HortScience 44(5):1390–1394. 2009.

Effects of Aminoethoxyvinylglycine Plus 1-Methylcyclopropene on 'Royal Gala' Apple Volatile Production After Cold Storage

Valeria Sigal Escalada[1]
Department of Horticulture, University of Kentucky, Lexington, KY 40546-0091

Douglas D. Archbold[2,3]
Department of Horticulture, University of Kentucky, Lexington, KY 40546-0091

Additional index words. aminoethoxyvinylglycine, 1-methylcyclopropene, postharvest, flavor

***Abstract*. Both aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP) are useful tools for managing apple fruit ripening, and their impacts on apple volatile production have been independently assessed. In this work, their combined effect, as might occur in commercial production and postharvest storage, on 'Royal Gala' apple volatile production at harvest and after air storage was compared with the effect of each alone. An aqueous solution of AVG was applied to 'Royal Gala' apple trees 4 weeks before the normal harvest date (H1) at 124 g·ha^{-1}/a.i. in 2004 and 2005. Control and AVG-treated fruit were treated at H1 or after harvest of AVG fruit 2 weeks later (H2) for 20 h at 30 °C with 1-MCP at a final headspace concentration of 1 μL·L^{-1}. Fruit were ripened for 7 d at room temperature immediately after harvest and treatment or after treatment and then cold storage at 4 °C for 6 or 12 weeks. Peel and cortex tissue of control and AVG plus 1-MCP-treated fruit was provided with butanol or hexanol and ester production was quantified. The combination of AVG plus 1-MCP was more effective in reducing internal ethylene concentration than either alone. Both total volatile production and that of the major individual esters, including hexyl acetate, butyl acetate, and 2-methylbutylacetate, which are considered key constituents of 'Gala' aroma, were consistently repressed by the combination of AVG plus 1-MCP after harvest and up to 12 weeks of cold storage. The effects of AVG plus 1-MCP were evident even with H2 fruit when the effects of AVG alone on fruit ripening were at least partially lost. Because alcohol–acyl transferase activity was unaffected by AVG plus 1-MCP, AVG plus 1-MCP-treated peel and cortex samples had similar total ester production when they were provided butanol or hexanol. Total alcohols showed recovery in most treatments except AVG plus 1-MCP, so precursor availability was likely the major factor limiting ester production. The results indicated a sustained adverse effect of the AVG plus 1-MCP treatment on aroma volatile production that could impact consumer acceptability.**

Techniques that stop premature fruit drop and slow ripening of climacteric fruit are valuable to growers, shippers, and retailers, permitting efficient harvest and orderly marketing. Aminoethoxyvinylglycine (AVG) is commercially used to stop apple (*Malus ×domestica* Borkh.) fruit drop with application 1 month before harvest (Drake et al., 2005, 2006; Greene, 2006; Greene and Schupp, 2004; Schupp and Greene, 2004). The compound acts by blocking ethylene biosynthesis. Postharvest use of 1-methylcyclopropene (1-MCP), which binds to ethylene receptors and blocks a response to the phytohormone, can significantly delay apple ripening (DeLong et al., 2004; Fan et al., 1999; Ferenczi et al., 2006; Mattheis et al., 2005; Moya-Leon et al., 2007; Rupasinghe et al., 2000). These compounds are commonly used along with cold storage to manage apple fruit ripening.

Several quality factors influence the acceptability of apples, including appearance, texture, and flavor. Flavor is a complex trait composed of sweetness, sourness, bitterness, saltiness, and aroma; the mix of sugars, acids, and volatile compounds play a primary role in quality composition (Baldwin, 2002). Aroma volatile compounds produced by apple fruit include esters, alcohols, aldehydes, acids, ketones, and terpenes (Fan and Mattheis, 1999; Lurie et al., 2002). The majority of the volatiles are esters (78% to 82%) and alcohols (6% to 16%), and the most abundant are even-numbered 2 to 6 carbon chains (Paillard, 1990). The changes in volatile production during ripening result from an increase in ester production that is regulated by ethylene (Fan and Mattheis, 1999). Ester synthesis involves the transfer of acyl moieties to alcohols from acyl-CoAs. This step, catalyzed by the enzyme alcohol–acyl transferase (AAT), is regulated by ethylene through an induction in transcription of *AAT* (Defilippi et al., 2005a; Fan and Mattheis, 1999). However, Echeverria et al. (2004) found that precursor availability is a more significant factor overall than enzyme activity for the development of aroma during on-tree maturation of 'Fuji' apples.

