Effect of Sprayable 1-MCP, AVG, and NAA on Ethylene Biosynthesis, Preharvest Fruit Drop, Fruit Maturity, and Quality of ‘Delicious’ Apples

Rongcai Yuan1 and Jianguo Li

Virginia Polytechnic Institute and State University, Alson H. Smith, Jr. Agricultural Research and Extension Center, 595 Laurel Grove Road, Winchester, VA 22602

Abstract. Effects of naphthaleneacetic acid (NAA), aminoethoxyvinylglycine (AVG), and sprayable 1-methylcyclopropene (1-MCP) alone or in combination on fruit ethylene production, preharvest fruit drop, fruit quality, and fruit maturation were examined in ‘Delicious’ apples (Malus ×domestica Borkh.). 1-MCP and AVG + NAA, when applied 15 days before anticipated harvest (DBAH) for untreated control trees, more effectively delayed preharvest fruit drop than AVG or NAA used alone. However, there was no significant difference in ethylene production between fruit treated with 1-MCP or AVG + NAA and those treated by AVG. Two applications of NAA increased fruit ethylene production and fruit softening, whereas AVG inhibited NAA-enhanced fruit ethylene production and fruit softening. There was no significant difference in fruit ethylene production, fruit firmness, and fruit drop control between one and two applications of 1-MCP. The concentrations of 1-MCP did not affect the efficacy of 1-MCP when applied 15 DBAH, but high concentration of 1-MCP more effectively delayed preharvest fruit drop than low concentration of 1-MCP when applied 7 DBAH. Both AVG and 1-MCP suppressed expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase gene MdACSI, ACC oxidase gene MdACO1, and polygalacturonase gene MdPG1 in fruit. Expression of AGS54 and MdACO1 but not MdACSI in fruit abscission zones was decreased by AVG and 1-MCP. 1-MCP more effectively suppressed expression of MdPG2 in fruit abscission zones than AVG alone.

‘Delicious’ is one of the most important apple (Malus ×domestica Borkh.) cultivars grown in the United States. Excessive preharvest apple fruit drop, which occurs before fruit develop optimum red color, maturity, or size, is one of its faults and usually causes a serious economic loss (Byers, 1997). Conversely, picking fruit before adequate maturity may lead to poor storability and poor fresh and processed fruit quality. Early harvest can also result in lower yields and prices because fruit will increase in weight 5% to 7% per week and price is based on larger fruit sizes (Byers and Eno, 2002). Ideally, plant growth regulators should hold fruit on the tree for an additional 3 weeks past optimum harvest date to improve fruit size, color, and crop value by as much as 20% while maintaining fruit quality (Byers and Eno, 2002).

Naphthaleneacetic acid (NAA), a synthetic auxin, and aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis are two compounds that commercial apple growers use to delay preharvest drop of apples since registrations of daminozide (Alar) and 2,4,5-TP were canceled in 1989 and 1986, respectively. One application of NAA may delay apple fruit drop for ∼10 to 14 d after treatment, and repeated applications of NAA delay fruit abscission more than single applications (Batjer and Moon, 1945; Marini et al., 1993). However, fruit softening is usually increased by two applications of NAA or warm weather after the first application (Smock and Gross, 1947; Yuan and Carbaugh, 2007). AVG suppresses fruit ethylene production, reduces preharvest fruit drop, and delays fruit ripening in apples (Auto and Bramlage, 1982; Bangerth, 1978; Greene, 2005; Schupp and Greene, 2004; Yuan and Carbaugh, 2007). Our preliminary results showed that the combination of NAA and AVG was more effective in delaying fruit drop than NAA and AVG alone in ‘Golden Supreme’ and ‘Golden Delicious’ apples while maintaining fruit quality (Yuan and Carbaugh, 2007).

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action, has been used to delay postharvest ripening of climacteric fruit such as apples (Blankenship and Dole, 2003). Recently, a sprayable formulation of 1-MCP became available for use in the field. Our preliminary results showed that sprayable 1-MCP more effectively delayed fruit drop than did AVG or NAA when they were applied 1 week before commercial harvest in ‘Golden Delicious’ apples (Yuan and Carbaugh, 2007). Elfving et al. (2007) reported that preharvest application of sprayable 1-MCP reduced ‘Delicious’ apple fruit ethylene production and slowed softening during and after storage, but they did not study its effect on preharvest fruit drop in ‘Delicious’ apples.

Apples are typical climacteric fruit characterized by a marked increase in ethylene production and respiration at ripening (Bianpied, 1972; Yuan and Carbaugh, 2007). It has been reported that concomitant with increased fruit ethylene production is increased expression of genes and activity of enzymes associated with cell wall degradation such as polygalacturonase (PG) (Bonghi et al., 2000; Brown, 1997). As a result, the middle lamellae of cells in fruit cortex and fruit abscission zone dissolves, and ultimately, the fruit becomes soft and abscises.

The purposes of this investigation were to: 1) study the effect of NAA and AVG alone or in combination on control of preharvest fruit drop and management of on-tree fruit quality in ‘Delicious’ apples; 2) evaluate the effect of concentration, timing, and frequency of application of sprayable 1-MCP on preharvest fruit drop and fruit quality in ‘Delicious’ apples; and 3) determine the effect of AVG and sprayable 1-MCP on the expression of genes related to ethylene biosynthesis and cell wall degradation in fruit cortex and fruit abscission zones.

