Maintaining Fruit Firmness of 'McIntosh' and 'Cortland' Apples with Aminoethoxyvinylglycine and 1-Methylcyclopropene during Storage

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Additional index words. 1-MCP, AVG, controlled-atmosphere storage, ethylene, growth substances, *Malus* ×*domestica*, maturity, postharvest handling, ReTain, SmartFresh, superficial scald

Summary. **The goal of this project was to evaluate the effectiveness of aminoethoxyvinylglycine (AVG) for increasing effectiveness of 1-methylcyclopropene (1-MCP) for maintaining firmness and preventing scald in 'McIntosh' and 'Cortland' apples (*Malus* ×*domestica*). AVG and 1-MCP used together maintained 'McIntosh' apple firmness more than 1-MCP used alone after 120 or 200 days of controlled-atmosphere (CA) storage. AVG and 1-MCP can be used to maintain firmness of 'McIntosh' when internal ethylene concentration (IEC) at harvest is as high as 240 μL·L^{-1}, but CA storage life is limited to 4 months. AVG was not effective at increasing efficacy of 1-MCP on 'Cortland' when IEC at harvest was not significantly different between AVG-treated and untreated fruit and IEC was less than 2 μL·L^{-1}. AVG increased efficacy of 1-MCP on 'Cortland' when IEC was 36 μL·L^{-1} in untreated fruit compared to undetectable in AVG treated fruit. 1-MCP prevented scald of 'Cortland' in 1 year and reduced it to 5% or less in another year when fruit were stored 120 days. 1-MCP reduced 'Cortland' scald to 34% or less after 200 days of storage.**

At a concentration of at least 1 μL·L^{-1}, 1-methylcyclopropene maintains firmness of 'McIntosh' up to 4 months in regular air and 7 months or longer in controlled-atmosphere (CA) storage, but a reduction in effectiveness occurs that is related to internal ethylene concentration at harvest (Watkins et al., 2000). To temporarily suppress ethylene biosynthesis in apple fruit, aminoethoxyvinylglycine (ReTain; Valent BioSciences, Libertyville, Ill.) is applied at a concentration of 50 g/acre 4 weeks prior to harvest (Bramlage et. al., 1980; Greene and Schupp, 2004). The combination of AVG and 1-MCP maintains firmness more than 1-MCP used alone in 'Scarletspur Delicious' and 'Gale Gala' (Drake et al., 2006). Preliminary studies have shown that AVG can extend the period in which 1-MCP can be used on 'McIntosh', a variety that is prone to excess softening in storage (Watkins et al. 2001; Weis and Bramlage, 2002). However, formal studies have not been conducted to document its consistency or the stage of maturity when this combination is most effective.

'Cortland', another popular variety in New England, is prone to superficial scald. 1-MCP can prevent scald in other varieties (Fan and Mattheis, 1999; Watkins et al. 2000), but has not consistently prevented it in 'Cortland'. Harvest at later stages of maturity or following cooler temperatures, which typically occur with later harvest, can reduce the incidence of this disorder (Barden and Bramlage, 1994; Meir and Bramlage, 1988; Merritt et al., 1961). AVG may be used to extend harvest and may thereby increase effectiveness in scald prevention.

The use of both AVG and 1-MCP can be very costly, so controlled studies are needed to determine when this strategy is most effective. The goal of this project was to evaluate the effectiveness of AVG and 1-MCP in maintaining firmness and preventing scald in 'McIntosh' and 'Cortland' apples.

Materials and methods

Four 'McIntosh' and two 'Cortland' orchards, located at the Maine Agricultural Experiment Station in Monmouth, were sprayed with the full, commercial rate of AVG on 27 Aug. 2003, 4 weeks before anticipated first harvest (Beaudry et al., 1993). Five 'McIntosh' orchards and four 'Cortland' orchards were sprayed with AVG on 26 Aug. 2004. In 2004, three of these orchards were located at the experiment station in Monmouth and the others in a commercial orchard in Turner, Maine. The trees were on semidwarfing rootstocks, 'Malling-Merton 106' and 'Malling-Merton 111', and ranged in age from 20 to 30 years old. Each orchard was a separate replicate. Two rows of trees in each orchard, approximately 40 trees per orchard, were left untreated and the rest, approximately 200 trees per orchard, were sprayed with AVG at 50 g/acre with a spray volume of 90 gal/acre. Untreated trees were separated from treated trees by one guard row. Organosilicone surfactant (Silwet L77; Helena Chemical Co., Collierville, Tenn.) was added at a rate of 0.05% v/v.

