Fruit quality in 'Pink Lady' apple, especially colour, as affected by preharvest sprays of aminoethoxyvinylglycine and ethephon

S.K. Whale[a], Zora Singh[a]*, M.H. Behboudian[b], J. Janes[a] and S.S. Dhaliwal[c]

[a]*Horticulture, Muresk Institute, Division of Resources and Environment, Curtin University of Technology, GPO Box U1987, Perth 6845, Australia and* [c]*School of Public Health, Division of Health Sciences, Curtin University of Technology, GPO Box U1987, Perth 6845, Australia*

[b]*Current address: Hort Science Group, Institute of Natural Resources (INR 433), Massey University, Palmerston North, New Zealand*

*Corresponding author. Tel.:+61-8-92663138; Fax: +61-8-92663063

Email address: Z.Singh@curtin.edu.au

Abstract

'Pink Lady' apple grown in Western Australia often develops poor colour at commercial harvest resulting in losses. To determine if fruit colour could be improved without advancing ripening, it was sprayed with aminoethoxyvinylglycine (AVG) alone, ethephon alone, or AVG followed by ethephon. The experiments were conducted at two different locations in Western Australia in 2002 and 2003. Fruit sprayed with AVG alone had retarded colour development at harvest. However, ethephon applied after AVG enhanced percent red blush, anthocyanin concentration and reduced chlorophyll concentration in the fruit skin in both locations. These fruit had similar colour to those treated with ethephon alone. Internal ethylene concentration and fruit firmness were unaffected by the different treatments in 2002.

However, in 2003 AVG with or without ethephon reduced internal ethylene concentration and maintained firmness compared to ethephon alone. In conclusion, AVG treatment alone delayed colour development and ripening of 'Pink Lady', while AVG application five weeks before harvest followed by an ethephon application two weeks later enhanced red colour at commercial harvest. This is therefore, an effective tool for improving colour of 'Pink Lady' apples at commercial harvest without adversely affecting other fruit quality attributes.

*Keywords:*fruit colour, storage, anthocyanins, AVG, ethyelene, apple, flavonoids and phenolic compounds

1. Introduction

Apple, in terms of tonnage, is the most important fruit crop in Western Australia (WA) and is grown in Donnybrook, Manjimup and the Perth Hills. Most of the apple produced in WA is exported, with 'Pink Lady' being the primary variety exported to Europe and South East Asian countries. 'Pink Lady', a WA bred variety, is highly popular because of its distinctive pink blush on a pale green background, crisp texture, and high sugar-acid ratio (Mackay et al., 1994). However, erratic and poor colour development is a serious problem in various countries including Australia. During 2003, poor colour development resulted in a 30% decline in the export of 'Pink Lady'. Poor colour was attributed to higher temperature prior to harvest (Department of Agriculture Western Australia, 2003) .

Some of the measures taken to improve fruit colour in apple include application of chemicals such as cultar and ethephon (Saure, 1990), overhead sprinkler irrigation

(Iglesias et al., 2002), use of reflective mulches (Layne et al., 2002) and fruit bagging (Fan and Mattheis, 1998).

Ethephon (2-chloroethylphosphonic acid) is widely reported to enhance colour in apple (Blanpied et al., 1975; Jones, 1979; Larrigaudiere et al., 1996). The mode of action of ethephon is through its property of releasing ethylene (Saure, 1990). However, ethephon has been reported to stimulate and advance ripening and reduce the storage potential of the fruit (Wang and Dilley, 2001; Stover et al., 2003).

Aminoethoxyvinylglycine (AVG) is an amino acid that inhibits ethylene production by inhibiting the activity of ACC (1-aminocylcopropane-1-carboxylate) synthase, a rate-limiting enzyme in the ethylene biosynthetic pathway (Yang and Hoffman, 1984). AVG also delays fruit ripening (Bangerth, 1978). This in turn delays colour development and fruit softening, a property that is useful for long-term cold storage of apples. The ability of AVG to retard ripening and maintain fruit firmness at harvest and after storage has been observed in different apple cultivars such as 'McIntosh', 'Spartan' and 'Spencer' (Bramlage et al., 1980), 'Gala' and 'Jonagold' (Wang and Dilley, 2001), and 'McIntosh' (Stover et al., 2003). Recently, Phan-Thien et al. (2004) reported delayed ripening of 'Gala' and 'Pink Lady' with AVG treatments under Australian conditions.