Materials and Methods

Expt. 1: Delicious (2006)

Plant material and treatments. To determine the effects of AVG and NAA alone or in combination, and concentration, timing, and frequency of sprayable 1-MCP on preharvest fruit drop and fruit quality, 66 10-year-old ‘Bisbee Delicious’/Mark located at the Alson H. Smith, Jr. Agricultural Research and Extension Center, Winchester, VA, were selected and grouped into six blocks of 11 trees each in Sept. 2006. A randomized complete block design was used. AVG (ReTain; Valen BioSciences Corp., Libertyville, IL) at 125 mg L−1 and NAA (Fruitone N; AMVAC Corp., Newport Beach, CA) at 20 mg L−1 alone or in combination were applied on 6 Sept., 15 d before anticipated harvest (DBAH), or on 14 Sept., 7 DBAH. A sprayable formulation of 1-MCP (Rohn and Haas Company, Spring House, PA) at 160 or 320 mg L−1 was applied on 6 Sept., 15 DBAH, or on 14 Sept., 7 DBAH. All spray solutions contained surfactant Silwet L-77 (Loveland Products, Greeley, CO) at 0.624 mL L−1. Spray treatments were applied to runoff with a low-pressure hand-wand sprayer. One tree in each block was not sprayed and served as a control.

Determination of fruit abscission, fruit ethylene evolution, fruit maturity, and fruit quality: To determine fruit abscission rate, two limbs of ∼30 fruit each on each tree were tagged. Fruit on tagged limbs were counted on 6 Sept. 2006 before any fruit abscission, and then fruit remaining on tagged limbs were counted weekly thereafter until 1 Nov.

Received for publication 8 Apr. 2008. Accepted for publication 15 May 2008.

1To whom reprint requests should be addressed; e-mail yuan@vt.edu

HortScience 43(5):1454–1460. 2008.
Six fruit were collected from each tree at 1- to 4-week intervals beginning on 1 Aug. and ending on 26 Oct. 2006. Fruit were enclosed in a 3.785-L container and incubated for 3 h. One milliliter of gas sample was withdrawn from the sealed container through the rubber septum affixed to the lid, and ethylene concentration was measured with a gas chromatograph equipped with an activated alumina column and an FID detector (model 3700; Varian, Palo Alto, CA) (Yuan and Carbaugh, 2007). To determine fruit maturity and quality, 10 fruit were sampled weekly from each tree beginning on 21 Sept. and ending on 26 Oct. 2006. Fruit red color was estimated visually on each fruit to the nearest 10%. Fruit firmness was measured on two sides of each fruit with an Effegi penetrometer (model FT 327; McCormick Fruit Tech, Yakima, WA) with an 11.1-mm tip. Soluble solids concentration (SSC) was measured with an Atago handheld refractometer (model N1; McCormick Fruit Tech) using a composite sample of juice resulting from penetrometer testing of 10 fruit from each treatment in each replicate. Each apple fruit was cut in half transversely, fruit with water core were counted, and then flesh starchy was evaluated by dipping half of each apple in iodine solution for ~15 s. The degree of staining was rated on a scale of 1 to 8, in which 1 = staining of the entire cut surface and 8 = absence of staining (Poapst et al., 1959). No phytotoxicity or other negative effects on trees were observed.

**Total RNA extraction and real-time quantitative polymerase chain reaction.** Forty fruit were collected from each tree, treated by sprayable 1-MCP at 0 or 160 mg L⁻¹ and AVG at 125 mg L⁻¹ on 6 Sept., 15 DBAH, of three blocks on 28 Sept. (all compounds were effectively inhibiting fruit abscission). The fruit were immediately separated into cortex and fruit abscission zone. Fruit abscission zones were collected by cutting 1 mm at each side of the abscission fracture plane. For fruit cortex, 10 fruit were randomly selected from the 40 fruit of each tree, peeled, cored, and sliced. Harvested tissues were immediately frozen in liquid nitrogen and stored at ~80 °C for extraction of RNA. Total RNA was extracted from fruit abscission zones and fruit cortex as described by Wan and Wilkins (1994). DNA was removed from each RNA sample using the TURBO DNA-free™ Kit (Ambion Inc., Austin, TX). Real-time polymerase chain reaction was performed using primers that span an intron in 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene MdACO1 to confirm that each RNA sample was free of genomic DNA contamination according to Dal Cin et al. (2005).

Two micrograms of total RNA was used to synthesize cDNA in a 20-µL reaction volume using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time quantitative polymerase chain reaction (PCR) was performed using the Power SYBR Green PCR Master Mix Kit (Applied Biosystems) on Applied Biosystems 7500 Real-Time PCR Systems (Applied Biosystems) according to the manufacturer’s instructions. Gene-specific primers were designed on nonconserved areas using Primer Expression 3.0 software (Applied Biosystems) synthesized by Integrated DNA Technologies (Coralville, IA); the primer sequences are listed in Table 1. Real-time samples were run in triplicate and the reaction volumes were 25 µL. Dissociation curves were run to determine the specificity of the amplification reactions. In addition, the amplified products by real-time quantitative PCR were sequenced as described by Dal Cin et al. (2005). After validation tests, normalization to actin was performed using the ΔΔCT method described by Applied Biosystems (“Guide to Performing Relative Quantitation of Gene Expression Using Real-time Quantitative PCR”; Applied Biosystems).