Cold storage alone can alter aroma volatile production (Plotto et al., 2000). In addition, AVG inhibited ethylene and volatile production (Bangerth and Streif, 1987; Fan et al., 1998; Halder-Doll and Bangerth, 1987; Mir et al., 1999), and 1-MCP reduced total volatile production of several apple cultivars after treatment or cold storage (Ferenczi et al., 2006; Kondo et al., 2005; Lurie et al., 2002; Rupasinghe et al., 2000). Thus, apple flavor can be altered by these common practices, and it is possible that these compounds may be used together along with cold storage. When used in combination as pre- and postharvest applications, respectively, AVG plus 1-MCP effects have been inconsistent, improving fruit firmness after cold storage more than either alone in some but not all studies (Argenta et al., 2006; Drake et al., 2006; Moran, 2006; Robinson et al., 2006). Although there is information about the effects of the chemicals individually and combined on some apple ripening traits, there have been no reports about their combined effects on aroma volatile production. Because fruit flavor is integral to consumer acceptability, and aroma volatile production is sensitive to ethylene levels, understanding the potential interaction of AVG and 1-MCP is essential to their effective use and to avoiding possible adverse consequences. Thus, the objectives of this study were to assess the effects of AVG plus 1-MCP on 'Royal Gala' apple aroma volatile production at harvest and after short-term cold storage.

Materials and Methods

Treatments and harvest. In 2004 and 2005, eight trees of 'Royal Gala/M7a', planted in 1993 at the University of Kentucky Horticultural Research Farm in Lexington, KY, and maintained following standard commercial horticultural practices for the region, were selected. Four of the trees, one per row and none adjacent to one another or to control trees, were treated with an aqueous solution of AVG (ReTain; Valent Biosciences, Libertyville, IL) containing 500 μL·L^{-1} Silwet L-77 (Helena Chemical Co., Collierville, TN) as surfactant 4 weeks before the expected normal harvest at the commercial rate of 124 g/a.i./ha (Commercial Tree Fruit Spray Guide, 2006), and four trees were not treated (controls). The AVG was applied to leaves and fruit with a hand pump sprayer to the point of runoff. Control fruit were harvested at the beginning of ripening (H1) based on starch

Received for publication 19 Feb. 2009. Accepted for publication 15 May 2009.
We thank Randall W. Collins for his assistance with aroma volatile analyses.
[1]Former Graduate Student.
[2]Professor of Horticulture.
[3]To whom reprint requests should be addressed; e-mail darchbol@uky.edu.

index and ethylene production, at 119 d after full bloom (DAFB) in 2004 and 123 DAFB in 2005. AVG-treated apples were harvested with the control fruit (H1) and were also harvested 2 weeks later (H2) in 2004 (133 DAFB) and 1 week later in 2005 (130 DAFB).

After harvest, fruit were allowed to equilibrate at ambient laboratory temperature (21 ± 0.5 °C) for 3 to 5 h. Half of each lot (≈150 fruit) was then placed in 26-L plastic containers for 20 h with 1-MCP (EthylBloc powder, 0.14% a.i.; Biotechnologies for Horticulture, Burr Ridge, IL) in a solution at pH 8.2 to produce an estimated final headspace 1-MCP concentration of 1 $\mu L \cdot L^{-1}$. There were four treatments at H1 (control, AVG, 1-MCP, and AVG plus 1-MCP) and two treatments at H2 (AVG and AVG plus 1-MCP). Fruit were ripened at 21 °C for 7 d after postharvest treatment or were stored in cold storage for 6 or 12 weeks at 4 °C and later ripened at 21 °C for 7 d.

Internal ethylene concentration. Internal ethylene concentration (IEC) was measured on 10 fruit per treatment on Days 1 and 7 after harvest or 6 or 12 weeks of cold storage. A gas sample was taken from the seed cavity by inserting a needle attached to a 10-mL syringe through the calyx end, and a 0.2-mL subsample was analyzed by gas chromatography (HP 5890; Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector (FID) and an alumina capillary column (AT-Alumina Plot GC Column, 30 m, 0.53 cm i.d.) containing activated alumina using N_2 as the carrier gas. Temperatures were 35, 175, and 125 °C for oven, injector, and FID, respectively. An external standard (100 $\mu L \cdot L^{-1}$ ethylene/helium; Alltech Associates Inc., Deerfield, IL) was diluted and used to quantify the amounts of ethylene.

Fruit tissue sampling. At 7 d after harvest or removal from 6 or 12 weeks of cold storage, the apples used for ethylene analyses were sectioned into peel and cortex, and tissue samples were frozen and stored at –80 °C. For analysis of volatile production and AAT activity, composite peel and cortex samples were derived from three fruit. There were three replications of composite samples for each treatment and storage period.

Aroma volatile analyses. Headspace volatile production was measured on three ≈9 g peel and three ≈9 g cortex composite samples per treatment after thawing as described in Hamilton-Kemp et al. (2003). Volatiles were identified from retention times matching those of authentic standards.

