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Research Article

Influence of 1-Methylcyclopropene Treatment on Postharvest Quality of Four Scab (*Venturia inaequalis*)-Resistant Apple Cultivars

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Scab (*Venturia inaequalis*) is a very serious disease for apples causing up to 80% of loss in yield but there are only a few studies on postharvest quality of scab-resistant cultivars. In this study we evaluated the effect of 1-methylcyclopropene (1-MCP) on fruit quality, total phenolic content, and antioxidant capacity after storage of four scab-resistant cultivars and compared to a standard cultivar, "Golden Delicious." In general, ethylene production and respiration rates significantly differed among cultivars, between control and 1-MCP-treated fruits, and between storage duration regimes. 1-MCP treatment retarded fruit softening and lowered juice pH but storage effect on soluble solids and acidity depended on cultivar and 1-MCP treatment. Total phenolic content was significantly affected by storage duration and 1-MCP treatment. Antioxidant capacity of the four scab-resistant cultivars was either similar to or significantly higher than that of "Golden Delicious" with the 1-MCP-treated fruits having significantly higher antioxidant capacity than the nontreated fruits after storage. Our results clearly show that the quality of four scab-resistant cultivars was comparable to that of "Golden Delicious" and 1-MCP effect differed among cultivars. These differences need to be considered in developing storage regime to minimize quality deterioration during long-term storage.

1. Introduction

The hazardous effects of pesticides on human health and the environment have contributed to the introduction of cultivars with effective resistance genes to major plant diseases. Yield losses from fungal diseases are significant in most apple producing countries worldwide. Apple scab, caused by the fungus *Venturia inaequalis*, is one of the most destructive diseases of apples throughout the world, causing up to 80% loss in yield without chemical intervention, which could include multiple classes of fungicides applied up to 22 times during the growing season [1]. In 1944 Hough, from University of Illinois, was the first to identify the scab-resistant gene V_f in *Malus floribunda* [2]. Since 1944, additional scab-resistant genes have been identified in other apple species [3]. In 1948, three US universities (Purdue, Rutgers, and Illinois, PRI)

formed a research team to develop apple cultivars resistant to scab, utilizing the V_f gene [3]. In recent years, several apple cultivars containing V_f have been introduced into commercial production from the PRI program, including "Dayton," "Prima," "Priscilla," and "Jonafree" [4]. Among the most promising introductions are "WineCrisp" [5], "CrimsonCrisp" [6], "Pixie Crunch" [7], and "GoldRush" [8]. In addition to the scab-resistant apple cultivars released by the PRI breeding program, several cultivars have also been released by breeding programs at Cornell University in NY, by Agriculture Canada, and by the National Institute of Agronomic Research in Angers, France, and Consorzio Italiano Vivaisti in Italy, and Breeding Initiative Niederelbe in Germany [9].

Despite the introduction of several apple scab-resistant cultivars, most have gained very little popularity, especially in

North America, because of lack of recognition by consumers [4, 10]. The overwhelming acceptance of "Honeycrisp" (a scab susceptible) cultivar by consumers in a relatively short-period of time has made it possible for newer cultivars to also be successful. "GoldRush," "WineCrisp," and "CrimsonCrisp" are becoming commercially popular in the USA, especially among organic and small acreage producers. However, there is very little scientific information published on the postharvest quality or storage potential of these cultivars. Abbott et al. [11] reported that the eating quality of "GoldRush" fruit slices was higher than "Granny Smith" and "Golden Delicious" and comparable to "Fuji" while there are two other studies that had ranked the sensory quality of scab-resistant cultivars from different sources [12, 13]. However, most other available information relies on nonscientific data to describe the quality of these cultivars.

The objectives of this research were to determine fruit quality after two periods of storage of four scab-resistant cultivars, "CrimsonCrisp," "GoldRush," "Pixie Crunch," and "WineCrisp," and compare the results to a standard cultivar, "Golden Delicious." In addition, we examined the effect of the ethylene inhibitor, 1-methylcyclopropene (1-MCP), on the quality of the four cultivars following storage. 1-MCP has been shown to maintain fruit storage quality of several apple cultivars [14].

2. Materials and Methods

2.1. Plant Material. Fruits were harvested from six-year-old "CrimsonCrisp," "GoldRush," "Pixie Crunch," "WineCrisp," and "Golden Delicious" trees grafted on "Budagovsky 9" rootstock and trained to the tall spindle system. Trees were grown at the Fruit Research Farm at University of Illinois, Urbana-Champaign, Illinois, USA. Cultural management of the trees was according to the 2013 Midwest Tree Fruit Pest Management Guide (https://ag.purdue.edu/hla/Hort/Pages/sfg_sprayguide.aspx).

Full bloom date for "Pixie Crunch" and "CrimsonCrisp" was 29 April, and that for "WineCrisp" and "Golden Delicious" was 2 May. The full bloom date for "GoldRush" was 6 May 2013. Fruits of "Pixie Crunch" and "CrimsonCrisp" were harvested on 2 September and "WineCrisp" and "Golden Delicious" on 10 September, and "GoldRush" was harvested on 2 October 2013. Harvest dates were determined by measuring flesh firmness, soluble solids, and starch levels, as described by Witney et al. [15], from 10 fruits of each cultivar from the middle of the canopy (3 trees per cultivar).

2.2. 1-MCP Treatment and Storage. A total of 240 uniform size fruits from each cultivar were randomly divided into two lots of 120 fruits, with each lot further divided into three replicates of 40 fruits each. One of the two lots of each cultivar was placed in a 117 L plastic container, tightly sealed; then 500 ppb of 1-MCP (SmartFresh®, AgroFresh, Collegeville, PA, USA) was injected with a hypodermic needle through a rubber septum installed in the wall of the container. Fruits were exposed to the 1-MCP for 24 hours at $0 \pm 1^{\circ}$C, following the manufacturer's instructions. Ten fruits per replicate were labeled and stored at $0 \pm 1^{\circ}$C and $90 \pm 5\%$ relative humidity

for up to 140 days. Treated fruits were sampled after 70 and 140 days of storage, placed at room temperature ($20 \pm 1^{\circ}$C) for seven days to simulate commercial handling practices, and then evaluated for their quality characteristics. Ten fruits from each replicate and treatment were analyzed for respiration, ethylene, firmness, skin color, soluble solids, and acidity as described next. Representative tissue samples (about 10 g) were collected from the sun- and shade-exposed sides of each fruit and freeze-dried in a Virtis freeze dryer (VirTis Comp. Inc., Gardiner, NY, USA) to analyze chemical composition and measure antioxidant capacity.

2.3. Fruit Firmness and Skin Color. Flesh firmness was measured in two perpendicular peeled sides from each fruit using a penetrometer (FT327, McCormick Fruit Tech, Yakima, Washington, USA) equipped with an 11 mm tip. Fruit firmness was presented in newton (N).

