et al., 1971). Injury can be manifested as large or small brown lesions, the largest of which are frequently surrounded by a narrow band of healthy tissue at the periphery of the fruit skin and resemble soggy breakdown (Pierson et al., 1971). CO_2 can cause an injury described by Snowdon (1990) as "brown heart," which is exacerbated by low O_2 (Plagge, 1929). Affected fruit are described as possessing small lesions of brown flesh distributed randomly between the skin and the core (Snowdon, 1990). Initially, the injured tissue is firm and moist, but after prolonged storage, they become spongy and dry developing cavities or lens-shaped voids (Plagge, 1929; Snowdon, 1990). 'Empire' apple develops both internal and external injuries and is known to be susceptible to CA storage atmospheres containing 3 to 5 kPa CO_2, and the damage is exacerbated by levels of O_2 of 1.5 kPa or below (Burmeister and Dilley, 1995). For 'Empire', the external CO_2 injury occurs early during storage (Burmeister and Dilley, 1995). Strategies and/or recommendations to control damage in 'Empire' include the use of DPA (Burmeister and Dilley, 1995; Fawbush et al., 2008; Wang et al., 2000; Watkins et al., 1997), use of CA storage with CO_2 levels below 2 kPa, and preferably nearer to 1 kPa (Watkins and Liu, 2010), and delaying CA in combination with 1-MCP to retard fruit softening (DeEll and Ehsani-Moghaddam, 2012a; Watkins and Nock, 2012b). CO_2 injury, expressed as internal browning, is also found in 'Braeburn' apples and is known as "Braeburn browning disorder" (BBD) per Elgar et al. (1998). BBD is triggered by elevated CO_2 and reduced O_2 during CA storage; hence, the recommendations are to store 'Braeburn' apples at less than 1 kPa CO_2 and 3 kPa O_2 (Elgar et al., 1998). The use of delayed CA is additionally recommended (Saquet et al., 2003).

Prestorage conditioning treatments have been used for the alleviation of storage disorders

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for nearly a century (Brooks et al., 1920; Harley and Fisher, 1930; Plagge, 1925, 1929). Prestorage conditioning treatments typically involve exposing fruit to elevated temperatures (i.e., elevated relative to storage temperatures) before storage and/or by delaying the application of refrigeration. Reports on prestorage conditioning effectiveness have been inconsistent with incidence of the disorder increasing, decreasing, or both, depending on cultivar (Brooks et al., 1920; Brooks and Harley, 1934; Harley and Fisher, 1930; Plagge and Maney, 1937). However, to our knowledge, no studies have shown that prestorage conditioning suppresses CA injury in 'Honeycrisp'.

The primary aim of this study was to determine the extent to which CA injury in 'Honeycrisp' was the result of low O_2 and/or elevated CO_2 and to evaluate the influence of prestorage conditioning and DPA treatment on the severity of CA injury of 'Honeycrisp'. A secondary objective was to evaluate whether the use of 1-MCP in air storage, as an alternative to CA storage, would compromise quality. The work was conducted in two phases. The first phase (Expt. 1) was conducted in 2008 and designed primarily to determine whether 'Honeycrisp' apples were susceptible to CA injury, to determine the relative influence of O_2 and CO_2, and to identify a treatment combination that would reliably generate symptoms so that control measures could be subsequently evaluated. The second phase (Expt. 2) was designed to evaluate whether prestorage conditioning treatments or the DPA could suppress CA injury and whether 1-MCP could be used as an alternative to CA storage, thereby avoiding CA injury. The latter experiment was conducted over 3 years (2009–11). An attempt was made to link accumulated heat units and rainfall to disorder incidence over the 3 years of the study.

Materials and Methods

Expt. 1

Plant material. Fruit were harvested from four grower cooperators at different locations in Michigan in the fall of 2008. Harvest dates ranged from 18 Sept. to 1 Oct., at the same time as the main commercial 'Honeycrisp' harvest at each farm. Fruit from each location were separated into 27 lots of 50 apples each for storage experiments. An additional 10 fruit were used for maturity analysis [fruit weight, red skin coloration, background color, internal ethylene concentration (IEC), fruit firmness, starch pattern index, and soluble solids concentration (SSC)] the day after harvest.