Alcohol acyl-CoA transferase activity. Alcohol acyl-CoA transferase activity was separately assayed on peel and cortex tissue of three 3-g composite samples per treatment, each composite from three apples. Tissues were frozen at –80 °C 7 d after harvest or removal from 6 or 12 weeks of cold storage and were later analyzed using a method described by Echeverria et al. (2004). AAT activity was expressed as $mU \cdot mg\ protein^{-1}$ in which a unit (U) of activity is the increase in one unit of absorbance per minute. Total protein content of the enzyme extract was determined spectrophotometrically at 595 nm using the Coomassie Plus™ Protein Assay Kit (Pierce, Rockford, IL) following the manufacturer's instructions and using bovine serum albumin (Fisher Scientific, Fair Lawn, NJ) as a standard.

Volatile substrate supplied. Alcohol substrates were separately provided in the headspace to apple peel and cortex tissues to assess capacity for volatile synthesis. The experiment was conducted in 2005 using control and AVG plus 1-MCP-treated fruit stored for 12 weeks at 4 °C. Fruit from storage were equilibrated at 21 °C for 3 h. Peel strips (10 to 12 mm wide) and cortex plugs (5 mm diameter, 50 to 80 mm length) were combined into nine separate 3-g composite samples, each composite from three fruit, per treatment and tissue type. All samples were placed on three layers of water-saturated filter paper in 15-mL glass jars sealed with Teflon-lined plastic screw caps. Three samples per treatment and tissue type were provided with 5 µL of 1-butanol or 1-hexanol (Fisher Scientific), and three samples not provided an alcohol were used as controls. Alcohol substrates were placed in small open glass containers inside the glass jars and allowed to evaporate to be available

Table 1. Internal ethylene concentration of 'Royal Gala' fruit in 2004 and 2005.[z]

	Internal ethylene concn ($\mu L \cdot L^{-1}$)					
	2004			2005		
Treatment	Harvest	6 weeks	12 weeks	Harvest	6 weeks	12 weeks
Control	6	0	0	9	96	173
AVG	0	3	1	6	44	71
1-MCP	1	2	21	1	1	4
AVG + 1-MCP	0	2	0	0	0	2
AVG H2	5	27	28	11	72	10
AVG + 1-MCP H2	0	1	1	0	6	1
LSD	2	9	11	10	49	111

[z]Fruit were treated with aminoethoxyvinylglycine (AVG) 1 month before harvest and/or 1-methylcyclopropene (1-MCP) immediately after harvest and ripened for 7 d at 21 °C after harvest or after 4 °C storage for 6 or 12 weeks. Ethylene was measured after 7 d. AVG-treated fruit were harvested with controls and 1 to 2 weeks later (H2). Fisher's least significant difference (LSD) at $P = 0.05$ is shown to compare means.

Table 2. Total volatile esters and alcohols produced by peel and cortex tissue from 'Royal Gala' fruit in 2004 and 2005.[z]

		AU × 10^{-3} per g fresh weight					
		Harvest		6 weeks		12 weeks	
	Treatment	Peel	Cortex	Peel	Cortex	Peel	Cortex
		2004					
Total esters	Control	2,207	979	722	286	677	246
	AVG	971	795	1,620	705	1,846	619
	1-MCP	1,149	697	2,185	1,052	2,794	1,738
	AVG + 1-MCP	362	143	736	336	691	275
	AVG H2	1,598	1,024	2,553	1,189	2,540	1,230
	AVG + 1-MCP H2	527	306	669	318	633	265
	LSD	604	216	957	460	647	487
Total alcohols	Control	51	51	19	16	18	19
	AVG	2	2	15	13	31	24
	1-MCP	16	16	36	40	62	63
	AVG + 1-MCP	1	1	4	3	3	2
	AVG H2	23	24	64	46	82	71
	AVG + 1-MCP H2	2	2	23	10	3	3
	LSD	10	4	33	26	21	19
		2005					
Total esters	Control	2,813	1,544	2,911	1,915	5,569	3,386
	AVG	1,691	992	1,751	1,068	5,376	3,128
	1-MCP	836	521	490	189	1,523	535
	AVG + 1-MCP	565	344	407	166	1,306	1,936
	AVG H2	1,940	839	2,357	1,027	4,350	2,320
	AVG + 1-MCP H2	569	335	361	514	756	273
	LSD	741	271	461	496	1,017	1,026
Total alcohols	Control	21	22	53	31	200	89
	AVG	12	9	33	23	189	85
	1-MCP	2	2	6	6	35	26
	AVG + 1-MCP	0	1	4	5	30	22
	AVG H2	32	24	61	35	174	52
	AVG + 1-MCP H2	2	2	1	5	21	18
	LSD	10	7	6	7	54	17

[z]Fruit were treated with aminoethoxyvinylglycine (AVG) 1 month before harvest and/or 1-methylcyclopropene (1-MCP) immediately after harvest and ripened for 7 d at 21 °C after harvest or after 4 °C storage for 6 or 12 weeks. AVG-treated fruit were harvested with controls and 1 to 2 weeks later (H2). Fisher's least significant difference (LSD) at $P = 0.05$ is shown to compare means.

