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Effects of Application Time and Rate of 1-Aminoethoxyvinylglycine Hydrochloride on Preharvest Fruit Drop, Quality, and Evolved Ethylene in 'Hi Early Delicious' and 'Law Rome Beauty' Apples

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ABSTRACT

The effects of time and rate of 1-aminoethoxyvinylglycine hydrochloride (AVG or Aviglycine HCl or ReTain) application on fruit retention and quality in 'Hi Early Delicious' and 'Law Rome Beauty' apples (*Malus domestica* Borkh.) were studied between 1991 and 1993. In 1991, the application of AVG once at 75 ppm, three times at 150 ppm, or once at 225 ppm, 3 weeks before harvest of 'Hi Early Delicious' or 4 weeks before harvest of 'Law Rome Beauty' increased fruit retention and reduced ethylene evolution. In 1991, a treatment consisting of three applications of AVG, each at 150 ppm applied at 4, 6, and 10 weeks before the anticipated harvest time, was more effective in reducing fruit drop than other treatments in 'Law Rome Beauty'. The time of AVG application was more critical than the rate on suppression of ethylene evolution in both apple cultivars. In 1992, the application of AVG at most tested rates and frequencies affected either fruit retention and/or fruit quality attributes in 'Law Rome Beauty'. The application of AVG at 100 ppm as a single application or multiple applications maintained firmness and reduced starch hydrolysis more than the untreated control or application of 10 ppm α-naphthaleneacetic acid (NAA) treatment in 'Law Rome Beauty' apple in 1992. Fruit retention, maturity and quality differences between AVG and the control or NAA treatments were more pronounced as the time past from the anticipated commercial harvest dates. Applications of AVG or NAA in 1992 did not affect the return fruit set in 'Law Rome Beauty' in 1993.

Keywords: aviglycine HCl, bio-regulator, ethylene inhibitor, ripening, *Malus domestica*, storage life
Abbreviations: AVG, aminoethoxyvinylglycine hydrochloride or **Aviglycine HCl**; **NAA**, α-naphthalene acetic acid; **SDP**, starch degradation pattern; **SSC**, soluble solids concentration

INTRODUCTION

Fruit drop prior to harvest is a major source of fruit loss in apples. Pre-harvest drop of apples is caused by a surge in endogenous ethylene at the abscission layer of the fruit stem. On the other hand, early harvest of apples will lead to low soluble solids concentration, scald development, and poor over-all fruit quality. Maintaining fruit quality attributes after harvest, particularly fruit firmness and starch, is the most important goal of fruit packing houses. Fruit endogenous and evolved ethylene can play a major role in ripening, and thus storage life and fruit quality of climactertic fruits such as apples (Fallahi *et al*. 1985a, 1985b).

The compound 1-aminoethoxyvinylglycine hydrochloride or Aviglycine HCl (AVG or ReTain, Valent BioSciences, Walnut Creek, Ca.) is an inhibitor of endogenous ethylene production in apples (Bangerth 1978). AVG was found to retain fruit firmness in apples (Wang and Mellenthin 1977; Bangerth 1978; Williams 1980) and pears (Wang and Mellenthin 1977). AVG also increased fruit set in 'Delicious' and 'Golden Delicious' apples (Williams 1980) and increased the number of productive fruit spurs in trees with "dead spur" disorder (Parish *et al*. 1994) in the following year. Since the initial works by Bangerth (1978) and Williams (1980) and our research reports and presentations at professional meetings during 1991-1994 (Fallahi, unpublished data), numerous studies have been conducted on apple preharvest drop (Stover *et al*. 2003), fruit maturity (Byers 1997; Greene 2002; Layne *et al*. 2002; Stover *et al*. 2003; Wang and Dilley 2001), fruit size, shape, color, firmness and postharvest quality (Halder-Doll and Bangerth 1987; Larrigaudiere *et al*. 1996; Wang and Dilley 2001; Beaudry and Jayanty 2003; Drake *et al*. 2005).