Maturity analysis. Fruit weight (g) was determined using a calibrated balance (Toledo Scale). Percent red coloration was estimated on individual fruit by trained assessors. IEC ($\mu L \cdot L^{-1}$) was determined by withdrawing a 1-mL gas sample from the interior of the apples using a disposable plastic syringe and subjecting the gas sample to gas chromatographic (Carle Series 400 AGC; Hach Company, Loveland, CO) analysis as previously described by Mir et al. (2001). Fruit firmness (N) was measured on opposite, paired sides of each fruit using a drill stand-mounted penetrometer (Effegi FT-327; McCormick Fruit Tree Inc., Yakima, WA) fitted with an 11.1-mm-diameter probe. SSC (°Brix) was measured using juice expressed from the penetrometer wound using a handheld refractometer (Atago N1; Atago Co. Ltd., Tokyo, Japan). Background color (1 to 4) was determined by comparison with a McIntosh background color index chart (Cornell University Cooperative Extension, 1948). The starch index (1 to 8) was determined by comparison of the iodine staining pattern of a transverse section of the fruit cut through the seed cavity according to the method of Blanpied and Silsby (1992).

Treatments. Of the 27 fruit lots from each of the four orchards, nine were stored for 1 month, nine were stored for 3 months, and the remaining nine held for 6 months. All fruit were stored at 3 °C, rather than 0 °C, to minimize CI and avoid confusion of soggy breakdown symptoms with CA-related injury symptoms. For each storage duration, each of the lots was given one of nine different storage treatments, which are described as the following O_2/CO_2 concentration (kPa) combinations: 1/0, 3/0, 1/3, 3/3, 21/3, 21/0 (air); 21/0 with 1-MCP (AgroFresh Inc., Spring House, PA); 21/0 conditioned 3 d at 10 °C; and 21/0 conditioned 5 d at 10 °C. At the altitude of the research site, roughly 270 m above sea level, 1% of atmospheric pressure equals 0.98 kPa. Fruit were placed into specially built 0.93-m^3 aluminum chambers (Storage Control Systems, Sparta, MI) and atmospheres were regulated with an automated atmosphere control system (ICA 61 Laboratory System; International Controlled Atmosphere Ltd., Paddock Wood, U.K.). All the CA treatments were imposed on the day of harvest; fruit were not cooled before placement in CA. The storage temperature was maintained by placing the CA chambers in controlled environment chambers held ≈0.5 °C cooler than the target temperature of the CA chamber. Chamber temperatures were monitored continuously. The ethylene action inhibitor, 1-MCP, was applied at an initial concentration of 1 $\mu L \cdot L^{-1}$ in a sealed CA chamber and held for 24 h. 1-MCP application took place 1 to 3 d after harvest while fruit were being held at 3 °C. Fruit given prestorage conditioning treatments were placed at 10 °C on the day of harvest and held for 3 or 5 d before the long-term storage at 3 °C in air. The intent was to determine if prestorage conditioning would lead to any problems with quality apart from any problems caused by CA. During prestorage conditioning, the fruit were covered with 3-mil (76.2-μm) thick low-density polyethylene (LDPE) bags to reduce moisture loss.

Post-storage evaluations. Fruit removed after each storage period were sorted to eliminate fruit with excessive decay, which might obscure disorder detection. Ten fruit from each lot were assessed for IEC, firmness, and SSC. The remainder were covered in LDPE bags as previously described and held for 7 d at 20 °C and afterward assessed for external and internal disorder incidence. External physiological disorders such as soft scald, lenticel breakdown, and bitter pit were evaluated visually and the incidence of the damage determined. CA-related injury to the cortex (brown lesions and lens-shaped cavities, the latter typically associated with smaller brown lesions) and senescence breakdown were evaluated by cutting apples transverse to the longitudinal axis at two points. The fruit section with the most extreme symptoms was used to assess the type, severity, and incidence of the disorder. Disorders were assessed after 1, 3, and 6 months of storage at 3 °C plus 7 d at 20 °C.