Skin color was measured at two different locations of equatorial regions of the fruit using a digital colorimeter (CR-200, Konica Minolta, Osaka, Japan) and expressed as averaged values. The hue angle values were calculated from the equation $\arctan(b^*/a^*)$.

2.4. Soluble Solids and Titratable Acidity. Juice extract from each replicate was used to measure soluble solids, titratable acidity, and pH. Soluble solids were measured using a temperature compensated refractometer (Leica 10430, Buffalo, NY, USA), while titratable acidity was determined by titrating juice samples from each replicate with a standardized 0.1 M NaOH and expressed as milligrams of malic acid per 100 mL juice using a pH meter (Fisher Science Education, Pittsburgh, PA, USA).

2.5. Organic Acids. A 1.0 g freeze-dried sample from each replicate was extracted with 5 mL of 0.004 N H_2SO_4 using a Polytron homogenizer (Kinematics, Switzerland) set at a speed of 4 for 1.0 min in the dark. The homogenate was centrifuged at a $27,000\,g$ at 5°C for 30 min. A 1.0 mL fraction of the supernatant was filtered through a $0.2\,\mu$m nylon filter and a $5\,\mu$L fraction was injected into an HPLC (Hitachi, Tokyo, Japan) equipped with a photodiode array detector and a REZEX $10\,\mu$ 8% H organic acid column (300×7.8 mm) (Phenomenex, Torrance, CA, USA). Sulfuric acid (0.0004 N) was used as a mobile phase at a flow rate of 0.7 mL/min. Organic acids were detected at 210 nm and quantified based on an external organic acids standard curve (malic, oxalic, and tartaric acids) as mg/100 g dry weight.

2.6. Ethylene and Respiration Measurement. Ethylene production and respiration rates were measured using a subsample of five fruits from each replicate. Fruits were weighed and placed in a 3.8 L glass jar and sealed for 1 h at 27°C. A 1.0 mL gas sample was withdrawn from each jar with a hypodermic syringe and injected into an AutoSystem gas chromatography (Perkin Elmer, Waltham, MA, USA) equipped with flame ionization detector (FID) and thermal conductivity detector (TDC). Ethylene was measured using FID, activated alumina column, and helium as carrier. The

oven, injector, and detector temperatures were 80, 100, and 200°C, respectively. Ethylene measurement data were expressed as μL/g/h. Respiration rate was measured as the amount of CO_2 generated. The amount of CO_2 was measured using TDC, Porapak R column (Agilent Technologies Santa Clara, CA, USA), and helium as carrier gas. One-milliliter air samples were collected from the same jars, as described above for ethylene, evaluated for CO_2 production, and expressed as mL/kg/h.

2.7. Total Phenolics and Antioxidant Capacity. For extraction of polyphenols, approximately 0.5 g of freeze-dried tissue samples was homogenized in 20 mL of 70% methanol using a Polytron homogenizer (Kinematica Ag, Luzern, Switzerland) set at a speed of 4 for 1 min. The homogenate was centrifuged twice for 10 min at 4,000 g. The supernatant was collected and used to determine total phenolic content using a colorimetric Folin-Ciocalteu method as previously described by Ku et al. [16]. Briefly, 10 μL of sample extracts was mixed with 0.2 N Folin-Ciocalteu phenol reagent (100 μL) in a 96-well plate. After 3 min, 90 μL of a saturated sodium carbonate solution was added to the mixture, followed by incubation at room temperature for 1 h. The resulting absorbance of the mixture was measured at 630 nm using a BioTek EL 808 microplate reader (Biotek Instruments Inc., Winooski, VT, USA). The total phenolic content was calculated on the basis of a standard curve using gallic acid (concentration range from 31.25 to 500 μg/mL). Results were expressed in milligrams of gallic acid equivalent (GAE) per g of dried apples.

Antioxidant capacity was evaluated using 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) methods according to Ku et al. [16]. Briefly, the ABTS method involved dissolving 7 mM ABTS (ammonium salt) in potassium phosphate buffer (pH 7.4) and combining with 2.45 mM potassium persulfate. The mixture was stored in the dark for 10 min. The dark blue solution was diluted with potassium phosphate buffer (pH 7.4) until the absorbance reached 1.0 ± 0.02 at 734 nm (BioTek EL 808 microplate reader, Biotek Instruments, Winooski, VT, USA). A 200 μL fraction of the resulting solution was mixed with 10 μL of the sample, and after 6 min of incubation under dark condition at room temperature, the absorbance was recorded. The results were expressed as Trolox equivalent (mM TE/g dry weight). All samples were analyzed in triplicate. For FRAP assay, 10 μL of the sample was mixed with 200 μL of freshly prepared FRAP reagent, consisting of 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl in distilled water, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water, and 25 mL of 0.3 M acetate buffer (pH 3.6). The absorbance was measured at 593 nm after 6 min of incubation on a microplate reader (BioTek EL 808 microplate reader, Biotek Instruments, Winooski, VT, USA). The results were expressed as Trolox equivalents (mM TE/g of dry weight). All samples were analyzed in triplicate.

2.8. Statistical Analysis. The experiment was set up as a randomized complete plot design with four replications. Analysis of variance and post hoc tests were performed using JMP Pro 12 (SAS Institute, Cary, NC, USA).

3. Results and Discussion

Results of this study show that the four scab-resistant cultivars have comparable fruit quality to the standard cultivar "Golden Delicious" after 70 and 140 days of storage. Fruit firmness differed significantly among the five cultivars. At harvest, "WineCrisp" was the firmest (102.5 N) compared to the other cultivars. Storage and 1-MCP treatment significantly affected the fruit firmness (Figure 1). Fruit firmness decreased over storage duration, in particular after 140 days at 0°C followed by 7 days at 20°C, in all cultivars except for 1-MCP-treated "WineCrisp." Generally, fruits treated with 1-MCP were firmer than nontreated fruits, except "GoldRush," where there was no difference between treated and nontreated fruits even after 140 days of refrigerated storage and then 7 days at room temperature. Several studies have shown that fruit firmness decreased during storage, but 1-MCP treatment retarded fruit softening [17, 18]. Watkins et al. [19] also showed differences among cultivars in response to 1-MCP, with 1-MCP-treated "Empire" and "Red Delicious" being about 20 and 10 N firmer than the control, respectively, while no significant differences in firmness were observed in 1-MCP-treated "La Rome" and "McIntosh" fruits. Firmness of most fruits is associated mainly with changes in pectin composition, especially galacturonic acid. Activities of enzymes involved in pectin degradation and fruit softening are generally ethylene-dependent [20, 21]. There is an agreement that 1-MCP treatment maintains fruit firmness through inhibition of ethylene action and reducing activities of cell wall hydrolases [22]. The variability in fruit softening in response to 1-MCP, among the five cultivars, may have been due to differences in the degree of ethylene inhibition. Several studies have reported different responses to 1-MCP among different apple cultivars [19, 23, 24]. For example, DeLong et al. [23] found that 1-MCP-treated fruits of "Redmax" and "Redcort Cortland" apples had significantly higher firmness than the control, while there was no difference in firmness between 1-MCP-treated and control fruits of "Summerland McIntosh" apples after nine months of storage. In the present study, we found a significant interaction between cultivar and 1-MCP treatment ($p < 0.0001$) and between cultivar and storage duration ($p < 0.0001$). These results suggest that textural change in response to 1-MCP treatment and storage is cultivar-dependent.

