Aminoethoxyvinylglycine, Combined with Ethephon, Can Enhance Red Color Development without Over-ripening Apples

Zhenyong Wang1 and David R. Dilley2
Department of Horticulture, Michigan State University, East Lansing, MI 48824-1325

Additional index words: ethylene, ethylene climacteric, fruit maturity, CA storage, flesh firmness, shelf-life, ReTain™, Malus ×domestica

Abstract. AVG, as ReTain™, an inhibitor of ethylene biosynthesis, was used alone or with a subsequent application of ethephon (Ethrel™), an ethylene-releasing chemical, to determine if red color development could be enhanced without over-ripening ‘Gala’ and ‘Jonagold’ apples. Treatments included: 1) AVG alone; 2) AVG followed by ethephon; 3) ethephon alone; and 4) control. Silwet L-77 surfactant was included in all treatments. Application of AVG delayed the onset of the ethylene climacteric and red color development of both cultivars. Application of AVG followed by ethephon similarly delayed the onset of the ethylene climacteric, but red color development at the commercial harvest date was only marginally reduced or not affected. The results were similar in both 1998 and 1999, although environmental stress during the growing seasons differed (1998—heat; 1999—moderate temperatures). The delay of fruit maturation and ripening observed at harvest following AVG +/- ethephon treatments improved storability of fruit in controlled atmosphere (CA) storage, as demonstrated by low internal ethylene levels after storage, and high retention of flesh firmness and shelf-life, while control fruit and those treated only with ethephon entered the ethylene climacteric during storage, and flesh firmness subsequently declined during shelf-life evaluation. Chemical name used: aminoethoxyvinylglycine (AVG).

Multiple harvests are often necessary to achieve maximum yield of well-colored, high-quality apples of cultivars, such as ‘Gala’ and ‘Jonagold’, adding to the cost of production. Red color development can be improved and preharvest fruit abscission delayed by applying naphthalenecacetic acid (NAA), but this may stimulate ethylene production and ripening. Ethephon, an ethylene-releasing chemical, can be used to improve red color development, but it also stimulates ripening, and fruit so treated have a very limited storage life. Earlier studies (Murphy and Dilley, 1988) with 2-chloroethyl-methylbis-(phenylmethoxy)silane (CGA 15281), another ethylene-releasing chemical, indicated that anthercyanin biosynthesis in apples could be enhanced without inducing other ripening changes. Marketed as ReTain™, AVG, an inhibitor of ethylene production, delays fruit abscission and development of watercore (Shafer et al., 1995), but also delays red color development and loss of chlorophyll (Bangerth, 1978; Wang and Dilley, 1998). This color development delay deters growers from using ReTain™ as a harvest management tool in spite of its other positive attributes. This investigation was conducted to test the hypothesis that red color development of AVG-treated apples may be enhanced by ethephon without overly stimulating other ripening processes. This could permit a once-over harvest (with attendant reduction in harvesting costs) of fruit with improved storability, although fruit matura-

Fruit samples from each treatment (30 to 40 fruit) were randomly harvested from five to eight trees of each cultivar in each block at 3- to 4-d intervals beginning 2 weeks prior to the estimated commercial harvest date for the control. Samples of fruit of each treatment at the commercial harvest time in 1999, including 10 replicates of ‘Gala’ and six replicates of ‘Jonagold’, were held separately under controlled-atmosphere (CA) storage, using a ventilated CA system under 1.5% O2 + 3% CO2 at 1 °C to evaluate fruit storability (CA storage studies were not conducted in 1998). The atmospheres in this CA system were generated with an air separator (Permea™, model 5; Permea, St. Louis) and distributed through a capillary flow board system for maintaining controlled atmospheres in a dynamic flow-through mode. Quality parameters evaluated at each harvest and after 1 week at 20 °C included: internal ethylene [gas chromatography (Hach Carle Series 100 AGC, Loveland, Colo.) with alumina column and flame ionization detector]; development of red color (percentage of red) and ground color index (1 = green and 5 = yellow); flesh firmness [Effegi penetrometer (Effegi, Alfonzine, Italy) with 11-mm tip]; starch index (1 = high and 9 = low) and Brix value.