Although several researchers have studied the effects of AVG on fruit drop of other 'Delicious' apple cultivars, limited or no similar studies are available for 'Hi Early Delicious' or 'Law Rome Beauty' apples. My objective in this experiment was to study the effects of time and rates of AVG applications on fruit drop, fruit quality attributes, and evolved ethylene in 'Hi Early Delicious' and 'Law Rome Beauty' apples.

MATERIALS AND METHODS

The experiments were started in 1991 and continued in 1992 and 1993. Trees were trained to the central leader system. Other than AVG treatments, all cultural practices were applied in a manner consistent with those of commercial apple orchards. In 1991, 10-year-old 'Hi Early Delicious' and 6-year-old 'Law Rome Beauty' apple trees on M.7 rootstock were sprayed with AVG at 75 ppm, 150 ppm, or 225 ppm. Each of these concentrations was sprayed either at 3, 5, or 9 weeks before anticipated harvest in 'Hi Early Delicious' and at 4, 6, or 10 weeks before harvest in 'Law Rome Beauty' apples. In addition to these treatments, one treatment received 150 ppm AVG at all three application times. In 1991, the commercial harvest time for 'Hi Early Delicious' was on 2 Oct. and for 'Law Rome Beauty' was on 4 Oct. The experimental design for each cultivar in 1991 was a complete randomized design with six single-tree replications per control or treatment. To study the effects of application times, data from all application rates

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within a given time were pooled together and were compared with the untreated control. To study the effects of application rates, data from all application times within a given rate were pooled together and were compared with the untreated control.

In 1992, only 7-year-old 'Law Rome Beauty'/M7 apple trees were used for this study, and treatments were: 1) Untreated control, 2) AVG at 25 ppm, applied at 1 week, 2 weeks, and 4 weeks before harvest (total of three times); 3) AVG at 50 ppm applied at 1 week, 2 weeks, and 4 weeks before harvest (total of three times); 4) AVG at 100 ppm applied at 1 week, 2 weeks, and 4 weeks before harvest (total of three times); 5) AVG at 100 ppm applied 4 weeks before harvest (once); 6) AVG at 200 ppm applied 4 weeks before harvest (once); 7) α-naphthalene acetic acid (NAA) at 10 ppm applied 7 days before harvest. All AVG applications were made at the rate of 935 $L \cdot ha^{-1}$ while NAA was applied at the rate of 1870 $L \cdot ha^{-1}$ with a handgun sprayer. In 1992, the experimental arrangement was a complete randomized design with six one-tree replications per treatment.

In each year, existing fruits under the trees were completely removed before application of chemicals. The dropped fruits under each tree were counted and discarded once a week from the beginning of the experiment until several weeks after anticipated harvest. In 1992, 10 fruits per tree from each treatment were sampled randomly at each sampling date on 24 Sept. (commercial harvest time), 13 Oct., and 26 Oct. Fruits were transported to the University of Idaho Pomology Laboratory in Parma, Idaho for complete quality and maturity evaluations. Crops of the experimental trees were not harvested at commercial time. This allowed us to continue our fruit drop and fruit quality evaluations several weeks after commercial harvest time. Remaining fruit from each tree was harvested after completion of the experiment, and the total number of fruit per tree at the beginning of the experiment was calculated.

For quality evaluations, fruits were weighed and fruit color was visually ranked on a scale of 1 to 5, with 1 = 20% red, progressively to 5 = 100% red. Soluble solids concentration (SSC) was measured by temperature-compensated refractometer (Atago N1, Tokyo, Japan). Fruit firmness was measured with a Fruit Texture Analyzer (Guss, Strand, Western Cape, South Africa). Starch degradation pattern (SDP) of equatorial slices of each fruit was recorded by comparison with the SDP standard chart developed for apples (Bartram *et al*. 1993).