Expt. 2

Plant material. During 2009, 2010, and 2011 seasons, 'Honeycrisp' apples were harvested from seven, eight, and six locations, respectively, across the state of Michigan and transported to the Post-harvest Physiology Laboratory at Michigan State University on the morning of harvest. Harvests were between 10 Sept. and 1 Oct. in 2009, 6 and 28 Sept. in 2010, and 13 and 28 Sept. for 2011. Every year for each harvest, fruit were distributed into 30 lots of 50 to 70 fruit and randomly assigned to the various prestorage conditioning treatment/storage condition combinations. Maturity at harvest and after 7 d at 20 °C was assessed on 20 fruit as described previously. Eleven different orchards were used in this study.

Treatments. From the 30 fruit lots, 15 were stored for 3 months and the remaining 15 were stored for 6 months. For each storage duration, five of the lots were conditioned at 3 °C, five lots were conditioned at 10 °C, and the remaining five lots were conditioned at 20 °C. Prestorage conditioning treatments were imposed the day of harvest and the duration was 5 d. During prestorage conditioning, fruit were covered with LDPE bags to reduce moisture loss as described. Lots from the prestorage conditioning treatment/storage duration combinations was subjected to one of the following five O_2/CO_2 partial pressure (kPa) combinations: 3/0, 3/3, 21/0, 3/3 with DPA and 21/0 with 1-MCP. After prestorage conditioning, the fruit were placed into custom-made CA chambers previously described and atmospheric conditions imposed. DPA treatments were applied the day of harvest. DPA-treated fruit were dipped in a DPA solution (No Scald, 31% a.i.; Elf Atochem) containing 1 $g \cdot L^{-1}$ a.i. for 1 min, allowed to drain and dry for 1 h, and then given one of the three prestorage conditioning treatments. 1-MCP was applied as described previously.

Post-storage evaluations. The evaluations performed for physiological disorders were carried out as for Expt. 1. Additionally, for 2011, the lens-shaped cavities and smaller lesion injuries were tracked independently. The purpose of this was to determine if these are different disorders and how they develop during storage.

Statistical analysis. For Expt. 1, the experimental design was completely randomized with a factorial combination of orchard (4), storage duration (3), and postharvest treatments/regimens (9). For Expt. 2, the experimental design was completely randomized with a factorial combination of orchard (6 to 8), storage duration (2), prestorage conditioning treatment (3), and postharvest storage regimens (5). Analysis of variance for both years was performed using SAS (Version 9.1; SAS Institute Inc., Cary, NC) software using PROC GLIMMIX. Tukey's test was used for multiple comparison analysis. An arcsine square root transformation was performed on percentage data before statistical analysis. Non-transformed data are presented. We explored whether relationships existed between the incidence of CA-induced lesions and growing degree-days (GDDs), accumulated precipitation, log IEC, and starch index using a stepwise multiple linear regression. All data from 2009, 2010, and 2011 were used for the regression.

Daily GDD and accumulated precipitation data were collected from Michigan Enviro-weather Automated Weather Station Network from nine stations across Michigan located near orchards used in the study. The weather stations corresponded to: Romeo, Benton Harbor/SWMREC, Sparta, Fremont, Ludington, Hart, Hartford, East Leland, and Berrien Springs, MI. The accumulated GDDs were calculated relative to base temperatures of 4, 10, 15, 21, and 26 °C. The number of GDDs between 4 and 10 °C, 10 and 15 °C, 15 and 21 °C, and 21 and 26 °C were also calculated. GDDs and rainfall data were collected from full bloom date until harvest date for each orchard. The PROC REG and STEPWISE selection method were performed in SAS (Version 9.1; SAS Institute Inc.) using a P value of 0.10. Variables in the model were log-transformed, tested for normality, and residual against predicted values plots were examined.