Table 3. Production of selected esters by peel tissue from 'Royal Gala' fruit harvested in 2004 and 2005.[z]

		AU × 10^{-3} per g fresh weight					
		Harvest		6 weeks		12 weeks	
Compound	Treatment	2004	2005	2004	2005	2004	2005
Ethyl-2-methyl butanoate	Control	490	614	302	2,383	225	371
	AVG	324	600	492	241	413	427
	1-MCP	287	381	428	164	399	296
	AVG + 1-MCP	175	304	465	165	431	352
	AVG H2	367	362	339	198	337	304
	AVG + 1-MCP H2	252	270	337	150	345	253
	LSD	124	146	89	55	48	103
2-Methylbutyl acetate	Control	380	393	74	413	46	554
	AVG	3	141	61	227	105	784
	1-MCP	191	131	162	47	243	203
	AVG + 1-MCP	5	46	10	39	7	153
	AVG H2	122	281	188	238	208	363
	AVG + 1-MCP H2	59	85	31	37	9	625
	LSD	102	153	125	69	86	216
Ethyl butanoate	Control	299	498	212	227	228	313
	AVG	293	320	280	182	318	316
	1-MCP	169	208	389	108	536	228
	AVG + 1-MCP	170	165	231	84	229	176
	AVG H2	225	226	375	168	402	256
	AVG + 1-MCP H2	150	130	223	89	254	121
	LSD	109	81	145	37	85	68
Butyl acetate	Control	190	190	29	295	35	840
	AVG	2	75	35	153	79	521
	1-MCP	44	17	206	25	376	72
	AVG + 1-MCP	0	4	2	18	1	57
	AVG H2	75	114	219	240	290	422
	AVG + 1-MCP H2	7	13	19	14	2	27
	LSD	53	99	162	70	121	109
Butyl hexanoate	Control	214	360	28	348	52	698
	AVG	2	122	80	224	160	768
	1-MCP	41	18	169	32	227	315
	AVG + 1-MCP	1	7	3	22	3	210
	AVG H2	115	232	258	306	302	650
	AVG + 1-MCP H2	11	12	10	13	0	71
	LSD	74	133	152	65	114	216
Hexyl acetate	Control	389	431	52	659	60	1,586
	AVG	20	247	89	309	166	1,059
	1-MCP	81	49	275	54	381	197
	AVG + 1-MCP	11	24	21	38	16	176
	AVG H2	222	361	357	578	392	960
	AVG + 1-MCP H2	33	36	39	34	16	105
	LSD	126	119	242	195	134	123

[z]Fruit were treated with aminoethoxyvinylglycine (AVG) 1 month before harvest and/or 1-methylcyclopropene (1-MCP) immediately after harvest and ripened for 7 d at 21 °C after harvest or after 4 °C storage for 6 or 12 weeks. AVG-treated fruit were harvested with controls and 1 to 2 weeks later (H2). Fisher's least significant difference (LSD) at $P = 0.05$ is shown to compare means.

for tissue uptake. The sealed containers were placed in an incubator at 22 °C for 24 h. Samples were then frozen at –20 °C. For volatile analysis, samples were thawed in 15-mL glass jars sealed with Teflon-lined plastic screw caps containing a three-layer septum. Samples were equilibrated in a water bath to 26 °C for 2 h and then placed at 21 °C. Headspace volatiles were sampled as described previously.

Experimental design and statistical analysis. Each experiment was conducted using a completely random design. All data were subjected to analysis of variance, and means were compared by Fisher's protected least significance difference ($P = 0.05$) using SAS Version 9.1 software (SAS Institute Inc., Cary, NC).

Results and Discussion

Internal ethylene concentration. Control fruit had an IEC close to 1 $\mu L \cdot L^{-1}$ 1 d after harvest both years, whereas all treated fruit had a lower IEC (data not shown). After 7 d at 21 °C, only control fruit and AVG-treated fruit from H2 had IEC values higher than 1 $\mu L \cdot L^{-1}$ in 2004, whereas control and AVG-treated apples from both H1 and H2 had IEC values over 1 $\mu L \cdot L^{-1}$ in 2005 (Table 1). IEC of fruit treated with 1-MCP or AVG plus 1-MCP was low both years. After 6 weeks of cold storage in 2004, AVG-treated fruit from H2 had the highest IEC, and controls and all other treatments from both harvests had low IEC values. In 2005, control and AVG-treated fruit from H1 and H2 had high IECs, whereas values for the other treatments were lower than controls. After 12 weeks of cold storage in 2004, 1-MCP- and AVG-treated fruit from H2 had the highest IEC. In 2005, control fruit had a higher IEC than any treatment other than AVG from H1. Overall, AVG plus 1-MCP consistently resulted in a low, or the lowest, IEC independent of harvest date and cold storage length. The effects of 1-MCP in 2004, AVG at H1 in 2005, and AVG at H2 both years may have been diminishing during cold storage as their IEC values increased.