**Expt. 2: Delicious (2007)**

Fifty-eight 11-year-old ‘Bisbee Delicious’/Mark located at the Alson H. Smith, Jr. Agricultural Research and Extension Center, Winchester, VA, were selected and placed into six blocks of eight trees each in Aug. 2007. A randomized complete block design was used. AVG at 125 mg L⁻¹ and NAA at 10 mg L⁻¹ were applied on 22 Aug., 28 DBAH, and 5 Sept., 14 DBAH, respectively. The combination of AVG at 125 mg L⁻¹ and NAA at 10 mg L⁻¹ were applied on 5 Sept., 14 DBAH. Sprayable 1-MCP at 80 or 160 mg L⁻¹ was applied on 5 Sept., 14 DBAH, or on 12 Sept., 7 DBAH, respectively. All spray solutions contained surfactant Silwet L-77 at 0.624 mL L⁻¹. Spray treatments were applied to runoff with a low-pressure hand-wand sprayer. One tree in each block was not sprayed and served as a control.

Fruit on two tagged limbs of each tree were counted on 5 Sept. 2007 before any fruit abscission, and then fruit remaining on tagged limbs were counted weekly thereafter until 16 Oct. Six fruit were collected from each tree on 19 Sept. and 3 Oct. to determine fruit ethylene production. Fruit ethylene production was measured as described previously. Ten fruit were sampled from each tree on 19 Sept. and 3 Oct. for fruit quality evaluations (fruit firmness, SSC, starch staining, and fruit red color). Fruit red color, firmness, and SSC were determined as described by Yuan et al. (2006). Fruit ethylene production was determined using a headspace gas chromatographic method as described by Yuan et al. (2006). Fruit firmness was measured on two sides of each fruit using a handheld penetrometer (model FT 327; McCormick Fruit Tech). Ethylene production was measured at harvest and at 15, 30, 45, 60, 75, and 90 min after harvest using a low-pressure hand-wand sprayer. One tree in each block was not sprayed and served as a control.

**Table 1. Gene-specific primers used for expression analysis of genes.**

| Gene Accession no. | Primer left | Primer right |
|-------------------|-------------|--------------|
| MdActin CN938023 | 5'-TGACCGAATGACAGGAAATTACT-3' | 5'-TACCTCAAGCTGTTGGCATTCCACATC-3' |
| MdACS1 L13147 | 5'-GCCTTGGTCTTCCTTGATG-3' | 5'-GCGGGGACACCCATTGTA-3' |
| MdACO1 AB034992 | 5'-GCCTTGGTCTTTGGATG-3' | 5'-TTGCAAGCTGTTGGCATTCCACATC-3' |
| MdPGA1 AB030589 | 5'-GACCTGCTGACAGGAAATTACT-3' | 5'-GCTTGGGTTTTCAGGCCAGCAG-3' |
| MdPG2 L27743 | 5'-TGACCGAATGACAGGAAATTACT-3' | 5'-ACCGTGAAGCAGGGAACCATGTA-3' |
| MdPG2 AB210897 | 5'-CGGTCAGCGCCAGAAGGTTG-3' | 5'-TACGAGTGGAGGAGGATGATCG-3' |

**Table 2. Effects of sprayable 1-MCP, AVG, and NAA on preharvest fruit drop of Bisbee Delicious apples (2006).**

| Treatment | Application time (DBAH) | Rate (mg L⁻¹) | 6 Sept. | 14 Sept. | 28 Sept. | 4 Oct. | 18 Oct. | 26 Oct. | 1 Nov. |
|-----------|-------------------------|--------------|--------|---------|---------|-------|--------|--------|-------|
| Control   | —                       | 0.0 a        | 3.8 a  | 19.7 a  | 27.8 a  | 43.4 a| 50.7 a  | 63.6 a  | 72.9 a |
| 1-MCP 15 and 7 160 | 0.0 a        | 2.7 a        | 5.3 c  | 8.6 de | 8.6 e   | 12.1 c| 14.8 d  | 26.9 d  | 64.6 b |
| 1-MCP 15 and 7 320 | 0.0 a        | 2.8 a        | 5.7 c  | 11.7 de| 15.2 c  | 21.5 c| 28.2 c  | 54.6 b  | 64.6 b |
| AVG 15 and 7 125 | 0.0 a        | 3.1 a        | 10.2 b | 13.4 c  | 23.6 b  | 27.0 b| 28.9 b  | 31.0 b  | 31.8 c |
| AVG 15 and 7 160 | 0.0 a        | 1.9 a        | 3.4 c  | 4.7 e   | 8.9 e   | 12.1 c| 20.4 c  | 34.1 b  | 68.6 b |
| AVG 15 and 7 320 | 0.0 a        | 0.8 a        | 10.5 b | 15.9 c  | 21.6 b  | 25.8 b| 32.8 b  | 33.7 b  | 45.0 b |
| AVG 15 and 7 160 | 0.0 a        | 0.7 a        | 2.7 b  | 21.5 b  | 27.8 b  | 33.6 b| 37.1 b  | 52.4 b  | 52.4 b |
| AVG 15 and 7 320 | 0.0 a        | 1.7 a        | 8.0 b  | 8.9 d   | 12.1 d  | 13.2 c| 22.4 c  | 23.7 c  | 51.7 b |
| AVG 15 and 7 160 | 0.0 a        | 0.0 a        | 6.4 bc | 14.6 c  | 21.2 b  | 23.2 b| 28.4 b  | 29.2 b  | 33.8 c |
| AVG 15 and 7 320 | 0.0 a        | 2.1 a        | 6.5 b  | 9.4 d   | 16.0 c  | 24.5 b| 35.4 b  | 40.5 b  | 53.3 b |
| AVG 15 and 7 160 | 0.0 a        | 1.1 a        | 5.7 c  | 6.7 d   | 11.0 d  | 13.1 c| 18.4 c  | 18.4 d  | 23.0 d |

aDays before anticipated harvest date, which was 21 Sept. 2006.

bMean separation within columns by Duncan’s multiple range test (P = 0.05).

