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Interactive Responses of Gala Apple Fruit Volatile Production to Controlled Atmosphere Storage and Chemical Inhibition of Ethylene Action

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Gala apples exposed to the ethylene action inhibitor 1-methylcyclopropene (1-MCP) for 12 h at 20 °C were stored at 1 °C in air or a controlled atmosphere (CA) maintained at 1 kPa O_2 and 2 kPa CO_2. Volatile compounds were measured after 4, 12, 20, and 28 weeks plus 1 or 7 days at 20 °C. Treatment with 1-MCP and then storage in air or CA or storage in CA without 1-MCP treatment reduced volatile production as compared to apples not treated with 1-MCP stored in air. The reduced production of esters, alcohols, aldehydes, acetic acid, and 1-methoxy-4-(2-propenyl)benzene was observed. Ester production by fruit stored in CA decreased throughout the storage period regardless of previous 1-MCP treatment. The production of esters, alcohols, aldehydes, acetic acid, and 1-methoxy-4-(2-propenyl)benzene by 1-MCP-treated fruit stored in air plus 7 days at 20 °C increased after 20 or 28 weeks of storage. Continuous exposure to 417 μmol m^{-3} ethylene for 7 days at 20 °C after 12 or 28 weeks of storage stimulated production of many volatile compounds, primarily esters and alcohols, by fruit stored in CA or 1-MCP-treated apples stored in air. However, exposure to ethylene had no effect on the production of aldehydes or acetic acid.

KEYWORDS: 1-Methylcyclopropene; ethylene; firmness; esters; alcohols; aldehydes; 1-methoxy-4-(2-propenyl)benzene

INTRODUCTION

Controlled atmosphere (CA) storage, used for decades for the commercial storage of apples, delays the loss in firmness, acidity, and many other quality attributes as compared to fruit stored in air (*1*). The optimum set points for O_2 (0.7−3 kPa) and CO_2 (0−2 kPa) vary with cultivar (*2*). It has been suggested that fruit responses to CA storage arise from inhibition of ethylene synthesis and action (*3*). Prolonged storage of apples in CA can also reduce fruit production of volatile compounds (*4*), an important contributor to apple flavor. This impact of CA on poststorage volatile production becomes more pronounced as O_2 and CO_2 concentrations decrease and increase, respectively, and as storage duration increases (*5*). A decrease in fruit sensitivity to ethylene as well as reduced ethylene production may contribute to the reduced production rate of volatile compounds that contribute to fruit aroma after CA storage (*6*, *7*).

Apple fruit in which ethylene action is inhibited following exposure to 1-methylcyclopropene (1-MCP) (*8*, *9*) exhibit alterations in ripening that are similar to those induced by CA storage. Apples treated with 1-MCP have reduced ethylene production, slower loss of firmness and titratable acidity, and decreased development of physiological disorders (*10*−*14*). Apples and other climacteric fruit exposed to 1-MCP also have reduced and/or delayed production of many volatile compounds (*15*−*20*). As the effects of 1-MCP on apple ripening can be enhanced when 1-MCP-treated fruit are stored in a CA (*14*), the objective of this study was to determine how Gala apple fruit volatile production is impacted in fruits stored in air or a CA following chemical inhibition of ethylene action with 1-MCP. The efficacy of poststorage fruit exposure to ethylene to stimulate volatile production was also examined.

MATERIALS AND METHODS

Gala [*Malus sylvestris* L. (Mill.) var. *domestica* Borkh. Mansf.] apple fruit was harvested at commercial maturity 138 days after full bloom in a commercial orchard near Manson, WA. Fruit analyses (firmness, starch index, titratable acidity, and internal ethylene concentration) were conducted at harvest and after 4, 12, 20, and 28 weeks of storage at 1 °C in air or a CA containing 1 kPa O_2 and 2 kPa CO_2, plus 1 or 7 days at 20 °C in air using methods reported previously (*21*). Means and standard deviations for indicators of fruit maturity and quality at harvest included the following ($n = 20$): firmness (N), 84.2 ± 5.7; starch index (*1*−*6*), 3.0 ± 1.0; soluble solids content (%), 12.1 ± 0.4; titratable acidity (%), 0.330 ± 0.015; weight (g), 204 ± 21; internal ethylene concentration (μmol m^{-3}), 50 ± 29; and respiration rate ($n = 3$, mmol CO_2 kg^{-1} h^{-1}), 0.54 ± 0.8. These values were consistent with the onset

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of the climacteric stage of fruit development and a maturity appropriate for long-term commercial CA storage (*2*).