Similar to firmness, soluble solids content differed among the cultivars (Table 1). At harvests, soluble solids content was similar in four cultivars but lower in "GoldRush." After storage, "Golden Delicious" fruits have the highest soluble solids, except for 1-MCP-treated fruit stored for 140 days at 0°C followed by 7 days at 20°C. Soluble solids content in "Golden Delicious," "GoldRush," and "WineCrisp" increased with storage but that in "Pixie Crunch" did not change with storage in both control and 1-MCP treatment. Soluble solid content in "CrimsonCrisp" decreased in control but increased in 1-MCP-treated fruits after 140 days at 0°C followed by 7 days at 20°C. These results indicate differential sugar metabolism during storage and response to 1-MCP among cultivars. Similarly, Watkins [25] found significant difference in soluble solids in response to 1-MCP treatment among cultivars, while DeLong et al. [23] reported that soluble solids

TABLE 1: Soluble solids content and acidity of apple fruit.

Cultivar[z]	SSC (°Brix)[z] Control	SSC (°Brix)[z] 1-MCP[y]	TA (mg malic acid/100 mL juice) Control	TA (mg malic acid/100 mL juice) 1-MCP	SSC/TA Control	SSC/TA 1-MCP	pH Control	pH 1-MCP
Day 0[z]								
GD	13.5 ± 0.2Ab[x]	13.5 ± 0.2Ab	0.47 ± 0.02Ca	0.47 ± 0.02Ca	29.0 ± 1.6Bc	29.0 ± 1.6Bb	3.61 ± 0.03Ab	3.61 ± 0.03Ab
GR	12.2 ± 0.3Bb	12.2 ± 0.3Bb	0.68 ± 0.01Aa	0.68 ± 0.01Ab	18.0 ± 0.8Cb	18.0 ± 0.8Ca	3.09 ± 0.03Cb	3.09 ± 0.03Cb
WC	13.3 ± 0.2Ab	13.3 ± 0.2Ab	0.54 ± 0.02BCa	0.54 ± 0.02BCa	24.7 ± 1.3Bc	24.7 ± 1.3Ba	3.23 ± 0.02Bc	3.23 ± 0.02Bb
PC	13.7 ± 0.1Aa	13.7 ± 0.1Aa	0.37 ± 0.01Da	0.37 ± 0.01Da	37.2 ± 0.5Ac	37.2 ± 0.5Ab	3.68 ± 0.04Ac	3.68 ± 0.04Ab
CC	13.9 ± 0.2Aa	13.9 ± 0.2Ab	0.57 ± 0.02Ba	0.57 ± 0.02Bab	24.3 ± 1.1Bc	24.3 ± 1.1Bab	3.32 ± 0.02Bc	3.32 ± 0.02Bb
Day 70								
GD	15.7 ± 0.2Aa	15.9 ± 0.2Aa	0.38 ± 0.01Cb	0.47 ± 0.02Ca*	41.0 ± 1.1Ab	34.1 ± 0.9Ab*	3.69 ± 0.05Bb	3.60 ± 0.00Bb
GR	14.0 ± 0.0Ba	14.3 ± 0.1Ca	0.56 ± 0.02Ab	0.80 ± 0.02Aa*	25.1 ± 0.8Ca	17.9 ± 0.5Da*	3.25 ± 0.01Da	3.12 ± 0.00Db*
WC	14.0 ± 0.0Ba	15.2 ± 0.0Ba*	0.44 ± 0.02BCb	0.59 ± 0.02Ba*	31.7 ± 1.2Bb	25.7 ± 0.7BDa*	3.42 ± 0.02Cb	3.32 ± 0.02Cb*
PC	13.5 ± 0.0Ca	13.8 ± 0.2Ca	0.29 ± 0.01Db	0.38 ± 0.01Da*	46.3 ± 1.9Ab	36.3 ± 0.6Ab*	4.18 ± 0.03Ab	3.82 ± 0.04Ab*
CC	13.3 ± 0.1Cab	14.4 ± 0.1Cab*	0.45 ± 0.01Bb	0.64 ± 0.02Ba*	29.5 ± 0.7BCb	22.5 ± 0.7Cb*	3.67 ± 0.01Bb	3.49 ± 0.04Ba*
Day 140								
GD	15.3 ± 0.1Aa	14.6 ± 0.7ABab	0.32 ± 0.01Cb	0.32 ± 0.01Cb	48.0 ± 1.6Ba	45.3 ± 1.8Aa	4.05 ± 0.04Ba	3.78 ± 0.00Ba*
GR	14.4 ± 0.2ABa	14.3 ± 0.3ABa	0.59 ± 0.02Ab	0.85 ± 0.03Aa*	24.7 ± 0.7Da	16.9 ± 0.7Ca*	3.35 ± 0.03Da	3.23 ± 0.02Ea*
WC	14.5 ± 0.3ABa	15.1 ± 0.0Aa	0.38 ± 0.01Bb	0.53 ± 0.04Ba*	38.2 ± 1.6Ca	29.2 ± 2.1Ba*	3.60 ± 0.03Ca	3.46 ± 0.02Da*
PC	13.5 ± 0.3BCa	13.4 ± 0.1Ba	0.21 ± 0.01Dc	0.29 ± 0.00Cb*	63.5 ± 0.7Aa	45.7 ± 0.5Aa*	4.40 ± 0.04Aa	4.05 ± 0.06Aa*
CC	13.0 ± 0.0Cb	14.9 ± 0.0ABa*	0.35 ± 0.01BCc	0.55 ± 0.01Bb*	37.2 ± 1.2Ca	27.3 ± 0.7Ba*	3.96 ± 0.02Ba	3.60 ± 0.01Ca*
Significance[x]								
Cultivar (C)	***		***		***		***	
1-MCP (T)	***		***		***		***	
Storage duration (S)	***		***		***		***	
C × T	***		***		***		***	
C × S	***		***		***		***	
T × S	***		***		***		***	
C × T × S	**		**		**		**	