To evaluate the effect of AVG on fruit maturity in 1991, five apples from each tree of selected treatments were weighed and then placed in 20 × 28 × 28.5-cm closed chambers. The temperature of the chambers was maintained at 22.8°C. Air samples with a constant flow rate of 80 $mL \cdot min^{-1}$ were drawn from the ripening chambers every 24 h to measure concentrations of evolved ethylene and carbon dioxide (CO_2) by gas chromatography. Samples were injected onto a gas chromatograph (Hewlett Packard 5890 Series II, Lionville, PA) equipped with a flame ionization detector and a HayeSep Q, 80/100 packed column (Alltech Inc., Deerfield, IL).

In spring of 1993, three 1.5-m branches on each the 'Law Rome Beauty' trees from the 1992 experiment were tagged and the number of flower clusters were counted. Number of fruits on this braches was later counted in June 1993 and "return fruit set" was calculated as: percent fruit set = (fruit number/number of flower clusters) x 100.

Assumption of normality was checked by computing univariate analyses for all treatments of this study. All data, including percentages and rankings, were normally distributed. Data were analyzed by general linear model (GLM) and LSD Test ($P \leq 0.05$) using SAS (SAS Institute Inc., Cary, N.C.) software.

RESULTS AND DISCUSSION

At harvest time in 1991, fruit drop was not affected by AVG treatments in either apple cultivar (**Tables 1, 2**). Endogenous ethylene levels in the apple fruit and abscission layer increase as the fruit matures and reaches a climacteric peak at the ripening stage (Fallahi *et al* 1985a, 1985b; Bangerth 2000; Silvereman 2004). At harvest time, the level of endogenous ethylene in none of the treatments or control has been sufficient to form an abscission layer, and thus no significant differences were seen in fruit drop. In the later

Table 1 Effects of time of AVG application (pooled over all rates) on the percentage of 'Hi Early Delicious' fruits retained on the tree at harvest and after harvest in 1991[z].

Treatment (weeks before harvest)	Fruit % at harvest (2 Oct.)	Fruit % on 16 Oct.	Fruit % on 28 Oct.	Fruit % on 8 Nov.
Control	94.5 a	33.2 b	24.6 c	16.7 c
3 Weeks	92.1 a	54.9 a	47.9 a	45.8 a
5 Weeks	94.9 a	46.0 ab	39.1 ab	36.4 ab
9 Weeks	85.0 a	41.9 ab	33.1 cb	26.2 cb

[z] Mean separation within column by LSD at 0.05.

Table 2 Effects of time of AVG application (pooled over all rates) on the percentage of 'Law Rome Beauty' fruits retained on the tree at and after harvest in 1991[z].

Treatment (weeks before harvest)	Fruit % at harvest (4 Oct.)	Fruit % on 28 Oct.	Fruit % on 8 Nov.
Control	83.8 a	28.2 b	24.3 b
4 Weeks	86.7 a	46.5 a	43.7 a
6 Weeks	85.7 a	42.3 ab	37.4 ab
10 Weeks	88.0 a	39.5 ab	36.3 ab

[z] Mean separation within column by LSD at 0.05.

sampling dates, however, applications of AVG at 3 or 4 weeks before harvest significantly increased fruit retention (reduced fruit drop) in 'Hi-Early Delicious' and 'Law Rome Beauty' apples, respectively (**Tables 1, 2**). Application of AVG 9 weeks before harvest in 'Hi-Early Delicious' and 6 or 10 weeks before harvest in 'Law Rome Beauty' did not affect fruit drop as compared to untreated control (**Tables 1, 2**). Fruit ethylene production was reduced with application of AVG at each rate to 'Hi Early Delicious' at 3 or 5 weeks before harvest (**Figs. 1-3**) and at 75 ppm or 150 ppm to 'Law Rome Beauty' at 4 weeks before harvest (**Figs. 4-6**). However, earlier applications of AVG at 75 or 150 ppm did not affect fruit ethylene in these cultivars. Since AVG was applied at 3, 5, or 9 weeks before harvest to 'Hi Early Delicious' but 4, 6, and 10 weeks before harvest to 'Law Rome Beauty', it is difficult to compare the response of each rate and time of AVG application to ethylene evolution in these two cultivars. However, it is noteworthy that in 'Law Rome Beauty', AVG at 75 ppm suppressed ethylene when applied only at 4 weeks before harvest, but not at 6 or 10