Approximately 60 'McIntosh' fruit were harvested from each orchard 24 Sept. and 13 Oct. 2003, coinciding with the end of harvest for CA storage (starch index = 5.8) and the end of harvest for immediate sale (starch index was not measured), respectively. A composite sample of

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.4047	acre(s)	ha	2.4711
29.5735	fl oz	mL	0.0338
3.7854	gal	L	0.2642
9.3540	gal/acre	L·ha^{-1}	0.1069
25.4	inch(es)	mm	0.0394
4.4482	lbf	N	0.2248
28.3495	oz	g	0.0353
1	ppm	μL·L^{-1}	1
(°F – 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

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fruit was harvested from two to seven trees in each orchard. An additional 60 fruit were harvested for treatment with 1-MCP. 'Cortland' apples were harvested 27 Sept. (starch index = 1.4) and 14 Oct. (starch index was not measured). In 2004, 'McIntosh' fruit were harvested 16 Sept. (starch index = 4.0) and 26 Sept. (starch index = 5.8), corresponding with the start and end of harvest for CA storage. 'Cortland' apples were harvested 29 Sept. (starch index = 1.8) and 7 Oct. (starch index = 4.2). Starch index was measured on 10 fruit using a visual rating where 1 = all starch remaining and 8 = no starch (Blanpied and Silsby, 1992). Internal ethylene concentration was measured on 10 fruit per replication to determine the efficacy of AVG (Table 1).

All treatments of 1-MCP (0.14% active ingredient, SmartFresh; AgroFresh, Springhouse, Pa.) were begun within 24 h of harvest. Fruit were exposed for 20 h to 1 $\mu L \cdot L^{-1}$ in 45-L portable, plastic beverage coolers (model no. 5248A718; Coleman Co., Wichita, Kans.). Fruit temperature during treatment ranged from 17 to 23 °C. 1-MCP, dissolved in water in a sealed vial, was placed inside the cooler, and the rubber septum was removed before shutting the cooler lid. A portable fan circulated the air inside the coolers. Fruit were then placed in cold storage at 3 °C. Controlled-atmosphere conditions were established 3 to 7 d following harvest. Large plastic bags and compressed nitrogen gas were used to maintain the concentration of oxygen in the range of 2.8% to 3.2% with occasional variations up to 3.5%. The concentration of carbon dioxide remained below 2%.

Twenty 'McIntosh' apples per replicate were removed from storage in late January, and remaining apples were removed from storage in late April. 'Cortland' apples were removed in early February and early May. After removal from storage, fruit were kept at 18 °C for 1 and 7 d, at which times firmness and IEC were measured on 10 fruit per replication. In 2004, fruit were segregated into large and small sizes for quality analysis with five fruit in each size category. This was done in order to determine if 1-MCP was less effective on larger-sized fruit. Fruit were weighed after storage. Mean fruit weight of "large" and "small" fruit is mentioned in the results section. Firmness of peeled flesh was measured on the green and red side of each fruit with an electronic firmness tester (model EPT-1; Lake City Technical Products, Kelowna, B.C., Canada). To measure IEC, a 25-mm-long stainless steel needle with syringe was inserted through the calyx end, and 1 mL of gas was removed and injected into a gas chromatograph with a flame ionization detector (model GC-8A; Shimadzu, Kyoto, Japan). Flame ionization detector temperature was 200 °C and column temperature 80 °C. An ethylene standard of 9.5 $\mu L \cdot L^{-1}$ was used for calibration. Peak area was determined with an integrator (model 3395; Agilent Technologies, Wilmington, Del.). The occurrence of superficial scald on 'McIntosh' in January and 'Cortland' in February was measured on 10 fruit after 7 d at 18 °C. Superficial scald was measured on all remaining fruit (approximately 40 per replication) in April or May.

The study had a randomized complete-block design with each orchard as a separate block or replication. The treatments were arranged as a 2 × 2 factorial of AVG and 1-MCP. The 'McIntosh' treatments were replicated four times in 2003 and five times in 2004. The 'Cortland' treatments were replicated two times in 2003 and four times in 2004. Data were analyzed by analysis of variance with mean separation by least significant difference test at $P \leq 0.05$ (LSMeans, SAS Release 8.1; SAS Institute, Cary, N.C.). Mean fruit weight was analyzed as a covariate in 2004.