1-MCP = 1-methylcyclopropene; AVG = aminooxyacetic acid; NAA = naphthaleneacetic acid.
SSC, and flesh starch were measured as described previously.

Statistical analyses. Statistical analyses included analysis of variance, Duncan’s multiple range test, and regression analysis using Statistical Analysis Systems Software for PC (SAS Inst., Cary, NC).

Results

Expt. 1

Delicious (2006). Preharvest fruit drop was reduced by 1-MCP, AVG, and NAA regardless of concentration, timing, and frequency of applications (Table 2). There was no difference in fruit drop control between NAA and AVG except on 28 Sept. and 4 Oct. when two applications of NAA more effectively reduced fruit drop than did AVG. There was no difference in fruit drop control between one and two applications of AVG either. AVG + NAA was more effective in the control of fruit drop than AVG or NAA alone. Overall, 1-MCP more effectively reduced fruit drop than either AVG or NAA alone until 26 Oct. except for 1-MCP at 160 mg L⁻¹ applied 7 DBAH, but there was no difference in fruit drop control between 1-MCP and AVG + NAA except for 1-MCP at 160 mg L⁻¹ applied 7 DBAH. 1-MCP and AVG were applied on 6 Sept., 15 d before anticipated harvest (DBAH), and NAA was applied on 6 Sept., 15 DBAH, and 14 Sept., 7 DBAH. There was also no difference in fruit drop control between one and two applications of 1-MCP except for 1-MCP at 160 mg L⁻¹ applied 7 DBAH, which had less effect in delaying fruit drop than other 1-MCP treatments.

Table 3. Effects of sprayable 1-MCP, AVG, and NAA on fruit ethylene production in Bisbee Delicious apples (2006).

| Treatment | Application time (DBAH) | Rate (mg L⁻¹) | Fruit ethylene production (µL kg⁻¹ h⁻¹ FW) |
|-----------|-------------------------|---------------|------------------------------------------|
| Control   | —                       | —             | 0.001 a 0.001 a 1.438 b 3.922 b 4.736 b 4.336 b 6.467 b 7.881 a |
| 1-MCP     | 15 and 7 160            | 0.001 a 0.001 a 0.018 c 0.047 c 0.132 c 0.859 c 7.433 b 7.926 a |
| AVG       | 15 and 7 320            | 0.001 a 0.001 a 0.017 c 0.099 c 0.125 c 1.571 c 6.878 b 7.803 a |
| NAA       | 15 and 7 20             | 0.001 a 0.001 a 0.021 c 0.093 c 0.076 c 0.023 d 0.035 e 0.055 c |

Fig. 1. Real-time quantitative polymerase chain reaction analysis of expression of *MdACS1*, *MdACS5A*, and *MdACO1* in fruit cortex (A, C, and E) and fruit abscission zones (B, D, and F) from ‘Bisbee Delicious’ apple trees treated with 1-methylcyclopropene (1-MCP) at 160 mg L⁻¹, aminoethoxyvinylglycine (AVG) at 125 mg L⁻¹, and naphthaleneacetic acid (NAA) at 20 mg L⁻¹. 1-MCP and AVG were applied on 6 Sept., 15 d before anticipated harvest (DBAH), and NAA was applied on 6 Sept., 15 DBAH, and 14 Sept., 7 DBAH. The mRNA levels of *MdACS1*, *MdACS5A*, and *MdACO1* were normalized using actin. Data are means ± SE (n = 3). Mean separation by Duncan’s multiple range test (P = 0.05). The values of *MdACS1*, *MdACS5A*, and *MdACO1* in fruit cortex and fruit abscission zones of untreated control ‘Delicious’ apples were arbitrarily set to 100%.
the onset of fruit ethylene climacteric. Concentration, timing, and frequency of applications of 1-MCP and AVG did not affect the efficacy of 1-MCP and AVG in inhibiting fruit ethylene production. Addition of AVG reduced fruit ethylene production stimulated by NAA. However, the effect of 1-MCP on fruit ethylene production decreased by 11 October and stopped by 18 October, whereas AVG alone or AVG + NAA inhibited fruit ethylene production through 26 October.

The expression of ACC synthase gene *MdACS1* in fruit cortex was suppressed by 1-MCP at 160 mg L⁻¹ and AVG on 28 September when all compounds were effectively inhibiting fruit abscission (Fig. 1A). However, *MdACS1* expression in fruit abscission zones was unaffected by either 1-MCP at 160 mg L⁻¹ or AVG applied 15 DBAH (Fig. 1B). 1-MCP and AVG had a slight or no effect on the expression of *MdACS5A* gene in fruit cortex (Fig. 1C). On the other hand, 1-MCP and AVG inhibited the expression of *MdACS5A* in fruit abscission zones (Fig. 1D). The expression of *MdACO1* gene in both fruit cortex and fruit abscission zones was reduced by 1-MCP and AVG (Fig. 1E–F). The expression of polygalacturonase genes *MdPG1* and *MdPG2* in both fruit cortex and fruit abscission zones was reduced by 1-MCP and AVG (Fig. 2A–D).