Aroma volatile production. Total volatile ester and alcohol production were derived from the sum of the area units of the identified ester and alcohol volatile compounds, including the esters (in descending order by amount from control fruit at harvest): ethyl-2-methylbutanoate, hexyl acetate, ethyl butanoate, 2-methylbutylacetate, butyl hexanoate, butyl acetate, hexyl hexanoate, hexyl 2-methylbutanoate, butyl 2-methylbutanoate, and butyl butanoate; and the alcohols: hexanol, 1-butanol, and 2-methyl-1-butanol. The total ester production by peel tissue of untreated control fruit was higher than that by cortex tissue both seasons (Table 2), but trends of controls over storage time differed between years. In 2004, total ester production of both tissues decreased over time in cold storage (peel total = $2301.4 - 385.7x + 21x^2$, $R^2 = 0.94$, $P < 0.01$; cortex total = $1024.2 - 177.7x + 9.5x^2$, $R^2 = 0.89$, $P < 0.01$; x is time in months; n = 9). In 2005, total ester production of

Table 4. Alcohol–acyl transferase activity of peel and cortex tissue from 'Royal Gala' fruit in 2004 and 2005.[z]

	mU·mg^{-1} protein					
	Harvest		6 weeks		12 weeks	
Treatment	Peel	Cortex	Peel	Cortex	Peel	Cortex
			2004			
Control	162	151	191	126	178	121
AVG	110	113	111	172	112	172
1-MCP	112	100	117	124	109	114
AVG + 1-MCP	111	107	160	118	127	115
AVG H2	114	135	119	136	149	198
AVG + 1-MCP H2	120	119	98	141	89	137
LSD	31	NS	26	NS	32	37
			2005			
Control	139	162	159	135	121	157
AVG	133	128	140	120	172	160
1-MCP	124	140	137	123	114	149
AVG + 1-MCP	125	122	145	148	115	140
AVG H2	143	161	168	178	198	160
AVG + 1-MCP H2	127	146	149	144	137	147
LSD	NS	26	24	47	37	NS

[z]Fruit were treated with aminoethoxyvinylglycine (AVG) 1 month before harvest and/or 1-methylcyclopropene (1-MCP) immediately after harvest and ripened for 7 d at 21 °C after harvest or after 4 °C storage for 6 or 12 weeks. AVG-treated fruit were harvested with controls and 1 to 2 weeks later (H2). Fisher's least significant difference (LSD) at $P = 0.05$; NS indicates nonsignificant difference among treatments.

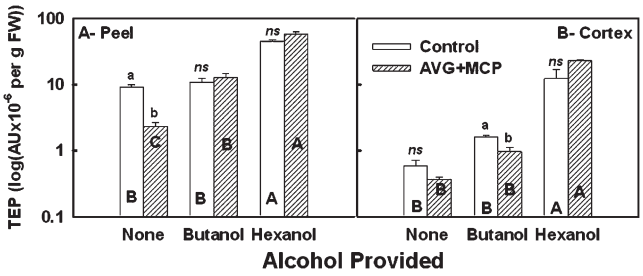

Fig. 1. Total ester production (TEP) by peel (**A**) and cortex (**B**) of 'Royal Gala' apples provided with alcohol substrates. Peel and cortex tissue of fruit that had been stored for 12 weeks at 4 °C were incubated with no alcohol (control), 1-butanol, or 1-hexanol for 24 h, and volatile ester profiles were subsequently measured. Different lower case letters indicate significant differences between control and aminoethoxyvinylglycine (AVG) plus 1-methylcyclopropene (1-MCP) peel or cortex tissue within alcohol substrate separated by least significant difference (LSD) at $P = 0.05$; different upper case letters indicate significant differences among alcohol substrates categories within tissue type and treatment (control or AVG plus 1-MCP) separated by LSD at $P = 0.05$ within date. *ns* = no significant difference.

both tissues increased over time in cold storage (peel total = $2874.5 - 196.6x + 37.5x^2$, $R^2 = 0.83$, $P < 0.01$; cortex total = $1565.9 - 32.2x + 15.9x^2$, $R^2 = 0.95$, $P < 0.01$). These patterns matched the patterns of ethylene production (Table 1).

Treatments reduced peel esters after harvest by 28% or greater in 2004 and by 32% or greater in 2005 (Table 2). By 6 and 12 weeks of cold storage in 2004, all the AVG and 1-MCP treatments were recovering ester production to levels greater than controls, but AVG plus 1-MCP values were still low irrespective of harvest date or storage duration. In 2005, 1-MCP and the AVG plus 1-MCP treatments failed to recover ester production through 12 weeks of cold storage. Although considerably lower than the total ester levels, total alcohols showed the same general responses.