[z]GD, "Golden Delicious"; GR, "GoldRush"; WC, "WineCrisp"; PC, "Pixie Crunch"; CC, "CrimsonCrisp"; SSC, soluble solids content; TA, titratable acidity; Day 0, at harvest; Day 70, 70 days of storage at 0°C followed by 7 days at 20°C; Day 140, 140 days of storage at 0°C followed by 7 days at 20°C. [y]Values for 1-MCP treatment before storage (Day 0) are the same as that for control. These values are kept due to statistical analysis and to assist with comparing the storage duration effects of each cultivar and each treatment. [x]Data are presented as mean ± standard error ($n = 4$). Capital letters indicate mean separation among cultivars of each treatment and each storage day and lowercased letters show significant difference among storage duration treatments of each cultivar and each treatment by Tukey's HSD at $p < 0.05$. Asterisks (*) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration treatment by Student's t-test at $p < 0.05$. [x]NS means nonsignificant. *, **, and *** mean significance at $p < 0.05$, 0.01, and 0.001, respectively.
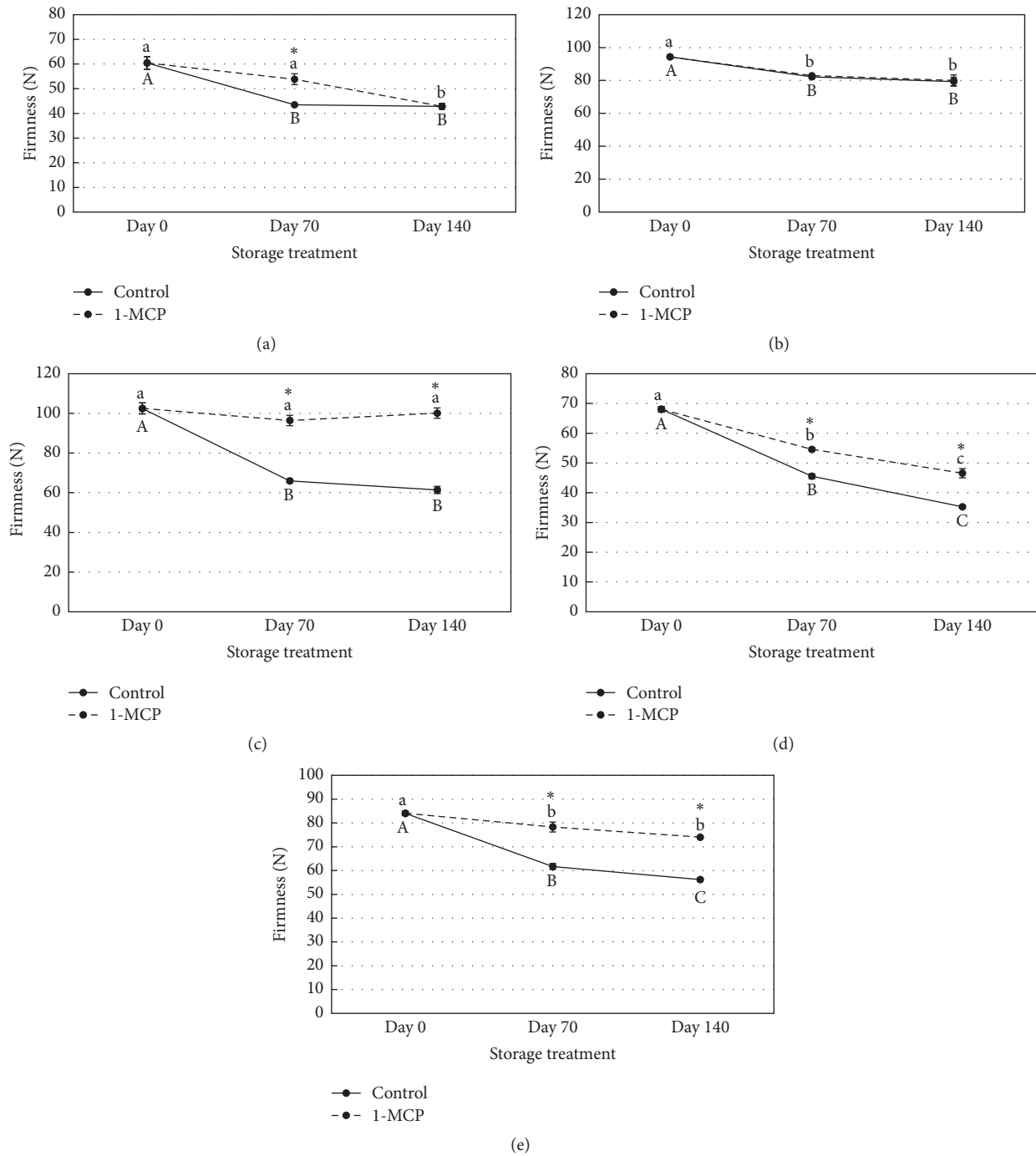
Figure 1: Firmness of apple fruit at (a) "Golden Delicious," (b) "GoldRush," (c) "WineCrisp," (d) "Pixie Crunch," and (e) "CrimsonCrisp" apples at harvest and 70 or 140 days of storage at 0°C followed by 7 days at 20°C. Day 0, at harvest; Day 70, 70 days of storage at 0°C followed by 7 days at 20°C; Day 140, 140 days of storage at 0°C followed by 7 days at 20°C. Capital letters and lowercased letters indicate mean separation among storage duration treatment of control and 1-MCP-treated apples, respectively, by Tukey's HSD at $p < 0.05$. Asterisks ($*$) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration using Student's t-test at $p < 0.05$. Vertical bars represent standard error ($n = 4$).

content in "Redmax," "Redcort Cortland," and "Summerland McIntosh" was not affected by 1-MCP treatment. Our study has identified a significant interaction between cultivar and 1-MCP treatment ($p < 0.0001$, Table 1) indicating that change in soluble solids content is cultivar-dependent.

Titratable acidity and pH also differed among cultivars. "GoldRush" was consistently highest in titratable acidity but lowest in pH while their change over storage differed between control and 1-MCP treatment (Table 1). Fruit acidity generally decreased with storage in the control group but
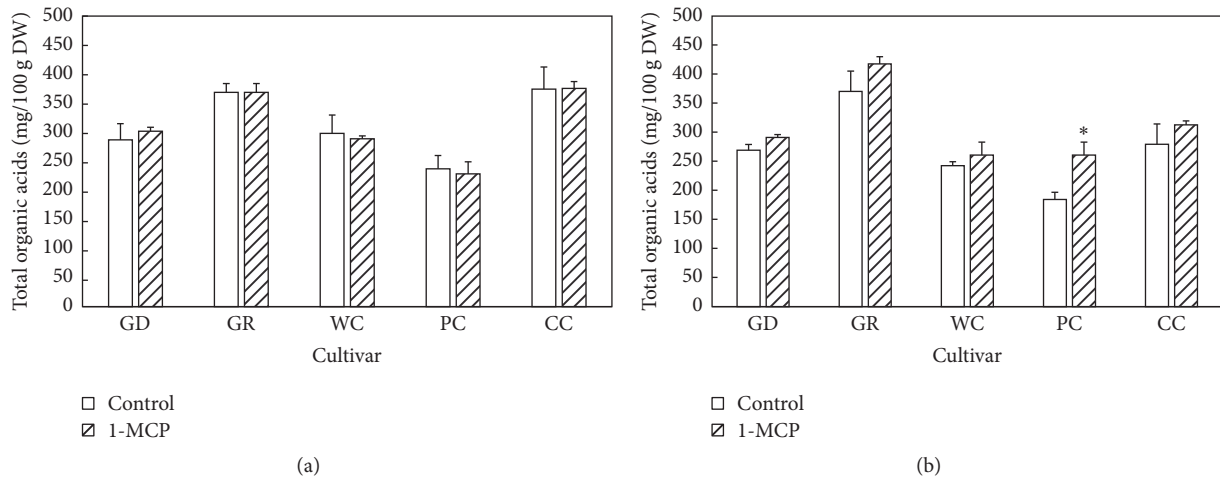
(a)