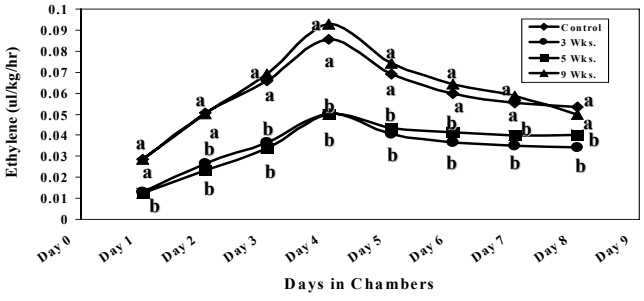

Fig. 1 The effect of 75 ppm AVG, applied at different periods prior to harvest, on ethylene evolution in 'Hi Early Delicious' apple. Mean separation within the same sampling date by LSD at 5%.

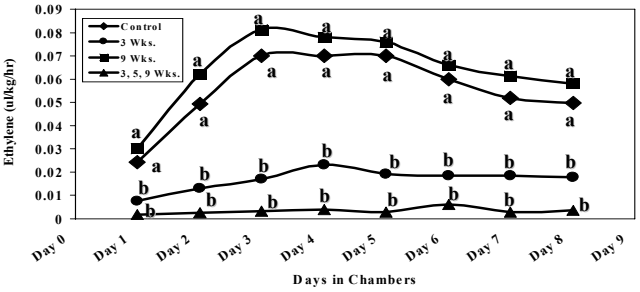

Fig. 2 The effect of 150 ppm AVG, applied at different periods prior to harvest, on ethylene evolution in 'Hi Early Delicious' apple. Mean separation within the same sampling date by LSD at 5%.

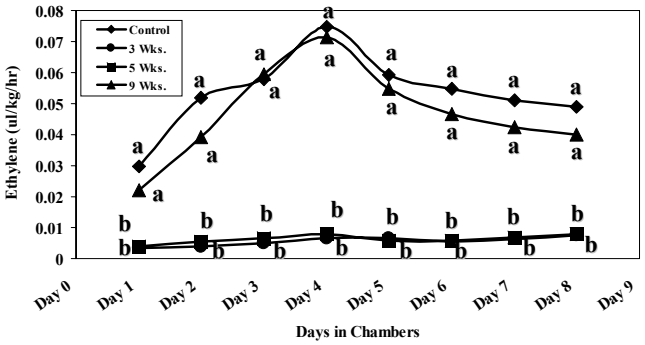

Fig. 3 The effect of 225 ppm AVG, applied at different periods prior to harvest, on ethylene evolution in 'Hi Early Delicious' apple. Mean separation within the same sampling date by LSD at 5%.

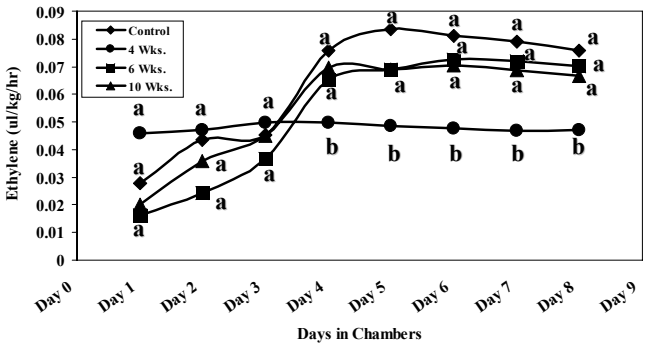

Fig. 4 The effect of 75 ppm AVG, applied at different periods prior to harvest, on ethylene evolution in 'Law Rome Beauty' apple. Mean separation within the same sampling date by LSD at 5%.