Materials and Methods

Plant materials. Our experiments were conducted in 1998 and 1999 with ‘Gala’ and ‘Jonagold’ apples at the Michigan State Univ. Clarksville Horticultural Experiment Station (CHES), based on results from a preliminary study conducted in 1997 (Wang and Dilley, 1998). The trees were planted in four main blocks in 1990 and managed as in commercial practice. Various combinations of rootstock/
(percentage of soluble solids) [hand refractometer (Atago ATC-1E; Cole-Parmer Instrument, Japan)] using 10 fruit per replicate. Fruit (20 per replicate) were examined for internal ethylene concentrations and quality parameters upon removal from storage and after 7 d in air at 20 °C. Internal ethylene concentration is an indirect measurement of ethylene production rate for apple (Burg and Burg, 1965). Since the fruit from each treatment were held separately during ventilated CA storage, this procedure provided a valid assessment of treatment effects on the development of the ethylene climacteric.

**Statistical analysis.** The data were analyzed based on a randomized block design. Each block consisted of 172 trees of three to five rootstocks and three training systems. Analysis of variance (ANOVA) was used to determine the significance of treatment differences by ANOVA at \( P \leq 0.05 \). Standard errors were calculated based upon three to five replicates of 10 fruit each.

**Results**

*Studies on ‘Gala’ in 1998 and 1999.* In 1998, the onset of the ethylene climacteric for ‘Gala’ apples from ethephon-only and control treatments occurred on 3 Sept., whereas it was greatly inhibited by the AVG and AVG + ethephon treatments, as measured at harvest and after holding fruit in air at 20 °C for 7 d (Fig. 1A and C). In 1999, AVG treatment, with or without a follow-up application of ethephon, clearly delayed the onset of the ethylene climacteric (Fig. 1E and G). No significant differences were observed between the ethephon-treated and control fruit, which had entered the climacteric by 30 Aug., whereas a slowly-developing climacteric commenced 10 d later in the AVG +/- ethephon-treated fruit (Fig. 1E and G), as in 1998.

In 1998, red color development was significantly delayed by treatment with AVG alone, and treatment with ethephon at 212 g·ha\(^{-1}\) did not fully overcome the AVG effect, even by the time of commercial harvest (14 Sept.) (Fig. 1B). In 1999, AVG applied alone delayed red color development relative to control and ethephon-only treatments, while AVG + ethephon-treated fruit were intermediate in color development, which was slightly improved relative to that of fruit treated with AVG alone (Fig. 1F and Table 1). By 9 Sept., red color development was between 73% to 81% for fruit from all treatments. Color development continued for fruit held in the dark at 20 °C for 7 d, but no treatment differences were significant (data not shown).

In 1998, flesh firmness of AVG +/- ethephon-treated fruit after 7 d at 20 °C was higher at the later harvest dates than that of fruit from all other treatments (Fig. 1D). At the commercial harvest date (14 Sept.), AVG +/- ethephon-treated fruit were 10 to 12 N firmer than ethephon-only or control fruit (Fig. 1D and Table 1). Starch conversion was delayed by 1 week in the AVG +/- ethephon-treated ‘Gala’ fruit (data not shown). The increase in Brix values reflected the trends observed for the decline in starch index (data not shown). In 1999, flesh firmness at harvest was similar regardless of treatment (data not shown). However, after holding the fruit in air at 20 °C for 7 d, AVG + ethephon-treated fruit was 4.5 N firmer than that of ethephon-only fruit and 3.7 N firmer than control fruit (Fig. 1H). Although the differences were not large, they were significant, confirming similar results in 1998. Starch conversion at commercial harvest was clearly delayed in fruit from the AVG +/- ethephon treatments relative to control and ethephon-only fruit (data not shown), and this delay persisted during 7 d in air at 20 °C, as judged by Brix values. After holding the fruit for 7 d at 20 °C, Brix values were inversely related to starch index. Brix values for AVG +/- ethephon fruit were 0.8% lower than those of control and ethephon-only fruit harvested on 25 Aug. (data not shown). The differences among treatments decreased as harvest was delayed.