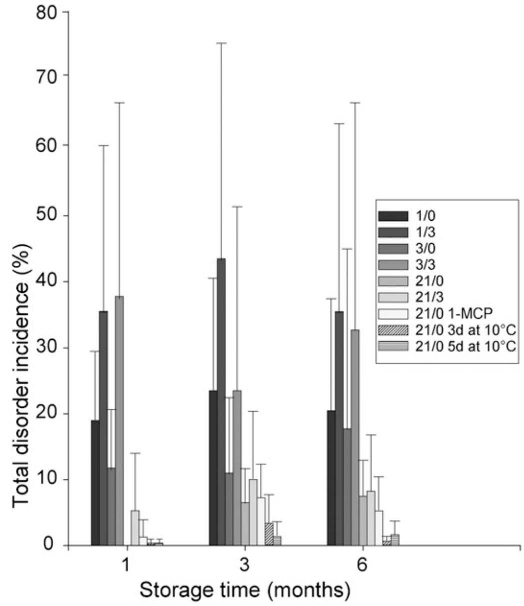

Fig. 1. Effect of controlled atmosphere (CA) storage and prestorage conditioning on CA injury incidence of 'Honeycrisp' apples after 1, 3, and 6 months of storage at 3 °C. For each storage duration, each of the lots was given one of nine different storage treatments, which are described as the following O_2/CO_2 concentration (kPa) combinations: 1/0, 3/0, 1/3, 3/3, 21/3, 21/0 (air); 21/0 with 1-methylcyclopropene (1-MCP); 21/0 conditioned 3 d at 10 °C; and 21/0 conditioned 5 d at 10 °C. Each data point represents the average of 50 fruit from each of four orchards (a total of 1800 fruit). Bars represent 1 SD.

Fig. 2. Transverse cross-sections of 'Honeycrisp' fruit depicting a range of severity of controlled atmosphere (CA)-related injury after 1 month of storage under 3 kPa O_2 and 3 kPa CO_2 at 3 °C.

Results and Discussion

Expt. 1. 'Honeycrisp' apple fruit exhibited a high sensitivity to both low oxygen and elevated CO_2 levels (Fig. 1). We found that the CA atmospheres used induced injuries typical of those associated with CO_2 (i.e., small brown lesions and associated lens-shaped cavities) and also larger dark brown lesions with often irregular margins (Fig. 2). The extent of the injury was higher for those fruit in an atmosphere with elevated CO_2 for each level of O_2. Watkins and Nock (2012b) also studied 'Honeycrisp' under CA conditions and reported this cultivar as highly susceptible to physiological disorders, especially internal CO_2 injuries, which were exacerbated with high levels of CO_2 during storage. After 1, 3, and 6 months of storage, the treatments 1/3 and 3/3 yielded the highest incidence of internal browning, coinciding with the findings of Watkins and Nock (2012b). In the current study, up to 70% of the fruit from individual lots suffered mild to extreme internal browning (data not shown) under low O_2 (both 1 and 3 kPa O_2) when the carbon dioxide level was at 3 kPa. Without CO_2, the internal browning severity was markedly reduced but still significant as long as the O_2 level was low. When the O_2 level was increased to 21 kPa, internal browning only occurred when CO_2 was present, although the degree of damage was relatively minor. 1-MCP and prestorage conditioning at 5 or 10 °C yielded fruit with a very low incidence of internal browning disorders. 1-MCP in air did not influence total disorders when compared with air storage alone. However, disorder incidence declined in air-stored fruit when prestorage conditioning was applied (Fig. 1).

Interestingly, only individual effects of O_2, CO_2, and orchard were significant for the development of disorders (Table 1). The interaction of oxygen and carbon dioxide was not significant, suggesting that these two gases do not have a synergistic effect, but rather are additive on the appearance of the disorders. Importantly, there was great variability between orchards, with some orchards producing susceptible fruit (up to 73% damaged fruit in the treatment 1/3) and others

Table 1. Individual effects and interactions of controlled atmosphere (CA) gases (1.5, 3, and 21 kPa O_2 with and without 3 kPa CO_2), orchard (four orchards) and storage time (1, 3 and 6 months) for CA injury incidence of 'Honeycrisp' apples stored at 3 °C in Expt. 1.