(b)

Figure 2: Total organic acid content in apple fruit at (a) 70 or (b) 140 days of storage at 0°C followed by 7 days at 20°C. GD, "Golden Delicious"; GR, "GoldRush"; WC, "WineCrisp"; PC, "Pixie Crunch"; CC, "CrimsonCrisp." Asterisks (∗) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration treatment by Student's t-test at $p < 0.05$. Vertical bars represent standard error ($n = 4$).

remained unchanged in the 1-MCP-treated "GoldRush" and "WineCrisp" in both storage durations (Table 1). In both storage durations, 1-MCP-treated fruits had higher titratable acidity, except for "Golden Delicious" stored for 140 days. Our results also show significant interaction between cultivar and 1-MCP treatment and between cultivar and storage duration for titratable acidity ($p < 0.0001$ for both interactions), indicating that treatments effects were cultivar-dependent. Similarly, Bai et al. [24] reported differential effect of 1-MCP on titratable acidity among several apple cultivars. Bai et al. [24] reported that the influence of 1-MCP on titratable acidity was less pronounced on 8-month stored fruits of "Delicious," "Fuji," and "Granny Smith" fruits than on "Gala." In general, our data show a positive correlation between total organic acids content and titratable acidity and a negative correlation between pH and titratable acidity ($p < 0.0001$, data not shown). However, changes in total organic acid content in response to 1-MCP treatment or storage duration were different from changes in titratable acidity or pH (Table 1). Previously, we reported different organic content among these five apple cultivars [26]. At harvest, we found that the total organic acid content was the lowest in "Pixie Crunch" [26]. After storage, total organic acid content was highest in "GoldRush" and lowest in "Pixie Crunch" (Figure 2). However, there was no difference in total organic acids between the two storage durations or between the control and 1-MCP treatment, except in "Pixie Crunch" where 1-MCP treated fruits had higher total organic acids compared to the control (Figure 2). The increase in total organic acids in "Pixie Crunch" treated with 1-MCP was not paralleled to changes in pH or titratable acidity (Table 1), which could be attributed to the fact that titratable acidity and pH are measured anion rather than direct analyses of acid molecules.

The SSC/TA has been suggested as an important indicator of fruit flavor and consumer acceptance [27]. The ratio of

soluble solids to titratable acidity (SSC/TA) was highest in "Pixie Crunch" and lowest in in "GoldRush" (Table 1). "Pixie Crunch" is gaining considerable popularity among organic growers for its balanced sugar to acid ratio. This ratio generally increased in fruits stored at 140 days in storage but was lower in fruits treated with 1-MCP. Jan and Rab [28] reported differences in SSC/TA among different cultivars and storage durations.

As expected, hue angle varied among cultivars depending on their color (Figure 3). At harvest, "Golden Delicious" and "GoldRush" apples, yellow-colored cultivars, had >110° of hue angle while red cultivars, "WineCrisp," "Pixie Crunch," and "CrimsonCrisp," had the hue angle of 23.6, 19.7, and 19.4°, respectively. Hue angle values changed differentially between control and 1-MCP treatment and between storage duration (Figure 3). Hue angle of "Golden Delicious" and "GoldRush" decreased during storage, while the other cultivars had similar hue angle values except for a significant decrease in 1-MCP-treated "CrimsonCrisp." The 1-MCP treatment affected hue angle of "GoldRush," "WineCrisp," and "Pixie Crunch" fruits stored for 70 days at 0°C, while that of "Golden Delicious," "GoldRush," and "WineCrisp" apples was affected after 140 days of storage. In general, 1-MCP treatment increased the hue angles of yellow cultivars and decreased them in red cultivars (Figure 3).