Overall, fruit weight increased linearly with time regardless of treatment (Table 4). Fruit weight increased 14.5% from 21 Sept. to 11 Oct. Fruit red color quadratically increased with time but was unaffected by 1-MCP, AVG, or NAA (Table 5). Fruit from trees treated with NAA were softer compared with fruit from control trees, whereas 1-MCP, AVG, or AVG + NAA slowed softening (Table 6). The efficacy of AVG and 1-MCP in slowing softening was not influenced by concentration, timing, and number of 1-MCP and AVG applications until 11 October. NAA alone significantly increased SSC except on 21 Sept. and 11 Oct., whereas only two applications of 1-MCP at 160 mg L⁻¹ reduced SSC on 21 Sept. (Table 7). Starch degradation as indicated by starch rating was increased by NAA but decreased by 1-MCP and AVG (Table 8). AVG reduced NAA-caused starch degradation. Water core was reduced by AVG and 1-MCP but increased by NAA on 4 Oct. (Table 9).

**Expt. 2**

*Bisbee Delicious (2007).* NAA, 1-MCP, AVG, and AVG + NAA delayed preharvest fruit

The values of *MdPG1* and *MdPG2* in fruit cortex and fruit abscission zones of untreated control ‘Delicious’ apples were arbitrarily set to 100%.

Fig. 2. Real-time quantitative polymerase chain reaction analysis of expression of *MdPG1* and *MdPG2* in fruit cortex (A and C) and fruit abscission zones (B and D) from ‘Bisbee Delicious’ apple trees treated with 1-methylcyclopropene (1-MCP) at 160 mg L⁻¹, aminoethoxyvinylglycine (AVG) at 125 mg L⁻¹, and naphthaleneacetic acid (NAA) at 20 mg L⁻¹. 1-MCP and AVG were applied on 6 Sept., 15 DBAH, and NAA was applied on 6 Sept., 15 DBAH, and 14 Sept., 7 DBAH. The mRNA levels of *MdPG1* and *MdPG2* were normalized using actin. Data are means ± se (n = 3).  *a* Mean separation by Duncan’s multiple range test (P = 0.05).

### Treatment

| Treatment | Application time (DBAH) | Rate (mg L⁻¹) | 21 Sept. | 28 Sept. | 4 Oct. | 11 Oct. | 18 Oct. | 26 Oct. | Significance |
|-----------|-------------------------|---------------|----------|----------|--------|--------|--------|--------|-------------|
| Control   | —                       | —             | 199.7    | 204.7    | 209.7  | 217.6  | 229.1  | 228.9  | L**Q***    |
| 1-MCP     | 15 and 7                | 160           | 194.3    | 205.2    | 206.5  | 228.2  | 238.9  | 246.1  |             |
| 1-MCP     | 15 and 7                | 320           | 206.0    | 215.3    | 225.2  | 242.2  | 243.1  | 257.5  |             |
| AVG       | 15 and 7                | 125           | 199.0    | 204.1    | 213.9  | 233.5  | 242.5  | 228.4  |             |
| 1-MCP     | 15                      | 160           | 193.9    | 207.3    | 208.6  | 217.8  | 226.7  | 232.1  |             |
| AVG       | 15                      | 125           | 202.1    | 205.8    | 216.4  | 231.6  | 242.5  | 241.1  |             |
| 1-MCP     | 7                       | 160           | 198.1    | 203.6    | 204.5  | 227.8  | 243.8  | 236.9  |             |
| AVG       | 7                       | 320           | 201.6    | 215.7    | 208.7  | 229.4  | 241.6  | 239.8  |             |
| NAA       | 15 and 7                | 20            | 201.9    | 209.0    | 220.5  | 243.3  | 262.4  | 244.7  |             |
| AVG + NAA | 15                      | 125           | 192.5    | 196.8    | 202.8  | 217.4  | 225.0  | 216.2  |             |
| NAA       | 15 and 7                | 20            | 199.8    | 206.8    | 211.9  | 228.8  | 241.2  | 239.2  |             |

Days before anticipated harvest date, which was 21 Sept. 2006.

Mean separation within columns by Duncan’s multiple range test (P = 0.05).

Non-significant or significant at P = 0.001, respectively.

1-MCP = 1-methylcyclopropene; AVG = aminoethoxyvinylglycine; NAA = naphthaleneacetic acid.
Table 7. Effects of sprayable 1-MCP, AVG, and NAA on soluble solids of Bisbee Delicious apples (2006).

| Treatment | Application time (DBAH) | Rate (mg L⁻¹) | Soluble solids (%) |
|-----------|-------------------------|---------------|--------------------|
| Control   | —                       | —             | 12.7 abc           |
| 1-MCP     | 15 and 7 160            | 12.0 d        | 13.0 bcde          |
| AVG       | 15 and 7 125            | 12.9 ab       | 13.3 bcd           |
| 1-MCP     | 15 160                  | 12.8 abc      | 13.0 bcde          |
| 1-MCP     | 7 320                   | 12.4 bcd      | 13.0 cde           |
| NAA       | 15 and 7 20             | 13.2 a        | 13.0 bcde          |
| AVG + NAA | 15 125                  | 12.8 abc      | 13.0 bcde          |

Days before anticipated harvest date, which was 21 Sept. 2006.

The concentration of 1-MCP did not affect the efficacy of 1-MCP in reducing preharvest fruit drop, but 1-MCP at 160 mg L⁻¹ more effectively reduced preharvest fruit drop than at 80 mg L⁻¹ when applied 7 DBAH. Fruit ethylene production was inhibited by AVG or 1-MCP, but one application of NAA at 10 mg L⁻¹ had no significant effect on fruit ethylene production (Table 11). 1-MCP, AVG, or AVG + NAA slowed softening, whereas NAA increased it. Starch degradation was delayed by AVG or 1-MCP. Only 1-MCP at 160 mg L⁻¹ applied 7 DBAH slightly reduced fruit red color on 3 Oct. AVG + NAA and 1-MCP, except 1-MCP at 80 mg L⁻¹ applied 14 DBAH, reduced fruit SSC.