Table 1. Harvest date and aminoethoxyvinylglycine (AVG) at 4 weeks prior to harvest affected internal ethylene concentration (IEC) of 'McIntosh', but not 'Cortland' apple fruit.

		IEC [$\mu L \cdot L^{-1}$ (ppm)]			
		McIntosh		Cortland	
Harvest	Treatment	2003	2004	2003	2004
late September	None	21 c[z]	12 b	0	1.1
	AVG	44 bc	0 b	0	0.0
mid October	None	475 a	56 a	36	0.9
	AVG	243 b	14 b	0	0.5
Significance					
Harvest		***	**	NS	NS
AVG		*	***	NS	NS
Harvest × AVG		**	*	NS	NS

[z]IEC data were log-transformed for analysis, but actual means are presented. Within a column, means followed by the same letter do not differ according to the least significant difference test ($P \leq 0.05$).
[ns, *, **, ***]Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Results and discussion

AVG reduced IEC in 'McIntosh', but not until the second harvest in each year (Table 1). AVG did not significantly lower IEC of 'Cortland' with either harvest. However, in 2003, 75% of the 'Cortland' fruit were preclimacteric (IEC < 1 $\mu L \cdot L^{-1}$) at the second harvest. Lack of significance with the second harvest may also have been due to insufficient replication of 'Cortland' in 2003. AVG did not affect firmness at harvest except in 2003 with the second harvest of 'McIntosh', which was 0.9 lbf firmer than untreated fruit ($P = 0.0503$).

In 2003, untreated 'McIntosh' softened in storage, losing 5.4 lbf of firmness after 120 d with the first harvest and 3.5 lbf with the second harvest (Fig. 1). Analysis of variance indicated that both AVG ($P = 0.0015$) and 1-MCP ($P = 0.0001$) maintained firmness with an interaction between the two ($P = 0.0072$). AVG maintained firmness in fruit from both harvests, but only when used with 1-MCP. The combination of AVG and 1-MCP maintained firmness more than 1-MCP used alone, but not in second-harvest fruit stored 200 d. This is not surprising since fruit from the second harvest were all climacteric at harvest and were not suitable for storage. Fruit were softer in 2003 because of more advanced maturity compared to 2004 and above average fruit size. By 200 d of storage, firmness of fruit from the first harvest remained above 12 lbf when treated with both AVG and 1-MCP. All other treatments were below 12 lbf firmness. The goal of growers and packers in Maine is to have firmness of 'McIntosh' of at least 12 lbf, since the number of consumers that reject apples increases as firmness decreases below 12 to 13 lbf (Harker, 2002; Kupferman et al., 2005).

In 2004, 1-MCP maintained firmness of 'McIntosh' with no AVG interaction. This occurred after both 120 and 200 d of storage with both harvests. Used alone, AVG maintained firmness by 0.7 to 1.9 lbf above the control ($P = 0.0001$) and 1-MCP by 3.0 to 5.7 lbf ($P = 0.0001$). The combination of AVG and 1-MCP maintained firmness by an additional 1.1 to 2.7 lbf. This was more apparent in fruit from the second harvest. Fruit size affected firmness after storage ($P = 0.0001$), but size did not interact with 1-MCP or AVG in their effects on firmness (data not shown). Smaller-sized 'McIntosh' fruit (128 g mean fruit weight) were 1.3 to 1.6 lbf firmer than larger fruit (198 g). Smaller-sized 'Cortland' (163 g) were 1.0 to 1.2 lbf firmer than large fruit (216 g).

In 2002, AVG had little influence on the efficacy of 1-MCP when used on preclimacteric 'McIntosh' fruit, indicating that this combination is best used on fruit from later harvests.

AVG by itself did not maintain firmness of 'Cortland' in either year or with either harvest (Fig. 2). 1-MCP maintained firmness above 12 lbf in both harvests and both storage durations ($P = 0.0001$ in 2003, $P = 0.0058$ in 2004). When used with AVG, 1-MCP maintained firmness by an additional 1.0 lbf, but this was significant only in 2003 after 200 d of storage when there was an interaction between AVG and 1-MCP ($P = 0.0361$). The effect of AVG and 1-MCP on firmness of 'Cortland' was not as great in 2004 and this may be attributable to the small differences in IEC at harvest.