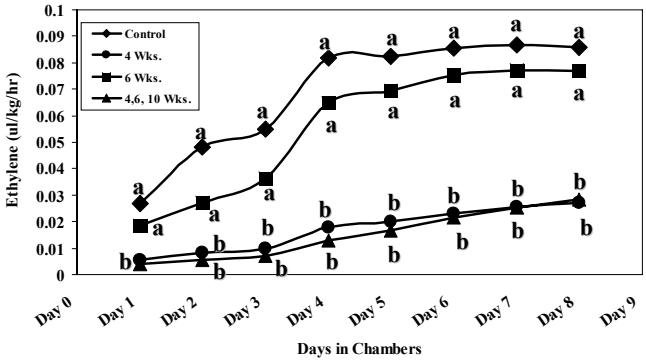

Fig. 5 The effect of 150 ppm AVG, applied at different periods prior to harvest, on ethylene evolution in 'Law Rome Beauty' apple. Mean separation within the same sampling date by LSD at 5%.

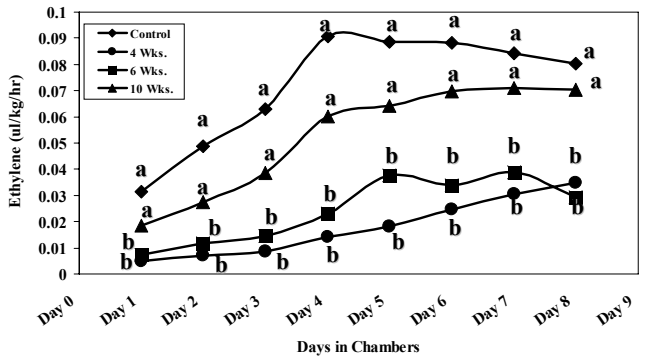

Fig. 6 The effect of 225 ppm AVG, applied at different periods prior to harvest, on ethylene evolution in 'Law Rome Beauty' apple. Mean separation within the same sampling date by LSD at 5%.

weeks before (**Fig. 4**). While, at 225 ppm rate, AVG suppressed ethylene when applied either at 4 or 6 weeks before harvest in 'Law Rome Beauty' (**Fig. 6**). In 'Hi Early Delicious', application of AVG at each rate reduced ethylene when applied either at 3 or 5 weeks before harvest (**Figs. 1-3**). Different cultivars may show different responses to AVG, perhaps because ACC synthase enzyme can react differently in different cultivars. Thus, the sensitivity of apple cultivars to this ethylene-inhibiting agent at comparable application rates and timings deserves further investigation. AVG inhibits 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, and thus blocks ethylene synthesis (Silverman *et al*. 2004). Since ethylene production in fruit tissue is low at early stages of fruit maturation, application of AVG at 9 to 10 weeks before the anticipated harvest time is too early to prevent ethylene production. The balance between various groups of hormones, particularly indole acetic acid (IAA) and endogenous ethylene also plays an important role in fruit senesce (Bangerth 2000). The lack of AVG response to fruit retention when applied 6 to 10 weeks before harvest in 'Law Rome Beauty' and 9 weeks before harvest in 'Hi Early Delicious' could be affected by the imbalance between IAA and endogenous ethylene. This speculation deserves further study.

Fruit respiration patterns in treatments were almost similar to those of ethylene evolution and thus not reported here. This study suggests that about 4 weeks before anticipated harvest is a desirable time for application of AVG.

In both 'Hi Early Delicious' and 'Law Rome Beauty' apples, fruit drop in over one month after commercial harvest time was reduced with all concentrations of AVG but three applications of AVG, each at 150 ppm, appeared to be more effective in retaining fruits on the trees in 1991 (**Tables 3, 4**).