Six esters comprised nearly 90% of the total esters produced by control fruit peel tissue after harvest. These individual esters generally exhibited the same treatment effects as observed with TVP (Table 3). Ethyl-2-methylbutanoate (sweet, strawberry aroma) and ethyl butanoate (fruity aroma) were the most abundant, even if repressed by the treatments. Three of the suppressed esters, hexyl acetate (ripe, pear aroma), butyl acetate (nail polish aroma), and 2-methylbutylacetate (solvent aroma), are considered key 'Gala' aroma volatiles (Plotto et al., 2000). Irrespective of the trends in control ester production and the recovery of AVG and/or 1-MCP treatments alone, the combined AVG plus 1-MCP treatments most consistently suppressed volatile production through 12 weeks of cold storage.

Alcohol-acyl transferase activity. AAT activity per milligram protein in peel and cortex tissue of 'Royal Gala' apples was similar (Table 4). Immediately after the 2004 harvest, peel AAT activity was equally reduced by all treatments, but there were no differences among control and treatments in 2005. No treatment reduced cortex AAT activity in 2004, whereas AVG at H1 and AVG plus 1-MCP reduced it in 2005. Peel AAT activity of AVG plus 1-MCP from H1 was greater than all other treatments but less than controls after 6 weeks in 2004, whereas no treatments differed from controls in 2005. By 12 weeks all treatments reduced peel AAT, except for AVG from H2 in 2004, but AVG from H1 and H2 were greater than controls in 2005. Cortex AAT activity of treatments did not differ from controls in 2004 or 2005 at 6 weeks, or at 12 weeks in 2005, and only AVG from H1 and H2 were greater than controls at 12 weeks in 2004. Overall, the AVG plus 1-MCP reduced peel AAT activity compared with controls in 2004 but not in 2005. Because total peel ester production was reduced both years, this suggests that substrate levels were affected by the treatments and impacted total ester production.

Volatile substrate supplied. Peel and cortex tissue of control and AVG plus 1-MCP-treated fruit stored at 4 °C for 12 weeks were provided butanol or hexanol, and their ester production compared. Overall, total ester production (TEP) was higher from peel than cortex tissue. TEP of control fruit peel tissue not supplied with alcohol substrates was almost four times higher than from AVG plus 1-MCP-treated peel (Fig. 1), whereas there were no differences between the respective cortex tissues. TEP by peel of control versus AVG plus 1-MCP-treated samples were similar when they were provided with either butanol or hexanol. Control peel provided with hexanol had fivefold higher TEP than peel not supplied with hexanol. When provided butanol, control fruit cortex tissue had higher TEP than AVG plus 1-MCP-treated cortex, but TEP by cortex of control and treated fruit was similar when samples were provided with hexanol.

In the present study, control fruit were harvested at similar days after bloom, similar starch indices, and IEC levels close to 1 $\mu L \cdot L^{-1}$ in both years and were starting to ripen. In addition, control IEC increased significantly within 7 d at 21 °C both years (data not shown). Although harvested at similar days after full bloom and apparent physiological status both years, IEC responses to AVG and 1-MCP individually and to cold storage duration differed between the two seasons. This suggests that production environment or other preharvest factors also affected the fruit. The average temperature from bloom (20 Apr.) to harvest (15 Aug.) was 5 °C higher in 2004 than in 2005. The combined treatment exhibited the lowest IEC levels through 12 weeks across seasons, however (Table 1).

Irrespective of the differing patterns of control fruit IEC and TVP production between years, AVG plus 1-MCP generally had a greater and more consistent impact on both IEC and TVP than either alone after harvest and through 12 weeks of cold storage both seasons, although synergistic effects were not observed (Tables 1 and 2). AVG and 1-MCP alone have suppressed ethylene production and slowed ripening for varying

periods of time after harvest and/or cold storage (Autio and Bramlage, 1982; DeLong et al., 2004; Drake et al., 2005, 2006; Fan et al., 1999; Johnson and Colgan, 2003; Mattheis et al., 2005; Moya-Leon et al., 2007; Rupasinghe et al., 2000; Schupp and Greene, 2004; Silverman et al., 2004), although their effects often diminished when used alone in the present work. The effects of 1-MCP diminished with storage time in 2004, and those of AVG did so in 2005. The combined treatment seemed to assure that ethylene and TVP were consistently suppressed.

Reduced TVP production by the AVG plus 1-MCP treatment was likely the result of substrate limitation, although AAT activity was also reduced in 2004. Ester synthesis can be limited by the concentration of substrates (Berger and Drawert, 1984; Echeverria et al., 2004). Peel and cortex tissues provided butanol or hexanol appeared capable of abundant volatile production (Fig. 1). Similar results from feeding precursors were observed after 1-MCP treatment alone (Ferenczi et al., 2006). Defilippi et al. (2005a, 2005b) indicated a clear regulation of AAT activity by ethylene, whereas alcohol dehydrogenase (ADH) and lipoxygenase (LOX), which provide volatile precursors, were not. However, Da-Peng et al. (2006) observed a reduction in both ADH and LOX after 1-MCP treatment, indicating substrate levels may be altered. Total alcohol production recovered in most treatments in the present study, although AVG plus 1-MCP levels remained low, if not the lowest, through 12 weeks (Table 2).