Fruit was treated on the day of harvest with air (control) or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C in 230 L steel chambers. 1-MCP gas was generated by the addition of EthylBloc powder (Floralife, Inc., Walterboro, SC) to water in a 5 L flask. The flask was connected to the treatment chamber in a closed loop, and then, 1-MCP gas was pumped from the flask into the treatment chamber for 15 min. The concentration of 1-MCP was measured by analysis of 0.5 mL chamber headspace using a gas chromatograph (HP 5880A; Hewlett-Packard, Avondale, PA) fitted with a 30 cm glass column (3.2 mm i.d.) packed with Porapak Q (80/100 mesh). Gas flows for N_2, H_2, and air were 30, 30, and 300 mL min^{-1}, respectively. Injector, oven, and flame ionization detector (FID) temperatures were 100, 130, and 200 °C, respectively. A response factor generated using the same GC system and a 1-butene standard (99.6 μL L^{-1} in N_2, Scott Specialty Gases, Plumsteadville, PA) was used to quantify 1-MCP. The response factor was generated using area counts obtained by injecting 1 μL of the standard with no dilution or after a 100-fold dilution using a gas buret.

After exposure to 1-MCP, apples (20 fruit per treatment per storage duration) were stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 1 °C. Chambers for CA storage were 0.145 m^3, and O_2 and CO_2 concentrations were monitored and adjusted at 90 min intervals (Techni-Systems, Chelan, WA). CA chamber atmospheres were static except when adjustments were performed. The four 1-MCP/storage treatment combinations were as follows: air, 1-MCP and then air (1-MCP), CA, and 1-MCP and then CA (1-MCP + CA).

Ethylene and CO_2 production were determined from apples placed in 10 L plexiglass chambers (five fruit per chamber, three chambers per treatment) through which compressed air flowed at 100 mL min^{-1}. Two 0.5 mL gas samples were collected from the outlet port of each chamber. One sample for ethylene analysis was injected into a gas chromatograph (HP 5880A; Hewlett-Packard) fitted with a 30 cm glass column (3.2 mm i.d.) packed with 80/100 mesh Porapak Q (Supelco, Bellefonte, PA). Gas flows for N_2, H_2, and air were 30, 30, and 300 mL min^{-1}, respectively. Oven, injector, and FID temperatures were 60, 60, and 150 °C, respectively. The concentration of CO_2 in the other 0.5 mL gas sample was determined using a gas chromatograph (HP5890, Hewlett-Packard) equipped with a methanizer (John T. Booker, Austin, TX) and a 60 cm stainless steel column (2 mm i.d.) packed with 80/100 mesh Porapak Q. Gas flows for N_2, H_2, and air were 65, 30, and 300 mL min^{-1}, respectively. Oven, injector, and FID temperatures were 30, 50, and 200 °C, respectively.

Volatile compounds were analyzed as described (*21*) at harvest and after 4, 12, 20, and 28 weeks of storage plus 1 or 7 days at 20 °C in air. Volatile compounds were collected using dynamic headspace sampling of three replicate samples of 4−5 intact fruit (approximately 1 kg) per treatment enclosed in 4 L glass jars with Teflon lids. Compressed air passed through cartridges containing $KMnO_4$, activated charcoal, molecular sieve, and Tenax TA (30−50 mesh, Alltech Associates, Deerfield, IL) prior to flowing through the jars at 100 mL min^{-1}. Volatile compounds in the jar outflow were adsorbed onto 50 mg of 30−50 mesh Tenax TA packed in glass tubing (17.5 cm × 0.4 cm i.d). Volatile compounds were desorbed from traps at 250 °C for 3 min using a Tekmar 6016 aerotrap desorber (Tekmar Co., Cincinnati, OH). The desorbed sample compounds were condensed at −120 °C, and then, the cryofocusing module was flash-heated to 250 °C under a stream of He carrier gas carrying the analytes into a Hewlett-Packard 5890A/5971A GC-MSD equipped with a DB-Wax column (J&W Scientific, Folsom, CA; 60 m × 0.25 mm i.d., 0.25 μm film thickness). The GC temperature program was as follows: initial oven temperature 35 °C held for 5 min, increased to 50 °C at 2 °C min^{-1}, increased to 200 °C at 5 °C min^{-1} and held 5 min. The linear velocity of the He carrier gas was 30 cm s^{-1}. Mass spectra were obtained by electron ionization at 70 eV. Transfer line and ion source temperatures were 280 and 180 °C, respectively. All compounds were identified by two methods: (i) comparison of spectra of sample compounds with those of authentic standards as well as spectra contained in the Wiley-NBS library and (ii) by comparing retention indices of sample compounds with those of authentic standards. Quantification was performed using selected ion monitoring for base peaks, and quantitative values were calculated using response factors generated with standards.