Discussion

Our results showed that AVG + NAA more effectively delayed preharvest fruit drop than AVG or NAA alone in ‘Bisbee Delicious’ apples although there was no difference in fruit ethylene production between AVG and AVG + NAA, suggesting that there is an additive effect between NAA and AVG in controlling fruit abscission. These results are in agreement with our previous reports in ‘Golden Supreme’ and ‘Golden Delicious’ apples (Yuan and Carbaugh, 2007). Fruit ethylene production and fruit drop were unaffected by one and two applications of AVG, which is consistent with previous report in ‘Golden Supreme’ apples (Yuan and Carbaugh, 2007). Two applications of NAA reduced fruit abscission although it enhanced fruit ethylene production and fruit softening. This indicates that NAA reduces preharvest fruit drop not through reducing ethylene production and plays a different role in fruit abscission and fruit softening. Similarly, NAA, but not AVG, reduced preharvest fruit drop caused by ethephon (Byers, 1997). Addition of AVG delayed NAA-enhanced fruit softening and starch degradation but did not affect fruit red color. This has been attributed to the action of AVG in inhibiting NAA-stimulated ethylene production (Yuan and Carbaugh, 2007). Therefore, the use of AVG + NAA to delay preharvest fruit drop while maintaining fruit quality may provide apple growers with flexibility to harvest their apples with high quality.

1-MCP counteracts ethylene action through blocking ethylene receptors (Blankenship and Dole, 2003). In this study, sprayable 1-MCP effectively inhibited fruit ethylene production, delayed softening, and more effectively delayed preharvest fruit drop than AVG or NAA alone in ‘Bisbee Delicious’ apples. This is consistent with previous reports in ‘Golden Delicious’ apples (McArtney et al., 2008; Yuan and Carbaugh, 2007). There was no difference in fruit ethylene production, fruit firmness, and fruit drop control between one and two applications of 1-MCP, indicating that the frequency of application of 1-MCP does not affect the effectiveness of 1-MCP. The concentration of 1-MCP did not affect the efficacy of 1-MCP when applied 15 DBAH, but the high concentration of 1-MCP more effectively delayed preharvest fruit drop than a low concentration of 1-MCP when applied 7 DBAH. A low concentration of 1-MCP applied at 7 DBAH did not affect preharvest fruit drop in the first 7 d after treatment. These suggest that more ethylene receptors are produced in apple fruit and higher concentration of...
Table 8. Effects of sprayable 1-MCP, AVG, and NAA on starch of Bisbee Delicious apples (2006).

| Treatment        | Application time (DBAH) | Rate (mg L\(^{-1}\)) | 21 Sept. | 28 Sept. | 4 Oct. | 11 Oct. | 18 Oct. | 26 Oct. |
|------------------|-------------------------|-----------------------|----------|----------|--------|---------|---------|---------|
| Control          | —                       | —                     | 4.3 d    | 4.6 c    | 5.4 d  | 6.0 c   | 6.5 d   | 7.6 a   |
| 1-MCP            | 15 and 7                | 160                   | 4.3 d    | 4.6 c    | 5.4 d  | 6.0 c   | 6.5 d   | 7.6 a   |
| AVG              | 15 and 7                | 125                   | 4.3 d    | 4.6 c    | 5.4 d  | 6.0 c   | 6.5 d   | 7.6 a   |
| 1-MCP            | 15 and 25               | 170                   | 4.6 c    | 4.9 d    | 5.6 d  | 6.2 d   | 6.7 d   | 7.8 a   |
| AVG              | 15 and 25               | 130                   | 4.6 c    | 4.9 d    | 5.6 d  | 6.2 d   | 6.7 d   | 7.8 a   |
| NAA              | 15 and 7                | 160                   | 4.9 d    | 5.5 d    | 6.0 d  | 6.5 d   | 6.9 d   | 7.8 a   |

*Days before anticipated harvest date, which was on 21 Sept. 2006.

Table 9. Effects of sprayable 1-MCP, AVG, and NAA on water core of Bisbee Delicious apples (2006).

| Treatment        | Application time (DBAH) | Rate (mg L\(^{-1}\)) | 21 Sept. | 28 Sept. | 4 Oct. | 11 Oct. | 18 Oct. | 26 Oct. |
|------------------|-------------------------|-----------------------|----------|----------|--------|---------|---------|---------|
| Control          | —                       | —                     | 0.0 b    | 0.0 c    | 0.0 d  | 0.0 e   | 0.0 f   | 0.0 g   |
| 1-MCP            | 15 and 7                | 160                   | 0.0 b    | 0.0 c    | 0.0 d  | 0.0 e   | 0.0 f   | 0.0 g   |
| AVG              | 15 and 7                | 125                   | 0.0 b    | 0.0 c    | 0.0 d  | 0.0 e   | 0.0 f   | 0.0 g   |
| 1-MCP            | 15 and 25               | 170                   | 0.0 b    | 0.0 c    | 0.0 d  | 0.0 e   | 0.0 f   | 0.0 g   |
| AVG              | 15 and 25               | 130                   | 0.0 b    | 0.0 c    | 0.0 d  | 0.0 e   | 0.0 f   | 0.0 g   |
| NAA              | 15 and 7                | 160                   | 0.0 b    | 0.0 c    | 0.0 d  | 0.0 e   | 0.0 f   | 0.0 g   |

*Days before anticipated harvest date, which was on 21 Sept. 2006.