Plotto et al. (1999) described a decrease in sensory scores for fruitiness of controlled atmosphere-stored 'Gala' apples that was correlated with a decrease in aroma volatile levels. At least some consumers may be able to distinguish 1-MCP-treated fruit by their low aroma volatile production (Marin et al., 2009). Because AVG plus 1-MCP repressed IEC and TVP the most, including key 'Gala' aroma volatiles, the treatment would negatively affect consumer acceptability. A longer period at 21 °C (Ferenczi et al., 2006), combined with sustained C_2H_4 treatment (Berger and Drawert, 1984; Fan et al., 1998; Johnson and Colgan, 2003), may be required for AVG plus 1-MCP-treated fruit to recover volatile production, although how the extended period and treatment may influence other quality traits would need to be determined.

Literature Cited

Argenta, L.C., M.J. Vieira, J.G. Krammes, J.L. Petri, and C. Basso. 2006. AVG and 1-MCP effects on maturity and quality of apple fruit at harvest and after storage. Acta Hort. 727:495–503.

Autio, W.R. and W.J. Bramlage. 1982. Effects of AVG on maturation, ripening and storage of apples. J. Amer. Soc. Hort. Sci. 107:1074–1077.

Baldwin, E.A. 2002. Fruit flavor, volatile metabolism and consumer perceptions, p. 89–106. In: Knee, M. (ed.). Fruits quality and its biological basis. Sheffield Academic Press Ltd., Sheffield, UK.

Bangerth, F. and J. Streif. 1987. Effect of aminoethoxyvinylglycine and low-pressure storage on the poststorage production of aroma volatiles by 'Golden Delicious' apples. J. Sci. Food Agr. 41:351–360.

Berger, R.G. and F. Drawert. 1984. Changes in the composition of volatiles by post-harvest application of alcohols to Red Delicious apples. J. Sci. Food Agr. 35:1318–1325.

Commercial Tree Fruit Spray Guide. 2006. Suggestions for growth regulators, p. 41–42. In: Bessin, R.T., R.T. Jones, J.G. Masabni, J.G. Strang, and J.R. Hartman (eds.). University of Kentucky Coop. Ext. Service, Lexington, KY.

Da-Peng, L., Y.-F. Xu, L.-P. Sun, L.-X. Liu, X.-L. Hu, D.-Q. Li, and H.-R. Shu. 2006. Salicylic acid, ethephon, and methyl jasmonate enhance ester regeneration in 1-MCP-treated apple fruit after long-term storage. J. Sci. Food Agr. 54:3887–3895.

Defilippi, B.G., A.A. Kader, and A.M. Dandekar. 2005a. Apple aroma: Alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. Plant Sci. 168:1199–1210.

Defilippi, B.G., A.M. Dandekar, and A.A. Kader. 2005b. Relationship of ethylene biosynthesis to volatile production, related enzymes, and precursor availability in apple peel and cortex tissues. J. Agr. Food Chem. 53:3133–3141.

DeLong, J.M., R.K. Prange, and P.A. Harrison. 2004. The influence of 1-methylcyclopropene on 'Cortland' and 'McIntosh' apple quality following long-term storage. HortScience 39:1062–1065.

Drake, S.R., T.A. Eisele, D.C. Elfving, M.A. Drake, S.L. Drake, and D.B. Visser. 2005. Effects of the bioregulators aminoethoxyvinylglycine and ethephon on Brix, carbohydrates, acid, and mineral concentrations in 'Scarletspur Delicious' apple juice. HortScience 40:1421–1424.

Drake, S.R., D.C. Elfving, M.A. Drake, T.A. Eisele, S.L. Drake, and D.B. Visser. 2006. Effects of aminoethoxyvinylglycine, ethephon and 1-methylcyclopropene on apple fruit quality at harvest and after storage. HortTechnology 16:16–23.

Echeverria, G., T. Fuentes, J. Graell, I. Lara, and M.L. Lopez. 2004. Aroma volatile compounds of 'Fuji' apples in relation to harvest date and cold storage technology. A comparison of two seasons. Postharvest Biol. Technol. 32:29–44.

Fan, X., S.M. Blankenship, and J.P. Mattheis. 1999. 1-Methylcyclopropene inhibits apple ripening. J. Amer. Soc. Hort. Sci. 124:690–695.

Fan, X. and J.P. Mattheis. 1999. Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. J. Agr. Food Chem. 47:2847–2853.