Authentic compounds purchased from Aldrich (Sigma-Aldrich, Milwaukee, WI) included 1-butanol, 1-hexanol, 1-pentanol, 1-propanol, 2-ethyl-1-hexanol, 2-furancarboxaldehyde, 2-methylbutyl acetate, 2-methyl-1-butanol, 2-methyl-1-propanol, 2-methylbutyl 2-methylbutanoate, 2-methylpropyl acetate, 2-propanol, 6-methyl-5-hepten-2-one, 1-methoxy-4-(2-propenyl)benzene, butanal, butyl 2-methylbutanoate, butyl butanoate, butyl hexanoate, butyl propanoate, decanal, ethanol, ethyl 2-methylbutanoate, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl propanoate, heptanal, hexanal, hexyl 2-methylbutanoate, hexyl acetate, hexyl butanoate, hexyl hexanoate, hexyl propanoate, methyl butanoate, methyl 2-methylbutanoate, nonanal, octanal, pentyl acetate, propyl acetate, and propyl propanoate. Authentic compounds purchased from Sigma (Sigma-Aldrich) included benzaldehyde, butyl acetate, ethyl butanoate, and pentanal. Acetic acid was purchased from Fisher Scientific (Pittsburgh, PA). Pentyl butanoate and propyl hexanoate were synthesized by combining 10 mL of pentanoic acid or hexanoic acid with 15 mL of 1-pentanol or 1-propanol, respectively, in a round bottom flask. Concentrated sulfuric acid (3 mL) was then slowly added, and the mixture was refluxed for 1 h. After the mixture was cooled, the solution was transferred to a separatory funnel containing 100 mL of deionized water (dH_2O). After the solution was partitioned, the organic phase was removed and partitioned two more times against dH_2O. The organic phase was removed and then added to a distillation apparatus, and the fraction boiling at 158.2 °C was collected. Compound identity was tentatively established by injecting 1 μL of the distillate into the same GC-MS described previously and matching sample spectra with spectra contained in the Wiley NBS library (**Table 1**). All reagents for ester synthesis were purchased from Aldrich.

Poststorage Ethylene Treatment. After 12 and 28 weeks of storage, apples from each treatment combination were placed into 10 L plexiglass chambers at 20 °C through which air or 420 μmol m^{-3} ethylene in air flowed at 100 mL min^{-1}. There were 20 apples per treatment combination (four chambers per treatment, five apples per chamber). After 7 days, the apples were removed from the chambers and volatile samples were collected as described.

Experimental Design and Statistical Analysis. The experiment was conducted using a completely random design. Data were analyzed using the SAS version 6.12 (SAS Institute, Raleigh, NC) analysis of variance procedure and means were compared with Fisher's protected least significant difference (LSD). Only significant results are presented and discussed.

RESULTS

Ethylene production by control fruit stored in air was similar throughout the storage period when analyzed 7 days after removal from storage (**Figure 1A**). Ethylene production by fruit exposed to 1-MCP and then stored in air was lower through 12 weeks in storage as compared to control fruit; then, production increased and was similar to controls after 28 weeks. Fruit stored in a CA with or without previous exposure to 1-MCP had significantly lower ethylene production as compared to control fruit stored in air during the 28 week storage period. After 4 and 12 weeks, fruit exposed to 1-MCP and then stored in air or a CA had lower ethylene production as compared to control fruit stored in a CA. Ethylene was not detected from 1-MCP-exposed apples stored in a CA after 20 and 28 weeks. Respiration of control fruit stored in air was the highest of all treatments throughout the 28 week storage period (**Figure 1B**). Fruit exposed to 1-MCP and then stored in air or a CA had the lowest respiration rate after 4 weeks, and values remained low throughout the evaluation period for all fruit stored in a CA. Respiration by 1-MCP-treated fruit stored in air approached that of control fruit stored in air after 28 weeks of storage. Exposure to 1-MCP and/or storage in a CA slowed the loss of fruit firmness and titratable acidity (**Figure 1C**,**D**). Treatment differences between fruit exposed to 1-MCP and/or storage in a CA were present after 20 and 28 weeks of storage.

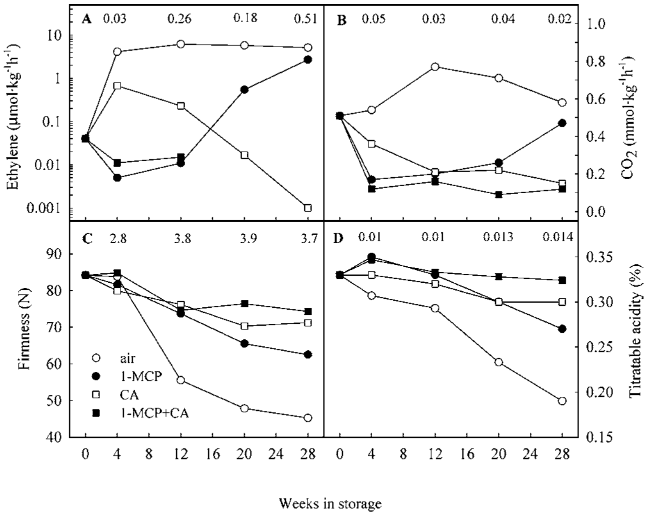

Figure 1. Ethylene production (**A**), respiration rate (**B**), firmness (**C**), and titratable acidity (**D**) of Gala apples held 7 days at 20 °C after removal from cold storage. Fruit were exposed to air or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C and then stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 1 °C. Numbers across the top of each graph are Fishers' LSD values, $p = 0.05$.