In 1992, all AVG treatments reduced fruit drop and delayed fruit SDP and retained fruit firmness of the 'Law Rome Beauty' fruits that were sampled on 13 Oct. or 26 Oct. (19 or 32 days after commercial harvest time, respectively) (**Table 5**). Retention of fruit firmness in this study is in agreement with previous reports in other apple cultivars (Wang and Mellenthin 1977; Bangerth 1978; Williams 1980) and pears (Wang and Mellenthin 1977). This fruit firmness retention is caused by suppression of ethylene evolution as reported in previous studies (Fallahi *et al*.1985a, 1985b; Silverman *et al*. 2004). The mechanism of SDP reduction in the AVG-treated trees in this study was later studied in a similar experiment with apples by Silverman *et al*. (2004). They investigated the enzymatic basis of starch mobilization in apple fruit and reported that amylase activi-

Table 3 Effects of rates of AVG (pooled over all application times) on the percentage of 'Hi Early Delicious' fruits retained on the tree at and after harvest in 1991[z].

AVG Treatments (over all application times)	Fruit % at harvest (2 Oct.)	Fruit % on 16 Oct.	Fruit % on 28 Oct.	Fruit % on 8 Nov. 8
Control (0 ppm)	94.5 a	33.2 b	24.6 b	16.7 b
75 ppm	92.6 a	41.0 a	29.6 ab	22.6 b
150 ppm	90.4 a	44.2 a	41.0 a	36.9 a
150 ppm (3, 5, & 9 Wks)	85.8 a	48.9 a	43.3 a	40.1 a
225 ppm	91.9 a	45.6 a	37.6 a	34.2 a

[z] Mean separation within column by LSD at 0.05.

Table 4 Effects of rates of AVG application (pooled over all application times) on the percentage of 'Law Rome Beauty' fruits retained on the tree at harvest and after harvest in 1991[z].

Treatment (weeks before harvest)	Fruit % at Harvest (4 Oct.)	Fruit % on 28 Oct.	Fruit % on 8 Nov.
Control	83.8 a	28.2 c	24.3 c
75 ppm	88.2 a	42.0 b	38.8 b
150 ppm	81.0 a	39.1 b	35.1 b
150 ppm (3, 5, & 9 Wks)	90.8 a	59.3 a	57.2 a
225 ppm	88.4 a	35.7 b	31.8 b

[z] Mean separation within column by LSD at 0.05.

Table 5 Effects of time and rates of AVG and NAA on 'Law Rome Beauty' fruit retention, firmness, color, and starch pattern in 1992 and return fruit set in 1993.

Treatment and application	Fruit retained on trees (% total fruit)			Fruit firmness (N)			Fruit Color (1-5 scale)			Starch pattern (1-6)			1993 Return fruit set
	24 Sept.	13 Oct.	26 Oct.	24 Sept.	13 Oct.	26 Oct.	24 Sept.	13 Oct.	26 Oct.	24 Sept.	13 Oct.	26 Oct.	(fruits/100 buds)
Control	63.6 cd	20.2 d	7.5 d	89.3 b	61.2 b	55.9 c	4.11 ab	4.08 a	4.15 ab	3.82 a	5.33 a	5.81 a	93.9 a
AVG 25 ppm, 3 times	66.7 cd	36.2 c	15.2 c	96.4 a	79.6 a	68.2 b	3.73 ab	4.08 a	4.30 a	3.26 a	4.41 b	5.40 b	72.5 a
AVG 50 ppm, 3 times	69.6 bcd	44.9 c	22.5 c	98.1 a	81.4 a	77.4 a	3.36 bc	3.61 a	3.96 ab	3.25 a	4.49 b	5.08 b	69.6 a
AVG 100 ppm, 3 times	81.7 a	70.5 a	55.8 a	94.6 ab	85.8 a	82.3 a	3.17 c	3.65 b	4.11 ab	3.07 a	4.04 b	4.59 c	98.0 a
AVG 100 ppm, 1 time	74.4 abc	40.0 c	18.2 c	99.4 a	77.0 a	67.3 b	4.02 a	3.98 ab	3.88 ab	3.40 a	4.57 b	5.36 b	88.2 a
AVG 200 ppm, 1 time	79.8 ab	56.1 b	36.1 b	98.6 a	83.6 a	81.0 a	3.17 c	3.58 b	3.72 b	3.94 a	4.40 b	5.13 b	98.6 a
NAA 10 ppm 1 time	59.0 d	40.2 c	21.4 c	94.6 ab	60.3 b	47.5 cd	3.73 ab	3.81 ab	3.68 b	3.98 a	5.17 a	6.00 a	72.8 a