Fan, X., J.P. Mattheis, and D. Buchanan. 1998. Continuous requirement of ethylene for apple fruit volatile synthesis. J. Agr. Food Chem. 46:1959–1963.

Ferenczi, A., J. Song, M. Tian, K. Vlanchonasios, D. Dilley, and R. Beaudry. 2006. Volatile ester suppression and recovery following 1-methylcyclopropene application to apple fruit. J. Amer. Soc. Hort. Sci. 131:691–701.

Greene, D.W. 2006. An update on preharvest drop control of apples with aminoethoxyvinylglycine (ReTain). Acta Hort. 727:311–319.

Greene, D.W. and J.R. Schupp. 2004. Effect of aminoethoxyvinylglycine (AVG) on Preharvest drop, fruit quality, and maturation of 'McIntosh' apples. II. Effect of timing and concentration relationships and spray volume. HortScience 39:1036–1041.

Halder-Doll, H. and F. Bangerth. 1987. Inhibition of autocatalytic C_2H_4 biosynthesis by AVG applications and consequences on the physiological behavior and quality of apple fruits in cold storage. Scientia Hort. 33:87–96.

Hamilton-Kemp, T.R., D.D. Archbold, K. Yu, and R.W. Collins. 2003. Emission patterns of wound volatile compounds following injury of ripe strawberry fruit. J. Sci. Food Agr. 83:283–288.

Johnson, D.S. and R.J. Colgan. 2003. Low ethylene controlled atmosphere induces adverse effects on the quality of 'Cox's Orange Pippin' apples treated with aminoethoxyvinylglycine during fruit development. Postharvest Biol. Technol. 27:59–68.

Kondo, S., S. Setha, D.R. Rudell, D.A. Buchanan, and J.P. Mattheis. 2005. Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. Postharvest Biol. Technol. 36:61–68.

Lurie, S., C. Pre-Aymard, U. Ravid, O. Larkov, and E. Fallik. 2002. Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. J. Agr. Food Chem. 50:4251–4256.

Marin, A.B., A.E. Colonna, K. Kudo, E.M. Kupferman, and J.P. Mattheis. 2009. Measuring consumer response to 'Gala' apples treated with 1-methylcyclopropene. Postharvest Biol. Technol. 51:73–79.

Mattheis, J.P., X. Fan, and L.C. Argenta. 2005. Interactive responses of Gala apple fruit volatile production to controlled atmosphere storage and chemical inhibition of ethylene action. J. Agr. Food Chem. 53:4510–4516.

Mir, N.A., R. Perez, P. Schwallier, and R. Beaudry. 1999. Relationship between ethylene response manipulation and volatile production in Jonagold variety apples. J. Agr. Food Chem. 47: 2653–2659.

Moran, R.E. 2006. Maintaining fruit firmness of 'McIntosh' and 'Cortland' apples with aminoethoxyvinylglycine and 1-methylcyclopropene during storage. HortTechnology 16:513–516.

Moya-Leon, M.A., M. Vergara, C. Bravo, M. Pereira, and C. Moggia. 2007. Development of aroma compounds and sensory quality of 'Royal Gala' apples during storage. J. Hort. Sci. Biotechnol. 83:403–413.

Paillard, N.M. 1990. The flavour of apples, pears and quinces, p. 1–41. In: Morton, I.D. and J. McLeod (eds.). Food favours, Part C. The flavour of fruits. Elsevier Science Publishing Company Inc., Amsterdam, The Netherlands.

Plotto, A., M.R. McDaniel, and J.P. Mattheis. 1999. Characterization of 'Gala' apple aroma and flavor: Differences between controlled atmosphere and air storage. J. Amer. Soc. Hort. Sci. 124:416–423.

Plotto, A., M.R. McDaniel, and J.P. Mattheis. 2000. Characterization in changes in 'Gala' apple aroma during storage using osme analysis, a gas chromatography–olfactometry technique. J. Amer. Soc. Hort. Sci. 125:714–722.

Robinson, T.L., C.B. Watkins, S.A. Hoying, J.F. Nock, and K.I. Iungerman. 2006. Aminoethoxyvinylglycine and 1-methylcyclopropene effects on 'McIntosh' preharvest fruit drop, fruit maturation and fruit quality after storage. Acta Hort. 727:473–480.

Rupasinghe, H.P., D.P. Murr, G. Paliyath, and L. Skog. 2000. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. J. Hort. Sci. Biotechnol. 75:271–276.

Schupp, J.R. and D.W. Greene. 2004. Effect of aminoethoxyvinylglycine (AVG) on preharvest drop, fruit quality, and maturation of 'McIntosh' apples. I. Concentration and timing of dilute applications of AVG. HortScience 39:1030–1035.

Silverman, S.P., P.D. Petracek, M.R. Noll, and P. Warrior. 2004. Aminoethoxyvinylglycine effects on late-season apple fruit maturation. Plant Growth Regulat. 43:153–161.