Previous results with 'Gale Gala' were similar to 'McIntosh', in which AVG and 1-MCP maintained firmness of 'Gale Gala' by an additional 1.1 to 1.3 lbf compared to 1-MCP alone (Drake et al., 2006) although IEC at harvest was lower than for 'McIntosh'. Previous results with 'Starkspur Delicious' were less dramatic, and AVG and 1-MCP maintained firmness by an additional 0.3 lbf after CA storage (Drake et al., 2006).

Superficial scald of 'McIntosh' occurred after 200 d of storage, but fewer than 20% of the untreated fruit and none of the fruit treated with 1-MCP were affected (data not shown).

'Cortland' developed scald by 120 d (Fig. 3). The later harvest reduced its occurrence to less than 1% in 2003 ($P = 0.0054$), but only after the shorter storage duration. There was little difference in scald occurrence between the two harvests in 2004. Harvest date had no significant effect on the occurrence of superficial scald after 200 d of storage even though 192 h below 10 °C had occurred between the two harvest dates in 2003 and 94 h in 2004. Previous research has shown that occurrence of scald in 'Cortland' is correlated with duration of exposure

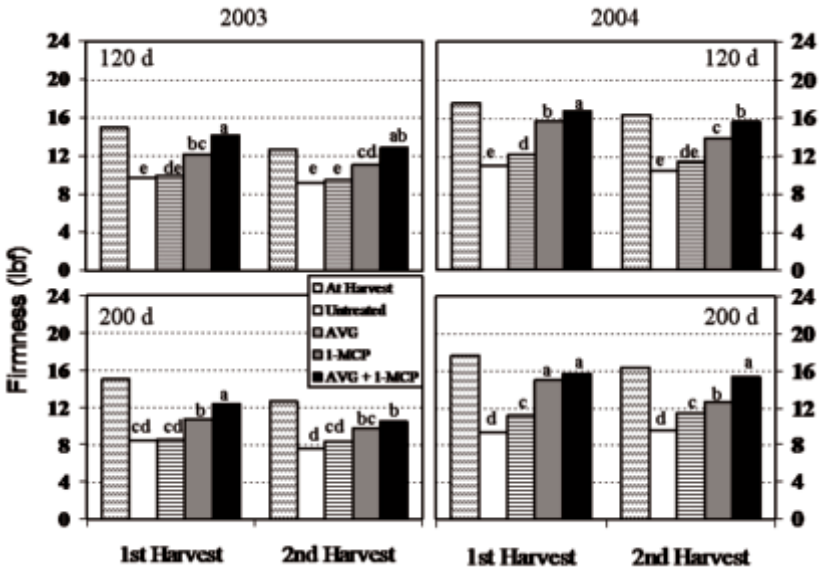

Fig. 1. Firmness of 'McIntosh' apples following 120 or 200 d of controlled-atmosphere storage and 7 d at 18 °C (64.4 °F). Treatments were application of aminoethoxyvinylglycine (AVG) at 4 weeks prior to harvest with or without subsequent application of 1-methylcyclopropene (1-MCP) to fruit from two harvest dates. Within each year and storage duration, means followed by the same letter are not significantly different according to the least significant difference test ($P \leq 0.05$); 1 lbf = 4.4482 N.