Source of variation	F Value	$P > F$
Oxygen	20.38	<0.0001
CO_2	13.70	0.0005
Orchard	30.79	<0.0001
Duration of storage	0.67	0.5182
Oxygen *CO_2	1.54	0.2231
Oxygen*duration	1.58	0.1950
CO_2*duration	0.35	0.7042
Oxygen *CO_2*duration	0.10	0.9807

producing fruit with little propensity for disorder development (as low as 5% incidence for the same treatment) (Table 2).

The extent of the CA injury seemed to be near its maximum after only 1 month in CA (Fig. 3). Total damage did not increase as the storage duration increased, but the distribution of the categories damage did change slightly (e.g., brown lesion injury decreased over time, whereas cavities increased).

Expt. 2. The CA injury seen in the preliminary study (2008) was reproduced the subsequent 3 years (2009, 2010, and 2011) but with varied intensity. High variability between orchards and years was observed (Table 3).

Brown lesions and cavities in the cortex were suppressed by prestorage conditioning at 20 °C only in 2009, when incidence was highest (Table 3). Unlike Watkins and Nock (2012b) who found high incidence of senescent breakdown during 2009 and 2010, we had a low incidence all 3 years and in all treatments and orchards. Prange et al. (2011) showed that senescent breakdown in 'Honeycrisp' increases with later harvest, especially in fruit heavier than 250 g. High temperatures (10 and 20 °C) during the prestorage conditioning period did not increase bitter pit incidence relative to 3 °C in any of the years. However, orchard affected bitter pit incidence markedly. Soft scald appeared only rarely in our 4 years of study (data not shown).

DPA effectively eliminated the CA injury each year of study (Table 3). DPA reduces CIs (soggy breakdown and soft scald) in 'Honeycrisp' only slightly (Watkins et al., 2004). However, CO_2-related injuries are known to be prevented by DPA (Argenta et al., 2002; de Castro et al., 2008; Mattheis and Rudell, 2008; Meheriuk et al., 1984; Wang et al., 2000; Watkins et al., 1997). In the case of 'Honeycrisp', we found that the brown lesions in the cortex were completely suppressed by DPA application, even when the prestorage conditioning temperature was 3 °C. The incidence of cavities ranged from 0.1% to 0.3% under the same DPA treatment. On the other hand, the most affected treatment was 3/3 followed by 3/0, 21/0, and 21/0 plus 1-MCP.

Although the effects of DPA in preventing disorders are well known, it is unclear how DPA prevents or reduces the disorders. Whitaker (2004) reported that its probable action is as a free radical scavenger, suggesting that oxidative reactions are involved in CO_2 injuries. Toivonen and Brummell (2008) suggested the antioxidative properties of DPA might be involved in protecting against membrane lipid degradation and inhibiting the release of phenylpropanoid substrates into the cytosol, where polyphenyloxidase catalyzes browning and polymerization of phenol substrates. Lee et al. (2012) showed in 'Braeburn' apples, an accumulation of amino acids, acetaldehydes, and ethyl ester compounds linked to appearance of internal browning, suggesting that DPA decreases the amino acid production or increases the protein degradation.

As pointed out previously, there was variation between orchards for brown lesions, cavities with associated small brown lesions, and bitter pit (Table 3). Incidence of damage for brown lesions in the orchards (averaged across all treatment combinations) varied from 0% to 14%, from 0% to 3%, and from 0.1% to 4% for 2009, 2010, and 2011, respectively. Cavity incidence ranged from 0.8% to 6%, 0% to 3% and 0.4% to 2% in 2009, 2010, and 2011, respectively. In 2011, the lens-shaped injury increased during storage up to 2%, whereas smaller brown lesions plateaued after 3 months with a low incidence of 0.3% damage (data not shown). The data may indicate that the smaller brown lesions convert to the lens-shaped cavities over time.