Table 10. Effects of sprayable 1-MCP, AVG, and NAA on preharvest fruit drop of Bisbee Delicious apples (2007).

| Treatment        | Application time (DBAH) | Rate (mg L\(^{-1}\)) | 5 Sept. | 12 Sept. | 19 Sept. | 25 Sept. | 2 Oct. | 9 Oct. | 16 Oct. |
|------------------|-------------------------|-----------------------|---------|----------|-----------|-----------|--------|-------|---------|
| Control          | —                       | —                     | 0.0 a   | 0.3 b    | 0.8 a    | 1.6 a    | 2.5 a  | 3.2 a  | 4.0 a   |
| 1-MCP            | 14 and 7                | 80                    | 0.0 a   | 0.2 a    | 0.8 a    | 1.6 a    | 2.5 a  | 3.2 a  | 4.0 a   |
| AVG              | 14 and 7                | 160                   | 0.0 a   | 0.2 a    | 0.8 a    | 1.6 a    | 2.5 a  | 3.2 a  | 4.0 a   |
| 1-MCP            | 7 and 16                | 80                    | 0.0 a   | 0.2 a    | 0.8 a    | 1.6 a    | 2.5 a  | 3.2 a  | 4.0 a   |
| AVG              | 7 and 16                | 160                   | 0.0 a   | 0.2 a    | 0.8 a    | 1.6 a    | 2.5 a  | 3.2 a  | 4.0 a   |
| NAA              | 14 and 7                | 80                    | 0.0 a   | 0.2 a    | 0.8 a    | 1.6 a    | 2.5 a  | 3.2 a  | 4.0 a   |

*Commercial harvest date was on 19 Sept. 2007.

Table 11. Effects of 1-MCP, AVG at 125 mg L\(^{-1}\), and NAA at 20 mg L\(^{-1}\) on fruit quality and maturity in Bisbee Delicious apples* (year 2007).

| Treatment        | Application time (DBAH) | Rate (mg L\(^{-1}\)) | 21 Sept. | 28 Sept. | 4 Oct. | 11 Oct. | 18 Oct. | 26 Oct. |
|------------------|-------------------------|---------------------|----------|----------|--------|---------|---------|---------|
| Control          | —                       | —                   | 17.993 a | 17.993 a | 17.993 a | 17.993 a | 17.993 a | 17.993 a |
| 1-MCP            | 14 and 7                | 80                   | 3.074 b  | 7.34 bc  | 7.0 a  | 2.8 c   | 5.5 e   | 2.8 f   |
| AVG              | 14 and 7                | 160                  | 3.074 b  | 7.34 bc  | 7.0 a  | 2.8 c   | 5.5 e   | 2.8 f   |
| 1-MCP            | 7 and 16                | 80                   | 2.634 b  | 7.30 c   | 6.90 a | 3.8 bcd | 5.8 cde | 3.8 cde |
| AVG              | 7 and 16                | 160                  | 2.634 b  | 7.30 c   | 6.90 a | 3.8 bcd | 5.8 cde | 3.8 cde |
| NAA              | 14 and 7                | 10                   | 0.05 a   | 11.504 a | 72.1 c | 62.3 c  | 4.7 a   | 7.1 a   |
| AVG              | 14 and 125              | 0.000 b              | 0.446 c  | 75.2 ab  | 68.5 a | 3.3 def | 5.7 de  | 3.3 def |
| NAA              | 14 and 125              | 0.000 b              | 0.446 c  | 75.2 ab  | 68.5 a | 3.3 def | 5.7 de  | 3.3 def |

*Commercial harvest date was on 19 Sept. 2007.

1-MCP are required to block ethylene receptors at 7 DBAH than 15 DBAH. Our results also showed that the effect of 1-MCP on fruit ethylene production, fruit firmness, and fruit drop sustained ~5 weeks in ‘Bisbee Delicious’ apples, which is similar to previous report in ‘Golden Delicious’ apples (Yuan and Carbaugh, 2007). Therefore, to effectively delay preharvest fruit drop, maintain fruit quality, and save the cost for 1-MCP, 1-MCP should be applied 15 DBAH instead of 7 DBAH in ‘Bisbee Delicious’ apples. It has been reported that the conversions of S-adenosyl methionine to 1-aminoacyclopropane-1-carboxylate and ACC to ethylene are the rate-limiting steps in ethylene biosynthesis and catalyzed by ACC synthase and ACC oxidase, respectively (Alexander and Grierson, 2002). In this study, AVG or 1-MCP only inhibited the expression of MdACS1 and MdACO1 but not MdACS5A in fruit cortex. These results indicate that MdACS1 and MdACO1 in fruit cortex are associated with climacteric ethylene production in apple fruit. In the fruit abscission zones, both AVG and 1-MCP inhibited the expression of MdACS5A and MdACO1 but not MdACS1. This suggests that both AVG and 1-MCP inhibit apple fruit drop by inhibiting expression of MdACS5A and MdACO1. On the other hand, NAA increases apple fruit ethylene production by increasing expression of MdACS1 and MdACO1 in fruit cortex (Li and Yuan, 2008). AVG and 1-MCP also decreased expression of MdPG1 and MdPG2 in fruit cortex. Our previous results showed that NAA increased expression of MdPG1 but decreased expression of MdPG2 in fruit cortex (Li and Yuan, 2008). These indicate that MdPG1 but not MdPG2 in fruit cortex is related to fruit softening. Both AVG and 1-MCP also inhibited expression of MdPG1 and MdPG2 in fruit abscission zones. However, our previous results showed that expression of MdPG1 in fruit abscission zones was effectively suppressed by 1-MCP and NAA although 1-MCP and NAA had no more effect on fruit ethylene production and fruit abscission (Li and Yuan, 2008). These results suggest that expression of MdPG2 but not MdPG1 in fruit abscission zones is related to fruit softening. In addition, 1-MCP more effectively inhibited expression of MdPG2 in
fruit abscission zones than did AVG. This may be related to the stronger inhibitory effect of 1-MCP on fruit abscission than when AVG was used alone in ‘Bisbee Delicious’ apples. Similarly, the expression of \textit{MdPG2} in fruit abscission zones was more effectively inhibited by NAA + AVG than by AVG or NAA alone (Li and Yuan, 2008). This has been attributed to the stronger inhibitory effect of NAA + AVG on fruit abscission than when AVG or NAA was used alone in ‘Bisbee Delicious’ apples (Li and Yuan, 2008).