Ester production by fruit stored in air was highest or in the group of treatments with highest production when evaluated 1 day after removal from storage (**Figure 2A**). Ester production by 1-MCP fruit was lower as compared to control fruit stored in air throughout the 28 week storage period; however, the difference in ester production between control and 1-MCP-treated fruit decreased between 20 and 28 weeks of storage. Ester production by CA control and 1-MCP + CA fruit was low relative to other treatments throughout the 28 week storage period with one exception, control fruit stored in a CA for 4 weeks plus 7 days at 20 °C (**Figure 2B**). Ester production by 1-MCP + CA fruit held 7 days at 20 °C after removal from storage was the lowest of all treatments throughout the 28 week storage period.

The production of both straight and branched chain esters was reduced following exposure to 1-MCP (**Figure 2C−F**). Esters with straight C chains detected were as follows: ethyl acetate, ethyl propanoate, propyl acetate, methyl butanoate, ethyl butanoate, propyl propanoate, butyl acetate, ethyl pentanoate, butyl propanoate, pentyl acetate, butyl butanoate, ethyl hexanoate, hexyl acetate, pentyl butanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate, hexyl butanoate, ethyl octanoate, and hexyl hexanoate. Esters with branched C chains detected were as follows: methyl 2-methylbutanoate, 2-methylpropyl acetate, ethyl 2-methylbutanoate, 2-methylbutyl acetate, butyl 2-methylbutanoate, 2-methylbutyl 2-methylbutanoate, and hexyl 2-methylbutanoate. Differences in production of both straight and branched chain esters decreased between control and 1-MCP-treated fruit stored in air after 20 and 28 weeks when the fruits were held for 7 days at 20 °C prior to analysis. A similar decrease occurred after 28 weeks for fruit analyzed 1 day after removal from storage. The production of butyl acetate, hexyl acetate, and 2-methylbutyl acetate, compounds previously identified as primary contributors to Gala apple aroma (*22*, *23*), reflected the trends of the straight and branched carbon chain groups (**Figure 3A−C**) for each compound.

Treatment effects on alcohol production were similar to those for esters (**Figure 4A**,**B**) except that control fruit stored in air or a CA produced similar amounts of alcohols after 4 weeks of storage plus 7 days at 20 °C. After 4 weeks of storage, control fruit stored in a CA produced less butanol after 1 or 7 days at 20 °C (**Figure 4C**,**D**) and less 2-methyl-1-butanol (**Figure 4E**,**F**), ethanol, pentanol, and hexanol (data not shown) after 7 days at 20 °C than control fruit stored in air. A higher production of ethanol detected after 4 weeks of CA storage plus 7 days at 20 °C was not apparent in 1-MCP + CA fruit.

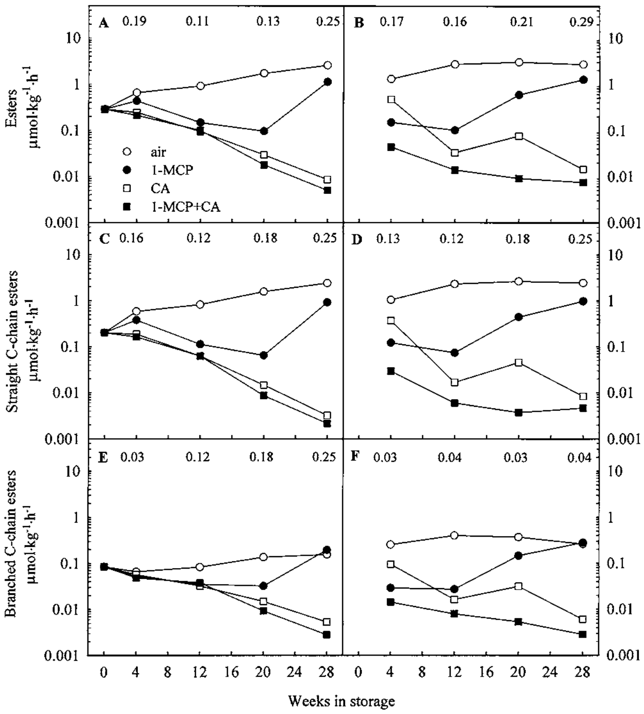

Figure 2. Production of all esters detected (**A**,**B**), straight chain esters including ethyl acetate, propyl acetate, methyl butanoate, ethyl butanoate, propyl propanoate, butyl acetate, ethyl pentanoate, butyl propanoate, pentyl acetate, butyl butanoate, ethyl hexanoate, hexyl acetate, pentyl butanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate, hexyl butanoate, ethyl octanoate, and hexyl hexanoate (**C**,**D**), and branched chain esters including methyl 2-methylbutanoate, 2-methylpropyl acetate, ethyl 2-methylbutanoate, 2-methylbutyl acetate, butyl 2-methylbutanoate, 2-methylbutyl-2-methylbutanoate, and hexyl 2-methylbutanoate (**E**,**F**) by Gala' apples after 1 (**A**,**C**,**E**) and 7 (**B**,**D**,**F**) days at 20 °C after removal from cold storage. Fruit was exposed to air or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C and then stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 1 °C. Numbers across the top of each graph are Fishers' LSD values, $p = 0.05$.