Weather data were extracted from weather stations near the orchards to explore whether CA injury susceptibility may have been linked to environmental influences. For instance, rainfall was found to influence firmness (Lachapelle et al., 2013) and susceptibility to soft scald (Moran et al., 2009). In 2009, the level of CA injury was highest yet accumulated GDDs were lowest (1891 GDDs, based on a 10 °C threshold) compared with the 2010 and 2011 growing seasons, which had 2378 and 2192 GDDs, respectively. However, stepwise regression resulted in GDDs, in addition to rainfall and starch index, being dropped from the model because they were not significant. Only one variable, ethylene, emerged to explain a significant portion of the model, revealing that disorder incidence declined as IEC at harvest increased. This was reminiscent of the findings of Ehsani-Moghaddam and DeEll (2013) in which a strong negative correlation was found between soft scald and IEC at harvest. DeEll and Ehsani-Moghaddam (2012b) found ethylene to be implicated in the sensitivity of 'Empire' apple fruit to CO_2-related storage injury. In 'Honeycrisp', we found the relationship between CA injury incidence and IEC was described by the following equation:

$$\text{Disorder incidence} = 37.71 - 8.27\log(\text{IEC}),$$
$$R^2 = 0.35,\ P\,\text{value} = 0.0043$$

The significant linear regression with the log of the IEC suggests that immature apples were more susceptible to CA-related physiological disorders (Fig. 4). According to our results, apples harvested with an IEC less than ≈20 μL·L^{-1} have a greater potential to develop CA-related injuries. This result suggests that a later harvest would help to minimize the risk of disorders. However, it is also known that soft scald increases with later harvest (Tong et al., 2003; Watkins et al., 2004), which would complicate 'Honeycrisp' handling for the fruit industry.

Harvest maturity is considered the most important factor affecting sensitivity of apples to physiological disorders during ripening and storage (Ferguson et al., 1999). Predictive equations have been used to estimate

Table 2. Controlled atmosphere injury incidence of 'Honeycrisp' apples for four Michigan orchards stored 6 months at 3 °C under different combinations of O_2 and CO_2 with additional treatments of prestorage conditioning (2 and 5 d at 10 °C) and 1-methylcyclopropene (1-MCP; 1 μL·L^{-1}) for fruit held in air.[z]

Treatment	Prestorage	Total disorder incidence (%)				
(O_2/CO_2)	treatment	Orchard 1	Orchard 2	Orchard 3	Orchard 4	Average (%)
1/0	None	39.33	15.67	24.66	1.66	20.33 ab
1/3	None	73.33	20.67	35.66	5.33	33.75 a
3/0	None	34.66	9.0	5.33	2.66	12.91 bc
3/3	None	71.33	28.0	21.66	4.33	31.33 a
21/0	None	7.33	7.67	2.66	1.0	4.66 c
21/3	None	15.33	11.67	4.33	0.0	7.83 c
21/0 1-MCP	1-MCP	9.0	5.67	4.0	0.0	4.66 c
21/0 3 d at 10 °C	3 d at 10 °C	1.0	10.5	3.33	0.0	3.70 c
21/0 5 d at 10 °C	5 d at 10 °C	2.0	5.0	1.33	0.0	2.10 c

[z]Treatment atmospheres are given in kPa. Tukey's test was used for multiple comparison analysis of averages. Numbers in the column followed by differing letters were different.

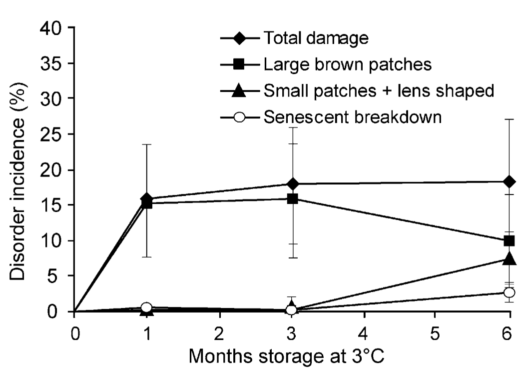

Fig. 3. Incidence of controlled atmosphere (CA)-related injury in 'Honeycrisp' apples after 1, 3, and 6 months of CA storage under 3 kPa O_2 and 3 kPa CO_2 at 3 °C. Each data point represents the average for fruit from all nine treatments noted in the caption for Figure 1 and all four orchards (a total of 1800 fruit). Bars represent 1 SD.