Hue angle is an indicator of color, which is a major determinant of appearance and marketability of most fruits. Skin color of apple fruits is affected mainly by three pigment groups, chlorophylls (green color), carotenoids (yellow color), and anthocyanin (red color) [29, 30]. Change in skin color of control "WineCrisp" fruits during storage indicates a degradation of chlorophyll and accumulation of anthocyanin. Similarly, a higher hue angle in the yellow cultivars after 140 days of refrigerated storage followed by 7 days at 20°C with 1-MCP treatment suggests an inhibition of chlorophyll degradation, while increased hue angle in "WineCrisp" with
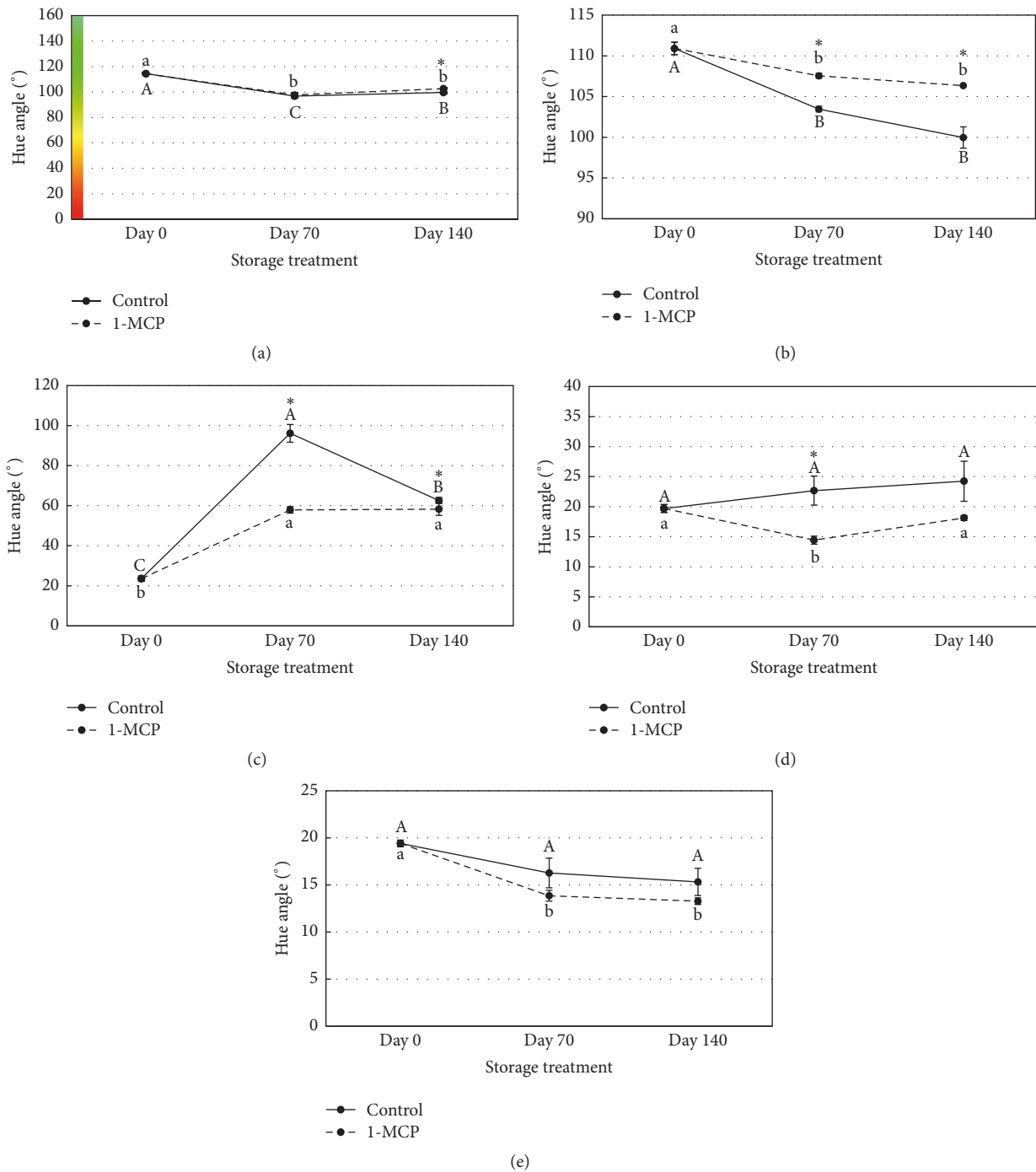
Figure 3: Hue angle (°) of apple fruit at (a) "Golden Delicious," (b) "GoldRush," (c) "WineCrisp," (d) "Pixie Crunch," and (e) "CrimsonCrisp" apples at harvest and 70 or 140 days of storage at 0°C followed by 7 days at 20°C. Day 0, at harvest; Day 70, 70 days of storage at 0°C followed by 7 days at 20°C; Day 140, 140 days of storage at 0°C followed by 7 days at 20°C. Capital letters and lowercased letters indicate mean separation among storage duration treatment of control and 1-MCP-treated apples, respectively, by Tukey's HSD at $p < 0.05$. Asterisks (∗) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration using Student's t-test at $p < 0.05$. Vertical bars represent standard error ($n = 4$).

storage may indicate anthocyanin degradation during storage. Except for "CrimsonCrisp," 1-MCP treatment resulted in significantly different hue angle at either storage duration, indicating that 1-MCP treatment may play a role in accumulation and degradation of pigments of apple skin during storage. However, the mechanism of how 1-MCP influences pigments in apple skin is not yet fully understood. Our study has identified significant interactions for hue angle between cultivar and 1-MCP treatment ($p < 0.0001$), cultivar and storage duration ($p < 0.0001$), and 1-MCP treatment and

Table 2: Ethylene production and respiration rate of apple fruit at 70 or 140 days of storage at 0°C followed by 7 days at 20°C.

Cultivar[z]	Ethylene production (μL/kg/h)		Respiration rate (mL of CO_2/kg/h)	
	Control	1-MCP	Control	1-MCP
Day 70				
GD	219.1 ± 4.0Aa[y]	32.6 ± 4.1Ab*	11.2 ± 0.4Aa	9.7 ± 2.8Aa
GR	102.2 ± 3.1Bb	19.2 ± 3.9Ba*	8.4 ± 1.3Aa	10.4 ± 2.3Aa
WC	67.4 ± 3.0Ca	4.5 ± 1.3Ca*	8.2 ± 0.1Aa	8.1 ± 1.0Aa
PC	48.5 ± 6.4CDa	4.5 ± 0.9Cb*	7.9 ± 1.0Aa	11.7 ± 1.2Aa
CC	42.9 ± 4.3Da	7.0 ± 1.2BCa*	9.1 ± 0.3Aa	7.2 ± 1.1Aa
Day 140				
GD	251.5 ± 15.4Aa	117.9 ± 1.7Aa*	8.5 ± 0.5Ab	8.6 ± 0.2Aa
GR	165.4 ± 6.6Ba	8.8 ± 0.4Ba*	8.1 ± 0.8Aa	3.8 ± 0.3Cb*
WC	59.0 ± 2.1Ca	4.5 ± 1.3BCa*	7.0 ± 0.6Aa	3.8 ± 0.1Cb*
PC	63.6 ± 4.7Ca	9.1 ± 0.8Ba*	8.4 ± 0.3Aa	5.8 ± 0.5Bb*
CC	46.2 ± 1.4Ca	0.7 ± 0.2Cb*	8.8 ± 0.9Aa	4.1 ± 0.2Cb*
Significance[x]				
Cultivar (C)	***		**	
1-MCP (T)	***		*	
Storage duration (S)	***		NS	
C × T	***		***	
C × S	***		NS	
T × S	NS		***	
C × T × S	***		NS	

[z]GD, "Golden Delicious"; GR, "GoldRush"; WC, "WineCrisp"; PC, "Pixie Crunch"; CC, "CrimsonCrisp". [y]Data are presented as mean ± standard error ($n = 4$). Capital letters indicate mean separation among cultivars of each treatment and each storage day by Tukey's HSD at $p < 0.05$. Lowercased letters show significant difference between storage durations of 70 and 140 days of each cultivar and each treatment, and asterisks (*) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration treatment by Student's t-test at $p < 0.05$; [x]NS means nonsignificant. *, **, and *** mean significance at $p < 0.05$, 0.01, and 0.001, respectively.

storage duration ($p < 0.0001$) and among all three factors ($p < 0.0001$), indicating that these factors were dependent on each other and treatment effects differed among cultivars. In agreement with our results, Vidrih et al. [31] reported that 1-MCP treatment affected skin color of "Jonagold" and "Golden Delicious" but has no effect on "Idared" skin color, indicating cultivar differences in response to 1-MCP.

Ethylene production was highest in "Golden Delicious," followed by "GoldRush," and lowest in "CrimsonCrisp," while "WineCrisp" and "Pixie Crunch" produced intermediate levels in both storage treatments (Table 2). However, there was no difference in ethylene production between the two storage durations, except in "GoldRush" where it was higher in fruits stored for 140 days at 0°C followed by 7 days at 20°C than for 70-day treatment, indicating that fruits of this cultivar require longer time to reach their climacteric peak than the other cultivars. Ethylene synthesis in the 1-MCP treatments was between 5.3- and 15-fold lower than the untreated fruits after 70 days of storage and between 2.1- and 66-fold lower than the untreated fruits after 140 days of storage at 0°C followed by 7 days at 20°C (Table 2).