For fruit stored longer than 12 weeks, aldehyde production was highest for control fruit stored in air (**Figure 5A**,**B**). Aldehydes detected included butanal, pentanal, hexanal, heptanal, octanal, nonanal, 2-furancarboxaldehyde, decanal, and benzaldehyde. Aldehyde production by control fruit held for 7 days after removal from a CA decreased to below that of 1-MCP fruit stored in air when fruit was stored longer than 4 weeks. The production of acetic acid was similar for all fruit evaluated 1 day after removal from storage through 12 weeks (**Figure 5C**,**D**). The production by control fruit stored in air longer than 12 weeks was highest for all treatments when fruit were evaluated 1 day after removal from storage. No acetic acid was detected in samples collected from 1-MCP-treated fruit stored in air for 12 weeks and evaluated 1 day after removal from storage and for 1-MCP + CA fruit stored 12 weeks plus 7 days at 20 °C. The production of 1-methoxy-4-(2-propenyl)benzene, a volatile phenylpropanoid that contributes a spicy note to apple aroma (*24*), was impacted by both 1-MCP treatment and storage in CA (**Figure 5E**,**F**). At each removal from storage, the

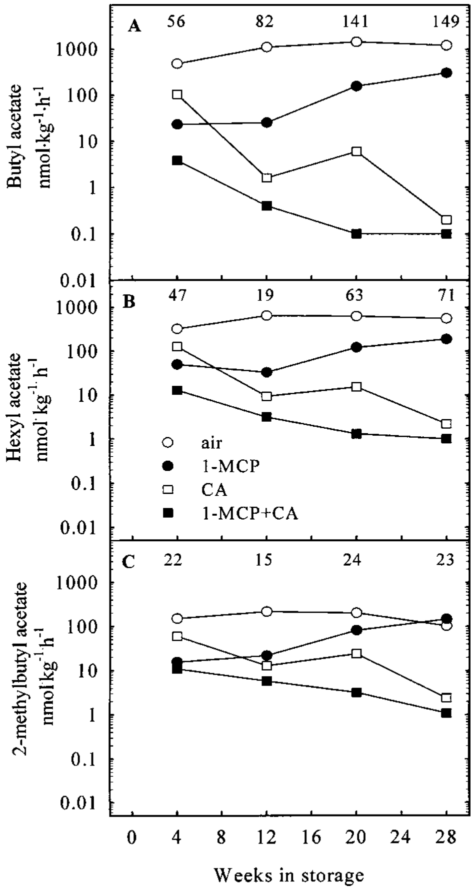

Figure 3. Production of butyl acetate (**A**), hexyl acetate (**B**), and 2-methylbutyl acetate (**C**) by Gala apples held for 7 days at 20 °C after removal from cold storage. Fruit was exposed to air or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C and then stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 1 °C. Numbers across the top of each graph are Fishers' LSD values, $p = 0.05$.

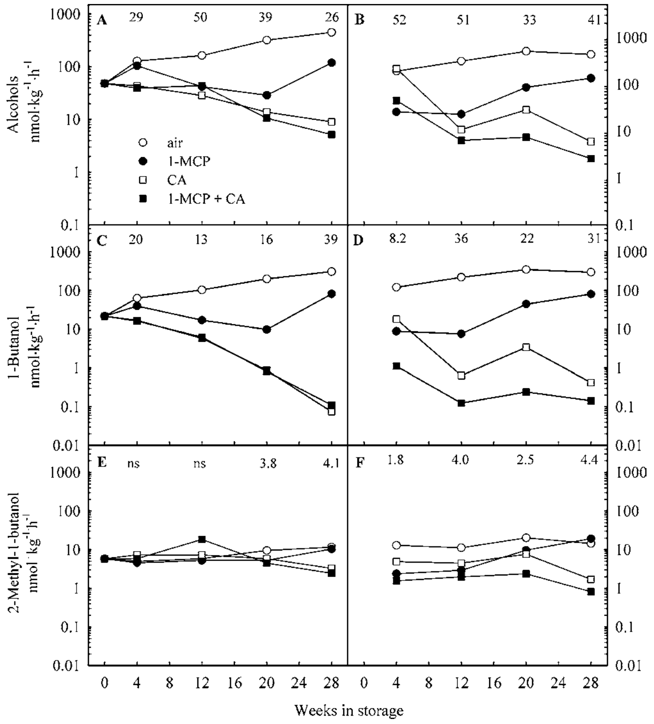

Figure 4. Production of all alcohols detected including 2-propanol, ethanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, and 1-pentanol (**A**,**B**), 1-butanol (**C**,**D**), and 2-methyl-1-butanol (**E**,**F**) by Gala apples after 1 (**A**,**C**,**E**) and 7 (**B**,**D**,**F**) days at 20 °C after removal from cold storage. Fruit was exposed to air or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C and then stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 1 °C. Numbers across the top of each graph are Fishers' LSD values, $p = 0.05$.

production of 1-methoxy-4-(2-propenyl)benzene was highest by control fruit stored in air and held for 7 days after removal from cold storage. The lowest production was by 1-MCP fruit stored in air (20 weeks or less) or a CA when evaluated 1 day after removal from storage or 1-MCP + CA fruit when evaluated 7 days after removal from storage. The amount of 1-methoxy-4-(2-propenyl)benzene produced by fruit stored in CA with or without 1-MCP treatment remained low throughout the storage and shelf life period.