Table 3. Total controlled atmosphere (CA) injury incidence of 'Honeycrisp' apples with or without prestorage conditioning treatments and stored 3 months at 3 °C under different CA treatments in 2009, 2010, and 2011.[z]

	Internal disorders									External disorders		
	Controlled atmosphere-related injury											
	Brown lesions (%)			Cavities (%)			Senescent breakdown (%)			Bitter pit (%)		
Effects	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
Prestorage conditioning temperature												
3 °C	10.3 a	1.3 a	1.9 a	4.1 a	1.1 a	1.4 a	0.0 a	0.8 a	0.3 a	5.9 a	13.6 a	8.4 a
10 °C	6.5 a	1.5 a	1.2 a	2.2 a	1.0 a	0.9 a	0.0 a	0.7 a	0.4 a	7.6 a	14.6 a	10.9 a
20 °C	0.6 b	0.5 a	0.6 a	0.7 a	0.3 a	0.6 a	0.0 a	0.7 a	0.2 a	6.2 a	18.8 a	6.7 a
Storage treatment (O_2/CO_2)												
3/0	7.7 b	0.8 b	0.3 b	2.3 b	0.3 b	0.9 b	0.0 a	0.4 a	0.0 a	7.3 a	16.7 a	5.0 a
3/3	13.6 a	3.3 a	5.8 a	6.3 a	3.0 a	3.7 a	0.0 a	0.6 a	0.4 a	4.1 a	14.5 a	10.3 a
3/3 + DPA	0.0 c	0.0 b	0.0 b	0.3 b	0.3 b	0.1b	0.0 a	0.3 a	0.1 a	6.6 a	13.8 a	7.8 a
Air	3.4 b	1.0 b	0.0 b	1.4 b	0.2 b	0.1 b	0.0 a	1.5 a	0.5 a	6.3 a	20.7 a	10.8 a
1-MCP	4.4 b	0.3 b	0.2 b	1.4 b	0.1 b	0.1 b	0.0 a	0.8 a	0.5 a	8.4 a	12.5 a	9.5 a
Orchard												
1	0.0 b	0.0 a	4.1 a	0.8 a	0.0c	0.7 a	0.0 a	0.0 b	0.0 b	15.3 a	31.1 b	25.3 a
2	0.0 b	1.1 a	2.2 a	5.7 a	0.3 bc	0.4 a	0.0 a	0.5 b	0.1 b	5.0 b	0.8 ef	3.2 d
3	9.7 a	1.9 a	0.1 a	0.8 a	3.2 a	1.2 a	0.0 a	0.0 b	0.0 b	4.2 b	22.6 b	0.3 d
4	3.0 b	3.1 a	0.7 a	1.5 a	0.0c	1.8 a	0.0 a	0.1 b	0.6 ab	15.2 a	13.5 c	14.5 b
5	11.6 a	0.4 a	0.3 a	4.0 a	0.0 c	0.8 a	0.0 a	0.0 b	0.0 b	0.6 c	0.0 f	7.8 c
6	14.3 a	0.0 a	0.1 a	2.5 a	0.2bc	0.9 a	0.0 a	1.4 b	1.2 a	0.3 c	47.0 a	1.1 d
7	2.2 b	0.0 a	—	0.9 a	0.3 bc	—	0.0 a	0.0 b	—	5.1 b	5.9 dc	—
8	—	2.2 a	—	—	2.2 ab	—	—	3.7 a	—	—	4.3 de	—

[z]Treatment atmospheres are in kPa. Tukey's test was used for multiple comparison analysis.
DPA = diphenylamine; 1-MCP = 1-methylcyclopropene.