Studies have shown that ethylene synthesis changes during long-term storage of apples [23, 32, 33]. However, results differed, depending on cultivar and storage conditions. Tsantili et al. [34] reported different levels of ethylene synthesis

between "Cortland," "Law Rome," and "Idared" apples during 25 weeks of storage at 0.5°C. The 1-MCP effect on ethylene production also slightly differed among cultivars. Although 1-MCP reduced ethylene production in many apple cultivars, the rate of synthesis started to increase after 5 weeks of storage in "Cortland" and "Law Rome" apples, while it stayed relatively constant in "Idared" fruits after 25 weeks of storage [34]. In the present study, 1-MCP treatment reduced ethylene production in all five cultivars. However, we observed significant differences in ethylene production among the five cultivars as well as differential response of these cultivars to the 1-MCP treatment in the two storage durations. Our results are in agreement with earlier observations made by Tsantili et al. [34]. Additionally, Watkins et al. [19] showed different sensitivity to 1-MCP among four apple cultivars, resulting in different degree of inhibition of ethylene production. They reported that fruits of "McIntosh" apples were less sensitive to 1-MCP, at three concentrations, compared to "Empire," "Delicious," and "Law Rome." These results are in agreement with the observed interactions in our data between cultivar and storage duration and among cultivar, 1-MCP treatment, and storage duration (Table 2), which indicate that these factors were dependent on each other and, therefore, 1-MCP treatment effect differed depending on cultivar and storage duration. Tatsuki et al. [35] reported that the expression levels
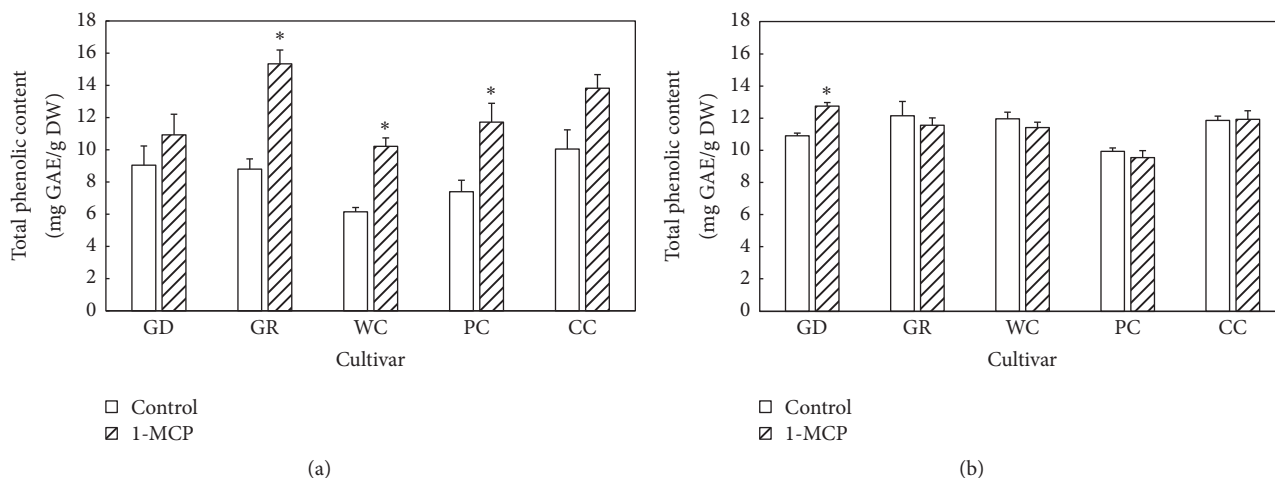
(a)

(b)

FIGURE 4: Total phenolic content of apple fruit at (a) 70 or (b) 140 days of storage at 0°C followed by 7 days at 20°C. GD, "Golden Delicious"; GR, "GoldRush"; WC, "WineCrisp"; PC, "Pixie Crunch"; CC, "CrimsonCrisp." Asterisks (∗) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration treatment by Student's t-test at $p < 0.05$. Vertical bars represent standard error ($n = 4$).

of the ethylene receptor genes, *MdERS2* and *MdERS2*, and the ACC-synthase gene, *MdACS1*, were inhibited in 1-MCP-treated "Fuji" apples even when the treatment was delayed for a week after storage. However, Tatsuki et al. [35] reported that in, "Orin," a high ethylene-producing cultivar, a delay in 1-MCP application after harvest resulted in less suppression of ethylene production and the level of gene expressions. These data also suggest cultivar difference in response to 1-MCP, similar to our result.

Unlike ethylene, respiration rates were not significantly different between the four disease resistant cultivars (Table 2). However, "Golden Delicious" treated with 1-MCP and stored for 140 days had higher respiration than similarly treated and stored disease resistant cultivars. Respiration rate of apple fruit has been reported to change during long-term storage, especially during first 1–3 months [36–38]. Although significant reduction of respiration rate by 1-MCP was reported for "Gala" and "Golden Delicious" apples during 5 months of storage at 0 or 1°C [37, 38], respiration rate of 1-MCP treated "Delicious" apples was not different from that of control after 50 days of storage at 0°C [36]. We found that the respiration rate slightly differed among cultivars, especially in the fruit treated with 1-MCP and stored for 140 days at 0°C and then 7 days at 7°C. The 1-MCP effect also differed depending on cultivar and storage duration. There was a significant interaction between cultivar and 1-MCP treatment and between 1-MCP treatment and storage duration. But there was no significant interaction between cultivar and storage duration, indicating that 1-MCP effect differed among cultivars and storage duration but the effect of storage duration was relatively consistent for cultivars used in this study.

We previously reported total phenolic content of theses apple cultivars at harvest [26]. In general, total phenolic content decreased with storage, except for 1-MCP-treated "Pixie Crunch." Total phenolic content in control fruits was not different among cultivars, except for lower content in "Pixie Crunch" after 140 days of storage (Figure 4). When treated with 1-MCP, "GoldRush" was the highest in total phenolic for 70 days storage treatment but "Golden Delicious," "GoldRush," and "CrimsonCrisp" were higher than the other cultivars after 140 days of refrigerated storage followed by 7 days at 20°C. 1-MCP-treated fruit of "GoldRush," "WineCrisp," and "Pixie Crunch" had higher total phenolic content compared to control after 70 days of storage whereas only "Golden Delicious" had higher total phenolic content with 1-MCP treatment after 140 days of storage.