Volatile Production after Exposure to Ethylene. Ethylene exposure following removal from storage had inconsistent effects on ester production by control fruit stored in air. Ester production by fruit exposed to ethylene after storage was lower (12 weeks) or higher (28 weeks) as compared to control fruit not exposed to ethylene (**Table 2**). While ester production by control fruit stored in CA was enhanced following exposure to ethylene after 12 weeks of storage, production was significantly lower as compared to control fruit stored in air. Exposure to ethylene resulted in enhanced ester production by 1-MCP-treated fruit stored in a CA for 12 or 28 weeks but did not affect ester production by 1-MCP-treated fruit stored in air for 28 weeks. Exposure to ethylene resulted in similar effects on alcohol production. The production of 1-methoxy-4-(2-propenyl)benzene was not altered by exposure to ethylene after 12 weeks of storage. After 28 weeks, 1-methoxy-4-(2-propenyl)benzene production decreased (control), did not change (1-MCP), or increased (CA, 1-MCP + CA) following fruit exposure to ethylene. No changes in aldehyde or acetic acid production were detected following exposure to ethylene (data not shown).

DISCUSSION

The production of volatile compounds by Gala apple fruit is impacted following inhibition of ethylene action by 1-MCP treatment at harvest and/or cold storage in a CA. Both processes reduce the production of a range of volatile compounds, but the dynamics of the effects differ. The inhibitory effect of CA storage on production of volatile compounds relative to fruit stored in air increased for each subsequent storage duration as previously reported (*5*) while the reduction in volatile compound production resulting from inhibition of ethylene action decreased over the same period for fruit stored in air. Although production of most volatile compounds did not reach that of control fruit stored in air over the same storage duration, after 28 weeks control fruits stored in air were senescent (firmness 45 N) while 1-MCP-treated fruits remained marketable (firmness 63 N). The production of esters, compounds contributing the majority of the characteristic Gala aroma (*22*, *23*), appears to be delayed following 1-MCP treatment. Ester production by 1-MCP-treated fruit stored in air for 28 weeks was comparable to that of control fruit stored in air for 4 weeks, a point at which control fruits have high market quality. The combination of treatment with 1-MCP followed by storage in a CA has the most impact for reduction of volatile production for compounds detected in this study.

Differential effects of 1-MCP on apple ester and alcohol production after storage were evident. Over the storage period, the production of branched but not straight chain esters by fruit

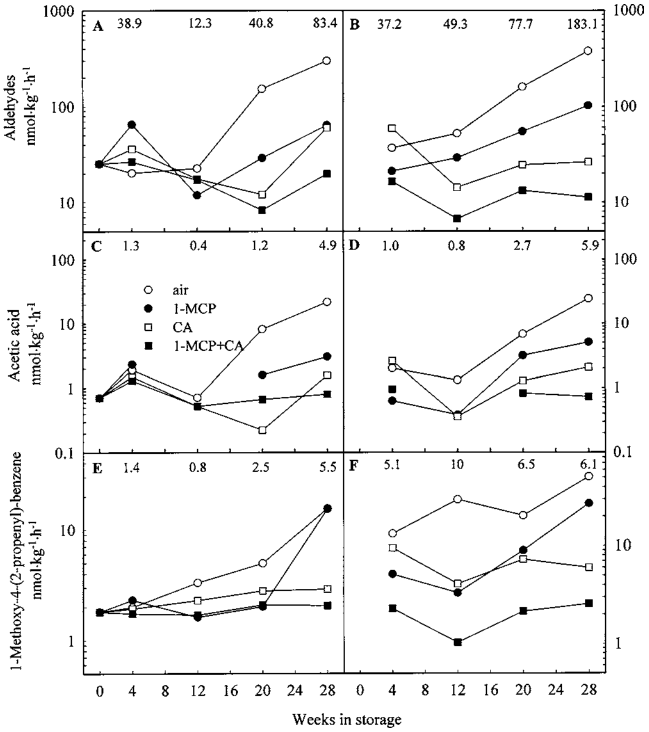

Figure 5. Production of all aldehydes detected including butanal, pentanal, hexanal, heptanal, octanal, nonanal, 2-furancarboxaldehyde, decanal, and benzaldehyde (**A**,**B**), acetic acid (**C**,**D**), and 1-methoxy-4-(2-propenyl)-benzene (**E**,**F**) by Gala apples after 1 (**A**,**C**,**E**) and 7 (**B**,**D**,**F**) days at 20 °C after removal from cold storage. Fruit was exposed to air or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C and then stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 1 °C. Numbers across the top of each graph are Fishers' LSD values, $p = 0.05$; ns, not significant.