*Studies on ‘Jonagold’ in 1998 and 1999.* In 1998, the onset of the ethylene climacteric of ‘Jonagold’ was strongly inhibited by treatment with AVG +/- ethephon (Fig. 2, A and C); these fruit were still preclimacteric on the day of commercial harvest (28 Sept.) as judged by low ethylene levels after 7 d at 20 °C. Control fruit and those treated only with ethephon entered the ethylene climacteric by 24 Sept. In 1999, the onset of the ethylene climacteric of ‘Jonagold’ fruit treated with AVG +/- ethephon was again significantly delayed at harvest (Fig. 2E) and this delay persisted after holding them in air at 20 °C for 7 d (Fig. 2G). Ethephon stimulated the onset of the ethylene climacteric prior to the first harvest on 10 Sept. 1999. Climacteric onset was evident in control fruit harvested on 20 Sept.

In 1998, red or yellow color (data not shown) development had not been affected by the treatments by the time of commercial harvest on 28 Sept. (Fig. 2B). Red color gradually increased from ≈20% to 40% from 14 to 28 Sept. In 1999, treatment with AVG alone delayed, whereas treatment with ethephon alone at 282 g·ha\(^{-1}\) enhanced, red color development (Fig. 2F and Table 1). More importantly, ethephon applied to AVG-treated fruit stimulated red color development (Fig. 2F) without inducing the ethylene climacteric (Fig. 2E and G). Fruit treated with AVG + ethephon were nearly as well-colored as the fruit from the ethylene-only treatment when harvested on 20 Sept., and were better colored than fruit treated with AVG only (Fig. 2F and Table 1).

In 1998, flesh firmness of AVG +/- ethephon-treated fruit was 3–5 N greater than that of ethephon-only or control fruit after holding them for 7 d at 20 °C after the commercial harvest date; firmness gradually decreased from ≈75 to 65 N over the harvest period (Fig. 2D). Starch conversion was similar for fruit from all treatments at the later harvest dates (data not shown). In 1999, no significant differences between the control and AVG + ethephon treatments were evident in the other maturity-linked development parameters measured, including firmness (Fig. 2H), and the values of these parameters generally fell between those for the ethephon-only and the AVG-only treatments (data not shown). This was most notable for Brix values (data not shown), which can be largely explained by ethephon stimulation of starch conversion and the retardation of this process by AVG as a direct consequence of enhancing or delaying the ethylene-induced stimulation of respiratory metabolism.

*Studies on ‘Gala’ and ‘Jonagold’ after CA storage in 1999.* After 6 months of CA storage plus 7 d at 20 °C, the ethylene production of ‘Gala’ fruit harvested on 9 Sept. (commercial harvest date), whether treated with ethephon or not, was still significantly inhibited by AVG treatments (Table 2). This effect was retained for ‘Gala’ fruit during the shelf-life period at 20 °C. These effects reflected differences between treatments noted for ‘Gala’ fruit measured at harvest. After 6 months of CA storage and 7 d at 20 °C, as at harvest time, red color of ‘Gala’ was significantly greater with AVG + ethephon than for AVG alone, but less than that for the control and for ethephon only. After 6 months of CA storage and 7 d at 20 °C, retention of flesh firmness of ‘Gala’ apples treated with AVG +/- ethephon was greater than that of control fruit or those treated with ethephon alone (Table 2). Retention of flesh firmness during storage was improved significantly by AVG treatment.

For ‘Jonagold’, after 6 months of CA storage and 7 d at 20 °C, ethylene production was still inhibited by AVG treatments (Table 2), however, red color was almost as well developed in AVG + ethephon-treated fruit as in control fruit, while apples treated with AVG alone had fair red color development (Table 2). Treatment with ethephon alone enhanced fruit softening (Table 2).