Studies have shown that change in phenolic content during storage was cultivar-dependent [39, 40]. Our results also show that change in total phenolic content during storage was different among cultivars. However, total phenolic content after storage was generally lower in all cultivars [26]. Increase in total phenolic content in response to 1-MCP treatment was reported in "Red Delicious" [41], while, in "Cripps Pink," Hoang et al. [42] reported a twofold increase in the flesh and about 10% decrease in the peel after 160 days of storage. Different results among studies including the present study could partially be due to different cultivar used and inconsistent 1-MCP treatment and storage conditions. Ethylene is known to increase activity of phenylalanine ammonia lyase, which is a key enzyme involved in earlier step of phenolic biosynthesis [43], which has been shown to increase flavonoid content in apples [44]. Our results of decreased total phenolic after storage indicate that the phenolic synthesis and degradation during storage might involve multiple factors, in addition to ethylene. Moreover, the total phenolic content analyzed by Folin-Ciocalteu reflects another compound having reducing capacity [45] and, therefore, different chemical composition among cultivars and their change during storage can affect the total phenolic content. Additionally, our results showed significant interaction between cultivar and storage duration ($p = 0.0028$) but not between cultivar and 1-MCP treatment,

TABLE 3: Antioxidant capacity of apple fruit at 70 or 140 days of storage at 0°C followed by 7 days at 20°C.

Cultivar[z]	ABTS (mM TE/g DW)		FRAP (mM TE/g DW)	
	Control	1-MCP	Control	1-MCP
Day 70[z]				
GD	26.2 ± 3.4Aa[y]	32.0 ± 3.16Ba	72.1 ± 17.2Aa	103.3 ± 11.7Aa
GR	26.6 ± 2.0Aa	40.6 ± 1.2ABa*	70.2 ± 7.6Ab	126.1 ± 3.9Aa*
WC	18.9 ± 2.0Ab	32.8 ± 1.7ABa*	73.5 ± 18.4Aa	95.6 ± 2.1Aa
PC	18.9 ± 2.2Aa	33.8 ± 1.9ABb*	78.6 ± 11.8Aa	107.2 ± 7.8Aa
CC	24.3 ± 2.7Ab	42.9 ± 2.7Ab*	93.4 ± 10.6Aa	108.2 ± 13.4Aa
Day 140				
GD	29.1 ± 0.1Aa	34.3 ± 1.0Ba*	102.4 ± 1.3Aa	105.4 ± 15.1Aa
GR	30.7 ± 2.2Aa	30.0 ± 0.8Bb	113.9 ± 6.4Aa	114.7 ± 3.6Aa
WC	31.5 ± 1.5Aa	43.5 ± 6.2ABa	114.1 ± 5.7Aa	98.6 ± 4.5Aa
PC	24.6 ± 3.4Aa	48.5 ± 1.5Aa*	115.8 ± 8.8Aa	99.2 ± 3.1Aa
CC	32.8 ± 0.2Aa	55.0 ± 1.3Aa*	112.1 ± 6.0Aa	106.9 ± 9.9Aa
Significance[x]				
Cultivar (C)	∗∗∗		NS	
1-MCP (T)	∗∗∗		∗∗	
Storage duration (S)	∗∗∗		∗∗	
C × T	∗∗∗		NS	
C × S	∗∗		NS	
T × S	NS		∗∗	
C × T × S	∗		NS	

[z]GD, "Golden Delicious"; GR, "GoldRush"; WC, "WineCrisp"; PC, "Pixie Crunch"; CC, "CrimsonCrisp"; Day 70, 70 days of storage at 0°C followed by 7 days at 20°C; Day 140, 140 days of storage at 0°C followed by 7 days at 20°C. [y]Data are presented as mean ± standard error ($n = 4$). Capital letters indicate mean separation among cultivars of each treatment and each storage day by Tukey's HSD at $p < 0.05$. Lowercased letters show significant difference between storage durations of 70 and 140 days of each cultivar and each treatment, and asterisks (∗) indicate significant difference between control and 1-MCP treatment in each cultivar at each storage duration treatment by Student's t-test at $p < 0.05$; [x]NS means nonsignificant. ∗, ∗∗, and ∗∗∗ mean significance at $p < 0.05$, 0.01, and 0.001, respectively.

suggesting that 1-MCP effect was relatively consistent for all cultivars but storage duration effect differed among cultivars.

"GoldRush" and "CrimsonCrisp" exhibited the highest total antioxidant capacity, as measured by ABTS and FRAP, in response to 1-MCP treatment (Table 3). These cultivars were also found to be the highest in antioxidant capacity at harvest [26]. However, antioxidant capacity measured by ABTS generally decreased while that analyzed by FRAP assay increased after storage. These differences may partially be related to the difference between assays [45]. Additionally, not only phenolic compounds but also some other compounds having reducing capacity can affect the result of these assays [45], indicating potential interference of those compounds and their change during storage on antioxidant capacity. The 1-MCP effect on antioxidant capacity was also dependent on cultivar and storage duration, similar to total phenolic content (Table 3). Hoang et al. [42] reported that total antioxidant activity increased by 40 and 70% in the peel and flesh of "Cripps Pink" apple, respectively, during storage with most of total antioxidants concentrating in the flesh. However, they found that 1-MCP significantly reduced the total antioxidant activity in peel after 160 days of storage at 0°C.

4. Conclusions

Data from this study have demonstrated that the four scab-resistant apple cultivars, "GoldRush," "Pixie Crunch," "WineCrisp," and "CrimsonCrisp," have excellent eating quality, compared to the most widely grown scab susceptible cultivar, "Golden Delicious," as judged by their flesh skin color, firmness, soluble solids, juice pH, and titratable acidity. Fruit quality, chemical composition, and antioxidant capacity of these apples were significantly affected by storage duration and 1-MCP treatment but these effects depended on cultivar. The favorable qualities of these four scab-resistant apple cultivars make them highly desirable for organic production and for production by small farmers and consumers who are conscious of the health risk of chemicals, their high cost, and their effect on the environment. Moreover, our results provide valuable information of these scab-resistant cultivars in developing storage regimes to minimize quality deterioration during long-term storage.

Conflicts of Interest

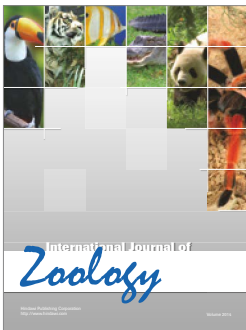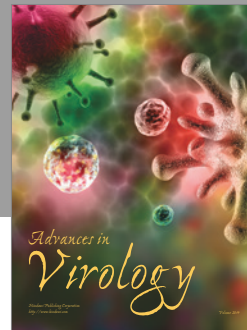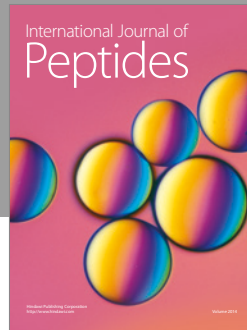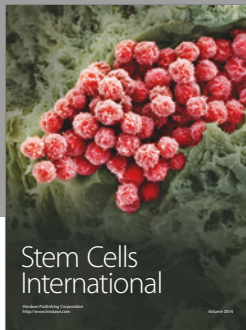The authors declare that there are no conflicts of interest.

Authors' Contributions

Moises Zucoloto and Kang-Mo Ku are co-first authors and equally contributed to this study.

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