Table 1. Volatile Compounds Detected in Headspace Samples Collected from Whole Gala Apple Fruit

no.	RT[a] (min)	RI[b]	compound	identification[c]
1	7.38	837	butanal	1, 2, 3
2	7.79	853	ethyl acetate	1, 2, 3
3	9.60	910	2-propanol	1, 2, 3
4	9.94	916	ethanol	1, 2, 3
5	11.49	942	propyl acetate	1, 2, 3
6	11.60	944	pentanal	1, 2, 3
7	12.30	983	methyl butanoate	1, 2, 3
8	13.32	1009	methyl 2-methylbutanoate	1, 2, 3
9	13.56	1020	2-methylpropyl acetate	1, 2, 3
10	14.98	1037	ethyl butanoate	1, 2, 3
11	15.22	1044	1-propanol	1, 2, 3
12	15.26	1045	propyl propanoate	1, 2, 3
13	15.70	1054	ethyl 2-methylbutanoate	1, 2, 3
14	16.87	1074	butyl acetate	1, 2, 3
15	17.09	1079	hexanal	1, 2, 3
16	18.25	1093	2-methyl-1-propanol	1, 2, 3
17	19.04	1123	2-methylbutyl acetate	1, 2, 3
18	19.67	1136	ethyl pentanoate	1, 2, 3
19	19.89	1143	butyl propanoate	1, 2, 3
20	20.55	1149	1-butanol	1, 2, 3
21	21.36	1174	pentyl acetate	1, 2, 3
22	21.84	1184	heptanal	1, 2, 3
23	23.24	1210	2-methyl-1-butanol	1, 2, 3
24	23.25	1220	butyl butanoate	1, 2, 3
25	23.82	1235	butyl 2-methylbutanoate	1, 2, 3
26	23.83	1236	ethyl hexanoate	1, 2, 3
27	24.85	1255	1-pentanol	1, 2, 3
28	25.48	1275	hexyl acetate	1, 2, 3
29	25.69	1283	2-methylbutyl 2-methylbutanoate	1, 2, 3
30	25.97	1290	octanal	1, 2, 3
31	26.94	1319	pentyl butanoate	1
32	27.00	1320	propyl hexanoate	1
33	27.67	1343	6-methyl-5-hepten-2-one	1, 2, 3
34	27.70	1344	hexyl propanoate	1, 2, 3
35	28.43	1360	1-hexanol	1, 2, 3
36	29.52	1398	nonanal	1, 2, 3
37	30.14	1418	butyl hexanoate	1, 2, 3
38	30.21	1420	hexyl butanoate	1, 2, 3
39	30.59	1433	hexyl 2-methylbutanoate	1, 2, 3
40	30.76	1437	ethyl octanoate	1, 2, 3
41	31.15	1453	acetic acid	1, 2, 3
42	31.70	1471	2-furancarboxaldehyde	1, 2, 3
43	32.73	1506	decanal	1, 2, 3
44	33.63	1539	benzaldehyde	1, 2, 3
45	35.76	1617	hexyl hexanoate	1, 2, 3
46	37.55	1679	1-methoxy-4-(2-propenyl)benzene	1, 2, 3

[a] Retention time. [b] Retention index. [c] Substance identification: 1, MS library search; 2, comparison of sample and reference compound spectra; and 3, comparison of sample and reference compound retention indices.

treated with 1-MCP and then stored in air increased to rates similar to those of control fruit. Similar trends existed for 1-butanol and 2-methyl-1-butanol. These results confirm that sensitivity to ethylene action varies among the pathways of ester production in apple fruit (*17*). Apple fruit synthesizes straight C chain alcohols or acids by β-oxidation of long chain fatty acids (*25*) and branch C chain alcohols or acids from amino acid degradation (*26, 27*). These compounds serve as substrates for ester synthesis, and their availability as well as activity of the enzyme catalyzing ester synthesis, alcohol acyl co-A reductase, limit ester production in many climacteric fruit (*28–30*). Exposure to ethylene for 7 days after removal from storage promoted the production of straight chain esters more than branched chain esters by fruit stored in a CA regardless of previous 1-MCP treatment (data not shown). The results differ from similar work where apples exposed to ethylene at harvest exhibited enhanced production of branched chain compounds (*31*). The production of the two groups of esters responded differently to inhibition of ethylene action. Straight chain ester production decreased more than that of branched chain esters in 1-MCP-treated fruit. This is similar to low O_2 responses of apple fruit where straight chain ester and alcohol production decreases with decreased O_2 concentration during storage (*32*).