**Discussion**

In two consecutive years, AVG applied alone delayed the onset of the ethylene climacteric and red color development of ‘Gala’ and ‘Jonagold’ apples, confirming other observations (Bangerth, 1978; Shafer et al., 1995; Wang and Dilley, 1998). Application of AVG followed by ethephon delayed the onset of the ethylene climacteric, and red color development at commercial harvest was significantly improved in comparison with that of fruit treated with AVG alone using a higher ethephon rate (282 g·ha\(^{-1}\)) as was done in 1999. This indirectly confirms the results of Bangerth (1978) who observed that AVG prevented autocatalytic ethylene production initiated by NAA or 2,4,5-trichlorophenoxyacetic acid (2,4,5-TP) if applied alone. Both NAA and 2,4,5-TP are known to promote red color development, but may stimulate ethylene production and ripening, whereas the ethylene-releasing compound CGA 15281 enhanced red color development without stimulating ripening (Murphy and Dilley, 1988). This was attributed to more rapid ethylene evolution from CGA 15281 than from ethephon at low fruit pH values. As expected, ethephon applied alone advanced maturity and stimu-
Fig. 1. Effects of AVG and/or ethephon on internal ethylene concentrations (A, C, E, G), red color (B, F), and flesh firmness (D, H) of ‘Gala’ apple in 1998 (A, B, C, D) and in 1999 (E, F, G, H) at harvest (A, B, E, F) and after an additional 7 d at 20 °C (C, D, G, H). Rate of ethephon applied (a.i.) was 212 g·ha⁻¹ in 1998 and 282 g·ha⁻¹ in 1999. AVG was used at 124 g·ha⁻¹. The bars indicate SE for data points. Note that scale for internal ethylene differs among graphs.

Fig. 2. Effects of AVG and/or ethephon on internal ethylene concentrations (A, C, E, G), red color (B, F), and flesh firmness (D, H) of ‘Jonagold’ apple in 1998 (A, B, C, D) and in 1999 (E, F, G, H) at harvest (A, B, E, F) and after an additional 7 d at 20 °C (C, D, G, H). Chemical treatments are as in legend to Fig. 1. The bars indicate SE for data points.

Table 1. The effect of AVG (ReTain™) on internal ethylene, red color, and flesh firmness of ‘Gala’ and ‘Jonagold’ apples at harvest (0 d) and after 7 d at 20 °C (7 d).

| Cultivar | Treatment | 0 d | 7 d | 0 d | 7 d |
|----------|-----------|-----|-----|-----|-----|
|          | Internal ethylene (µL·L⁻¹) | Red color (%) | Flesh firmness (N) | Internal ethylene (µL·L⁻¹) | Red color (%) | Flesh firmness (N) |
| Gala     | AVG       | 1.45 a    | 75.8 a | 78.5 b | 1.18 a    | 80.6 a | 83.0 c |
|          | AVG+Eth   | 1.28 b    | 63.40 b | 71.0 a | 0.93 a    | 45.7 a | 60.1 b |
|          | Ethephon  | 3.12 c    | 76.90 b | 69.0 a | 2.94 c    | 106.00 b | 80.8 c |
|          | Control   | 8.25 b    | 43.20 b | 65.4 ab | 2.21 b    | 104.00 b | 76.2 ab |

1998

| Gala     | AVG       | 0.27 ab   | 42.5 a | 68.5 b | 0.17 a | 31.8 a | 73.1 b |
|          | AVG+Eth   | 0.19 a    | 41.2 b | 73.3 b | 0.18 a | 28.50 b | 49.3 b |
|          | Ethephon  | 0.68 c    | 48.3 a | 63.7 a | 0.86 b | 81.10 b | 67.0 a |
|          | Control   | 0.52 bc   | 42.3 a | 65.4 ab | 0.63 ab | 57.30 b | 71.1 ab |

1999

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The concentration of AVG was 124 g·ha⁻¹ and the concentrations of ethephon were 212 g·ha⁻¹ in 1998, and 282 g·ha⁻¹ in 1999.