[z] Mean separation within column by LSD at 0.05. Fruit color rank: 1= least red color, 5 highest red color. Starch degradation pattern: 1=least starch degradation; 6= highest starch hydrolysis.

vity was somewhat reduced in AVG-treated fruit. Amylase activity was less in AVG-treated fruit during the early stages of starch mobilization. Starch phosphorylase activity increased dramatically during the later stages of starch mobilization, but was not affected by AVG treatment (Silverman *et al*. 2004). Soluble starch synthase activity was also unaffected by AVG treatment and remained constant throughout the eight-week harvest period. Moreover, AVG did not affect the levels of amylopectin, fructose, malate, ascorbate, citrate, or anthocyanin. Silverman *et al*. (2004) conclude that apple fruit ripening has both ethylene-dependent and -independent processes occurring simultaneously.

Three applications of AVG, each at 100 ppm, signifycantly reduced the number of 'Law Rome Beauty' fruit drop at second and third samplings as compared to all other treatments in 1992. There was no consistent effect of AVG treatments on fruit size (data not shown) or color (**Table 5**).

As compared to control, application of NAA at 10 ppm to 'Law Rome Beauty' trees reduced fruit drop on 13 Oct. and 26 Oct. but did not affect fruit quality attributes of any time in 1992 (**Table 5**).

Over all, application of AVG was more effective in reducing pre-harvest fruit drop (increasing fruit retention) and delaying fruit maturity as compared to untreated control or NAA at 10 ppm. Suppression of ethylene evolution, and/or delaying apple fruit maturity as a result of AVG application in this study was later confirmed by several researchers (Nazir *et al*. 1999; Zhiguo and Bramlage 2001; Drake *et al*. 2005; Moran 2006).

This finding has a major impact on both harvest and postharvest strategies in apples. By reducing pre-harvest fruit drop and delaying maturity, apple growers will have a longer time for harvest. This is particularly beneficial for growers who have large fruit operations and need longer time for harvest. They also can prolong the storage life of apple fruits treated with AVG and market them at a higher price.

In the recent years, researchers have focused on combing 1-methylcyclopropene (1-MCP) with AVG for maintaining firmness and preventing scald in different apple cultivars (Drake *et al*. 2005; Moran 2006). It has been reported that combination of 1-MCP with AVG was more effective in maintaining fruit firmness and reducing scald than the use of AVG alone in 'McIntosh' and 'Cortland' apples (Moran 2006). In that report, Moran (2006) suggested that AVG and 1-MCP can be used to maintain firmness of 'McIntosh' when internal ethylene concentration at harvest was as high as 240 $\mu L \cdot L^{-1}$, but control atmosphere storage life was limited to 4 months. Synergistic effects of AVG and 1-MCP deserves further study as the effects could vary with apple cultivars and environmental conditions.

CONCLUSIONS

Fruit retention, maturity and quality attributes measured in this study indicate that AVG delays fruit maturity in both 'Hi Early Delicious' and 'Law Rome Beauty' apples. This delay is caused by retarding ethylene evolution and respiration. Based on the results of this study, application of AVG at a concentration between 75 ppm and 150 ppm, 4 weeks before anticipated harvest, is essential for reducing pre-harvest drop and retaining fruit starch and firmness, and thus extending the postharvest fruit life in a medium to long-term storage.

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