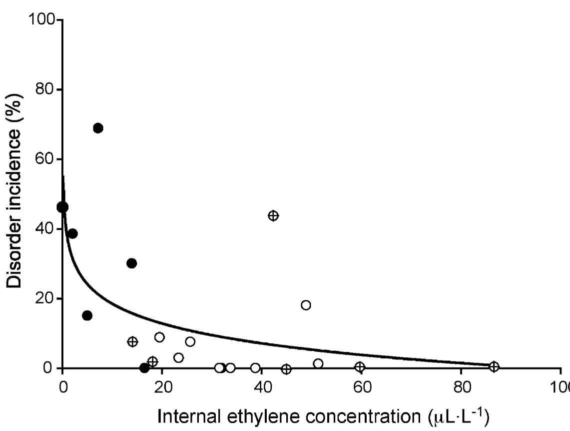

Fig. 4. Relationship between controlled atmosphere (CA)-related injuries (brown flesh lesions only) and internal ethylene concentration (IEC) at harvest for 'Honeycrisp' apples after 3 months of CA storage under conditions of 3 kPa O_2 and 3 kPa CO_2 at 3 °C. Each symbol corresponds to the average of 50 fruit from one orchard. Open symbols correspond to year 2009; closed symbols to 2010; and open cross symbols to 2011.

the incidence of disorders (Autio et al., 1986; Moran et al., 2009). In addition to the influence of maturity, many studies have also demonstrated a strong relationship between disorder susceptibility and orchard practices and/or environmental conditions (Ferguson et al., 1999). Watkins and Liu (2010) suggested mineral nutrition could be used to predict susceptibility to CO_2 injury, but Watkins and Nock (2012a) found no correlations between mineral concentrations and incidence of CO_2 injury in 'Honeycrisp' apples. A better understanding of the factors involved in fruit storage disorders and how they relate to each other will allow optimization of storage quality and development of methods for predicting disorder risk.

Although it can take several years to establish a storage protocol resulting from validation for fruit harvested in different years, optimizing handling protocols for the new cultivars have been successful in the past to reduce the incidence of physiological disorders to a manageable level (Johnston and Brookfield, 2012). Development of such protocols has allowed, for example, reducing the incidence of soft scald in 'Honeycrisp' by managing appropriate storage temperatures (Watkins et al., 2004; Watkins and Rosenberger, 2000).

The development of recommendations for the storage of this cultivar in CA is progressing (Beaudry et al., 2010; Contreras and Beaudry, 2010), however additional considerations are warranted. For instance, although the 7-d prestorage conditioning treatments provided some protection against the development of CA injury, shorter durations should be investigated to prevent quality loss resulting from excessive ripening, which could cause increased skin greasiness and undesirable flavor profile (DeLong et al., 2009). Given the rapid rate of formation of CA injury, it seems advisable to minimize the exposure to elevated CO_2 during room loading and prestorage conditioning in addition to the first month or so of CA storage. If 'Honeycrisp' behaved like 'Empire' apple fruit with regard to CA injury development, it may be possible to increase CO_2 after first 4 to 6 weeks of CA storage. Use of DPA can be used to avoid CA injury and with concentrations of CO_2 as high as 3%, but fruit may still have to be conditioned and would need to be held at 3 °C to minimize the risk of CI. Finally, in-air storage has merit in extending the storage duration of 'Honeycrisp' in that its use avoids the issue of CA injury altogether.

Extended storage of 'Honeycrisp' in CA or with 1-MCP application in air storage requires additional considerations not directly related to the storage atmosphere. Given the propensity of 'Honeycrisp' to develop bitter pit (Rosenberger et al., 2004; Watkins et al., 2005), storage and handling recommendations should probably include preharvest management of bitter pit (e.g., through Ca^{+2} applications and crop thinning). Although the current study suggests that prestorage conditioning does not exacerbate this disorder, previous work does (Watkins et al., 2004) and therefore vigilance is probably justified. Although decay was not specifically evaluated in the current study, the decay rate was ≈5% to 10% in 3- and 6-month-stored fruit. There is some concern, therefore, that prestorage conditioning and elevated storage temperatures may promote decay, so a good preharvest decay control strategy would be advisable for this decay-susceptible cultivar (Rosenberger, 2004; Watkins et al., 2005). Although starch index was not linked to CA injury, low ethylene production was. Watkins et al. (2005), found a starch index above 5 was associated with increasing ethylene levels, suggesting this might be an appropriate target for harvest maturity for fruit destined for CA storage.

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