1Mean separation within columns, cultivars, and years by ANOVA at P ≤ 0.05.

lated ethylene production, ripening, and red color development each year. Similar results were obtained with ‘Jonagold’. Results with AVG and AVG + ethephon treatments for both cultivars in 1998 and 1999 were more dramatic than in 1997 (Wang and Dilley, 1998) presumably because the trees experienced drought stress in 1997, whereas they were irrigated in 1998. The 1999 growing season was “typical” with ample rainfall and moderate temperatures.

The results from 1999 experiment largely confirm those obtained in 1998, as well as in 1997 (Wang and Dilley, 1998), when an experiment was conducted in a similar manner in the same research plot. The experimental design for these experiments included at least three different rootstocks for each cultivar, plus different training systems and tree spacings for each replication. Despite this variability among trees, our results have been similar in most respects in three very different growing seasons. Based on our 1999 data, the ethephon usage rate was an important factor in improving red color development. This indicates that predictable responses can be obtained using a combination of these plant
growth regulators to improve red color development while not overly stimulating fruit ripening.

The fruit maturity/ripening-related effects of the treatments were reflected in the storability of ‘Gala’ and ‘Jonagold’ fruit held in CA storage in 1999 (Table 2), where the action of ethylene on ripening can be attenuated. Fruit treated with AVG, with or without ethephon, were still at preclimacteric ethylene levels after 6 months in CA storage and remained so after holding in air at 20 °C for 7 d, and flesh firmness and shelf-life were still excellent, especially for ‘Gala’. Ethephon-only and control fruit had higher ethylene levels and softened during CA storage and shelf-life evaluation. These results confirmed our earlier observations (Wang and Dilley, 1998).

Collectively, our results show that AVG treatment at the commercially recommended time according to the label for ReTain™ (≈4 to 6 weeks before normal harvest) and rate of application (124 g·ha⁻¹), followed by an ethephon application at 282 g·ha⁻¹ ≈2 weeks later, may improve fruit firmness and slow maturation without reducing red color development, whereas using AVG alone inhibits color development. Delaying the timing of the ethylene climacteric by AVG (Bangerth, 1978; Boller et al., 1979; Bufler, 1984, 1986; Halder-Doll and Bangerth, 1987) allows the least mature fruit to continue to mature while delaying that of the more mature fruit. We attribute the promotion of red color development of AVG + ethephon-treated fruit to the action of ethylene in stimulating anthocyanin biosynthesis. Once initiated by ethylene, the anthocyanin synthesis pathway appears to remain engaged, while the ethylene released from ethephon rapidly dissipates. This transient ethylene was apparently sufficient in some cases to promote anthocyanin biosynthesis without initiating autocatalytic ethylene production. This suggests that ethylene action may elicit expression of specific genes by affecting different ethylene receptors in a concentration- and time-regulated manner (Lashbrook et al., 1999), or by gene-specific promoters (Kneissl and Deikman, 1999).

Improved storability of ‘Gala’ fruit treated in 1999 with AVG alone and AVG + ethephon over that of the ethephon-only and control fruit is attributed to the same ethylene-related phenomena. Similar results were obtained with ‘Gala’ in 1997 (Wang and Dilley, 1998) and ‘Jonagold’ in 1999. Our results suggest that, when used in combination with ethephon, the benefits of AVG as a tool to manage maturation and ripening of apples can be achieved without significantly sacrificing red color development and storability in CA storage. Moreover, the combined use of these plant growth regulating substances would favor a once-over harvest of well-colored, high-quality fruit, reducing harvest labor input; this would help offset chemical costs. Commercial trials employing a staggered AVG/ethephon application schedule have yielded similar results with several cultivars (A. Swindeman, Sela Heights, Wash., personal communication).

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