In conclusion, our results showed that 1-MCP or NAA + AVG was more effective than NAA or AVG alone in reducing preharvest fruit drop and extending the harvest season while maintaining fruit quality. 1-MCP applied 15 DBAH more effectively delayed preharvest fruit drop than when applied 7 DBAH in ‘Delicious’ apples. 1-MCP more effectively inhibited expression of \textit{MdPG2} in fruit abscission zones than AVG alone.

\textbf{Literature Cited}

Alexander, L. and D. Grierson. 2002. Ethylene biosynthesis and action in tomato: A model for climacteric fruit ripening. J. Expt. Bot. 53: 2039–2055.

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.

Bangerth, F. 1978. The effect of a substituted amino acid on ethylene biosynthesis, ripening, and preharvest drop of apple fruits. J. Amer. Soc. Hort. Sci. 103:401–404.

Batjer, L.P. and H.H. Moon. 1945. Effect of naphthaleneacetic acid sprays on maturity of apples. Proc. Amer. Soc. Hort. Sci. 46:113–117.

Blankenship, S.M. and J.M. Dole. 2003. 1-Methylcyclopropene: A review. Postharv. Biol. Technol. 28:1–25.

Blanpied, G.D. 1972. A study of ethylene in apple, red raspberry, and cherry. Plant Physiol. 49: 627–630.

Bonghi, C., P. Tonutti, and A. Ramina. 2000. Biochemical and molecular aspects of fruitlet abscission. Plant Growth Regulat. 31:35–42.

Brown, K.M. 1997. Ethylene and abscission. Physiol. Plant. 100:567–576.

Byers, R.E. 1997. Effects of aminoethoxyvinylglycine (AVG) on preharvest fruit drop and maturity of ‘Delicious’ apples. J. Tree Fruit Production 2:53–75.

Byers, R.E. and D.R. Eno. 2002. Harvest date influences fruit size and yield of ‘York’ and ‘Golden Delicious’ apple trees. J. Tree Fruit Production 3:63–79.

Dal-Cin, V., M. Danesin, A. Boschetti, A. Dorigoni, and A. Ramina. 2005. Ethylene biosynthesis and perception in apple fruitlet abscission (\textit{Malus domestica} L. Borck). J. Expt. Bot. 56:2995–3005.

Elfving, D.C., S.R. Drake, A.N. Reed, and D.B. Visser. 2007. Preharvest applications of sprayable 1-methylcyclopropene in the orchard for management of apple harvest and postharvest condition. HortScience 42:1192–1199.

Greene, D.W. 2005. Time of aminoethoxyvinylglycine application influences preharvest drop and fruit quality of ‘McIntosh’ apples. HortScience 40:2056–2060.

Li, J. and R. Yuan. 2008. NAA and ethylene regulate expression of genes related to ethylene biosynthesis, perception and cell wall degradation during fruit abscission and ripening in ‘Delicious’ apples. J. Plant Growth Regul. (in press).

Marini, R.P., R.E. Byers, and D.L. Sowers. 1993. Repeated applications of NAA control preharvest drop of ‘Delicious’ apples. J. Hort. Sci. 68:247–253.

McArtney, S.J., J.D. Obermiller, J.R. Schupp, M.L. Parker, and T.B. Edington. 2008. Preharvest 1-methylcyclopropene delays fruit maturity and reduces softening and superficial scald of apples during long-term storage. HortScience 43:366–371.

Poapst, P.A., G.M. Ward, and W.R. Philips. 1959. Maturation of McIntosh apples in relation to starch loss and abscission. Can. J. Plant Sci. 39:257–263.

Schupp, J.R. and D.W. Greene. 2004. Effect of aminoethoxyvinylglycine (AVG) on preharvest drop, fruit quality, and maturation of ‘McIntosh’ apples. 1. Concentration and timing of dilute applications of AVG. HortScience 39:1030–1035.

Smock, R.M. and C.R. Gross. 1947. The effect of some hormone materials on the respiration and softening rates of apples. Proc. Amer. Soc. Hort. Sci. 49:67–77.

Wan, C.Y. and T.A. Wilkins. 1994. A modified hot borate method significantly enhances the yield of high-quality RNA from cotton (\textit{Gossypium hirsutum} L.). Analytical Bioch 223:7–12.

Yuan, R. and D.H. Carbaugh. 2007. Effects of NAA, AVG, and 1-MCP on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of ‘Golden Supreme’ and ‘Golden Delicious’ apples. HortScience 42:101–105.