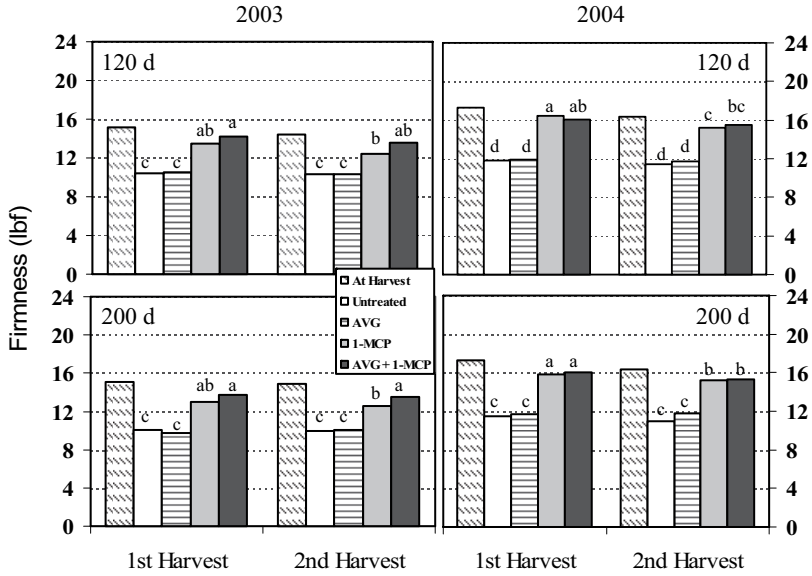

Fig. 2. Firmness of 'Cortland' apples following 120 or 200 d of controlled-atmosphere storage and 7 d at 18 °C (64.4 °F). Treatments were application of aminoethoxyvinylglycine (AVG) at 4 weeks prior to harvest with or without subsequent application of 1-methylcyclopropene (1-MCP) to fruit from two harvest dates. Within each year and storage duration, means followed by the same letter are not significantly different according to the least significant difference test ($P \leq 0.05$); 1 lbf = 4.4482 N.

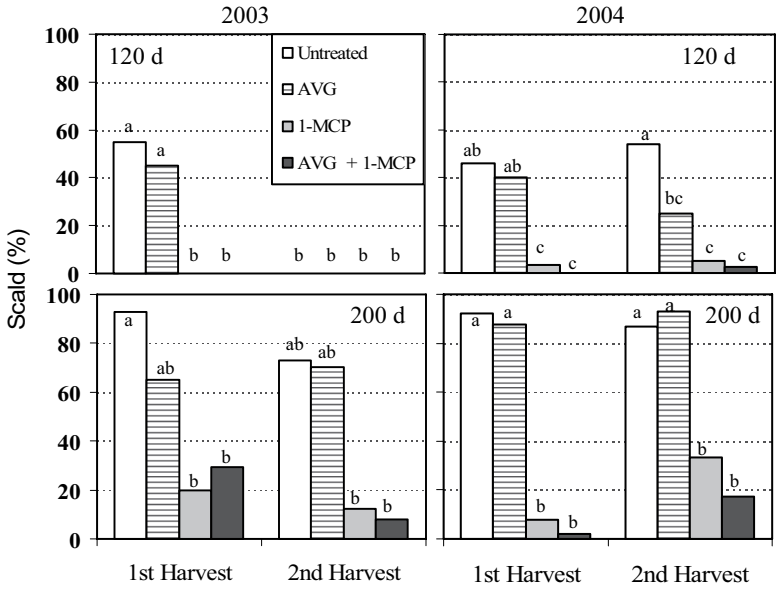

Fig. 3. Superficial scald of 'Cortland' apples following 120 or 200 d of controlled-atmosphere storage and 7 d at 18 °C (64.4 °F). Treatments were application of aminoethoxyvinylglycine (AVG) at 4 weeks prior to harvest with or without subsequent application of 1-methylcyclopropene (1-MCP) to fruit from two harvest dates. Within each year and storage duration, means followed by the same letter are not significantly different according to the least significant difference test ($P \leq 0.05$).

to low temperature (Barden and Bramlage, 1994). However, most of our fruit were harvested from trees with dense canopies, so fruit were grown under lower light levels. Fruit grown in shaded conditions are more prone to scald, with little difference between harvest dates (Barden and Bramlage, 1994), and this may explain why scald after long-term storage was not affected by harvest date in our study.

1-MCP was effective in preventing scald after 120 d storage in 2003 ($P = 0.0001$) and reducing it to less than 5% in 2004 ($P = 0.0001$). By 200 d, severe scald occurred, and this was reduced by 1-MCP ($P = 0.0021$ in 2003; $P = 0.0001$ in 2004), but not as effectively as after 120 d.

Results of this study were based on 1-MCP application within 1 d of harvest. However, application at later times can reduce efficacy, particularly in mature fruit (Watkins and Nock, 2005).

AVG reduced ethylene biosynthesis at harvest and improved the efficacy of 1-MCP on 'McIntosh' fruit. Although they were larger in size and not as firm at harvest, fruit from the second harvest treated with both 1-MCP and AVG remained as firm as fruit from the first harvest treated with only 1-MCP. However, CA storage duration of late-harvested fruit may be limited to 4 months since AVG and 1-MCP were not as effective in maintaining firmness of 'McIntosh' after 7 months, when IEC at harvest was as high as 243 $\mu L \cdot L^{-1}$.

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