The production of 1-methoxy-4-(2-propenyl)benzene, a product of the shikimic acid pathway (*33*) that imparts a spicy character to apple aroma (*24*), is also reduced following CA storage (*21*, *34*) and inhibition of ethylene action with 1-MCP. This response indicates that the production of 1-methoxy-4-(2-propenyl)benzene is regulated at least in part by ethylene action. The mechanism for this response is unknown but may be a result of lower fruit metabolic activity, lack of substrate, and/or less capacity for synthesis. Exogenous ethylene activates phenylalanine ammonia-lyase (EC 4.3.1.5) a regulatory enzyme of the shikimic acid pathway in citrus fruit and lettuce leaves (*35*, *36*). Exposure to ethylene stimulated 1-methoxy-4-(2-propenyl)-benzene production only after 28 weeks of storage for apples previously stored in CA.

Reduced sensitivity to ethylene has previously been suggested as the mechanism by which volatile production is decreased by CA storage (*6*). Previous research with ethylene action inhibitors indicates that continuous ethylene action is required for maximum production of volatile compounds, alcohols and esters in particular, by apple fruit (*17, 33*). Similarities in the impacts of CA storage, which has been suggested to inhibit ethylene action (*3*), and 1-MCP suggest a common basis by which both technologies inhibit fruit ripening.

The storage of apple fruit in a CA imparts residual effects on apple volatile production (*34*). During the poststorage 7 days

Table 2. Effect of Ethylene Exposure on Gala Apple Volatile Production[a]

treatment	12 weeks			28 weeks		
	$- C_2H_4$	$+ C_2H_4$	*b*	$- C_2H_4$	$+ C_2H_4$	*b*
			esters			
control	2707 a[c]	2046 a	**	2724 a	3274 a	*
1-MCP	101 b	211 b	**	1265 b	1355 b	NS
CA	33 b	214 b	*	14 c	130 c	***
1-MCP + CA	14 b	25 c	**	8 c	81 c	**
			alcohols			
control	313 a	218 a	*	436 a	660 a	*
1-MCP	23 b	37 b	**	135 b	151 b	NS
CA	11 b	37 b	**	6 c	21 bc	***
1-MCP + CA	6 b	10 b	**	3 c	11 c	*
			1-methoxy-4-(2-propenyl)benzene			
control	30 a	21 a	NS	51 a	41 a	*
1-MCP	3 b	4 bc	NS	27 b	41 a	NS
CA	4 b	8 bc	NS	6 c	42 a	*
1-MCP + CA	1 b	2 c	NS	3 c	8 b	***

[a] Fruit was treated with air (control) or 63 μmol m^{-3} 1-MCP for 12 h at 20 °C and then stored in air or a CA (1 kPa O_2, 2 kPa CO_2) at 0 °C. Fruits were continuously exposed to air ($- C_2H_4$) or 417 μmol m^{-3} ethylene ($+ C_2H_4$) for 7 days at 20 °C during the poststorage period. Esters: ethyl acetate, propyl acetate, methyl butanoate, ethyl butanoate, propyl propanoate, butyl acetate, ethyl pentanoate, butyl propanoate, pentyl acetate, butyl butanoate, ethyl hexanoate, hexyl acetae, pentyl butanoate, propyl hexanoate, hexyl propanoate, butyl hexanoate, hexyl butanoate, ethyl octanoate, hexyl hexanoate, methyl 2-methylbutanoate, 2-methylpropyl acetate, ethyl 2-methylbutanoate, 2-methylbutyl acetate, butyl 2-methylbutanoate, 2-methylbutyl 2-methylbutanoate, and hexyl 2-methylbutanoate. Alcohols: 2-propanol, ethanol, 1-propanol, 2-methyl-1-propanol, 1-butanol, 2-methyl-1-butanol, and 1-pentanol. Values (nmol kg^{-1} h^{-1}) are means, $n = 4$. [b] Ethylene effect not significant (NS) or significant at $p < 0.05$ (*), 0.01 (**), or 0.001% (***). [c] Means with the same letters within columns for each volatile are not significantly different ($p = 0.05$).

in air, production of most volatile compounds after CA storage did not increase. The residual effect may be largely due to the effect of CO_2 in CA storage since high CO_2 has a longer lasting effect on aroma production after storage than at low O_2 (*5*). The fruits did respond to ethylene exposure, suggesting that low ethylene production is also a factor in the residual suppression of volatile compounds.

In summary, fruit stored in a CA had a reduced production of esters, alcohols, and 1-methoxy-4-(2-propenyl)benzene. Volatile production was delayed in apples with ethylene action inhibited by 1-MCP and then stored in air. Inhibition of ethylene action by 1-MCP followed by storage in a CA resulted in a further decrease in production of many volatile compounds, indicating that a CA may prolong the impact of 1-MCP. Poststorage ethylene exposure promoted ester and alcohol production by fruit stored in a CA regardless of previous treatment with 1-MCP. The production of all compounds previously identified as significant contributors to Gala apple aroma was reduced for some period of storage following treatment with 1-MCP and/or storage in a CA. Impacts on fruit organoleptic and other attributes, particularly firmness, that are important contributors to apple sensory quality are likely to vary depending on storage duration after 1-MCP treatment.

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