One of the recent approaches for improving apple colour has been combining AVG and ethephon. This involves treating fruit with a preharvest application of AVG followed by ethephon a few weeks later (Wang and Dilley, 2001; Stover et al., 2003). Wang and Dilley (2001) reported that in 'Gala' and 'Jonagold', a combination spray of AVG followed by ethephon delayed ethylene climacteric at commercial harvest. However, red colour development was not inhibited. The treated fruit had better

quality after controlled atmosphere (CA) storage compared to control fruit and fruit treated with ethephon alone. Similarly, Stover et al. (2003) reported enhanced colour development in 'McIntosh' apples treated with AVG followed by ethephon compared to AVG treatment alone.

The effects of preharvest spray of AVG and AVG in combination with ethephon on colour development and fruit quality at harvest are yet to be investigated in 'Pink Lady' grown in Australia. The objectives of this study were to investigate the effects of preharvest applications of AVG alone and AVG in combination with ethephon on colour development, internal ethylene concentration, changes in pigment concentrations in fruit skin, and fruit quality at commercial harvest.

2. Materials and Methods

2.1. Plant materials

Trials were conducted in Western Australia on 'Pink Lady' apple [Malus sylvestris (L.) Mill. var. domestica (Borkh.) Mansf.] at Mullalyup (lat. 33º45´S; long. 115º57´E) in 2002 and at Carmel (Perth Hills, lat. 31º57´S; long. 115º50´E) in 2003.

2.2. Experiment 1: 2002

In Mullalyup, the trial was based at Fruit Projects Australia. Seven-year-old apple trees grafted on MM 104 and planted in the north-south direction maintaining row distances of 5 m and plant distances of 2.5 m were used.

The treatments included: (a) unsprayed control, (b) aqueous solution containing active ingredient (a.i.) ethephon (280 g·ha^{-1}) applied on 27 March 2002, which was five weeks before harvest (149 DAFB), (c) aqueous solution containing a.i. AVG (124.5 g·ha^{-1}) applied on 27 March 2002 and, (d) AVG (124.5 g·ha^{-1}) sprayed five

weeks before harvest (149 DAFB) followed by ethephon (280 g·ha^{-1}) 18 days later on 15 April 2002 (167 DAFB). All preharvest sprays contained 0.1% v/v Nufarm Freeway Gold Penetrant (1020g·L^{-1} polyether modified polysiloxane). The chemical solutions were sprayed using a knapsack sprayer (Jacto X-16, nozzle-JD-12P) at the rate of 1000 L·ha^{-1} till run off. Ethrel® 48 (a.i. 480 g·L^{-1} ethephon) was used as the ethephon source and ReTain™ (a.i. 15% w/w AVG) was used as the AVG source. The experiment had a randomised complete block design with a single tree representing an experimental unit and replicated four times. Full bloom for the 2001–02 growing season occurred on 30 October 2001.

2.3. Experiment 2: 2003

This trial was conducted at a commercial orchard (A. Giumelli and Sons) at Carmel in Perth Hills. Six-year-old apple trees grafted on MM 109 and planted in the east-west direction maintaining row distances of 5 m and plant distances of 2.5 m were selected for the trial.

Treatments in 2003 were similar to those in 2002. AVG and ethephon alone were sprayed approximately five weeks before harvest (155 DAFB) on 24 March 2003. The follow up ethephon spray for the AVG followed by ethephon treatment, was applied two weeks later on 7 April 2003 (169 DAFB). Unsprayed trees served as control. Full bloom for the 2002–03 season occurred on 20 October 2002. The experimental design was a randomised complete block design with a single tree representing an experimental unit and replicated three times.

In both trials, uniform trees free from pests and diseases were selected and all the experimental trees received similar cultural practices except the experimental treatments. During the trials, orchard temperatures at Mullalyup and Carmel were

recorded using a data logger (Tinytag*Plus* Gemini Data Logger, U.K.). Temperature data were obtained using Gemini Logger Manager Software (Version 2.8) and are presented in Fig. 1. Average day temperature was calculated between sunrise and sunset, while average night temperatures were calculated between sunset and sunrise. Sunset and sunrise times during the experimental period were obtained from (Geoscience Australia, 2005).

2.4. Harvesting and fruit quality analysis

In both locations, at commercial harvest, 182 DAFB at Mullalyup (29 April 2002) and 189 DAFB at Carmel (26 April 2003), mature apples were harvested at random from all parts of the tree canopy from each experimental tree. In addition, in 2003, five fruit per tree were randomly harvested immediately after application of the treatments (zero days) and at intervals of three to 10 days until commercial harvest for monitoring changes in colour, internal ethylene concentration (IEC), respiration, and fruit firmness. At commercial harvest fruit were evaluated for colour development; respiration rate; firmness; and concentrations of internal ethylene, soluble solids, titratable acidity (TA), different pigments, and flavonoids.

2.5. Fruit colour

Percent blush on fruit surface was assessed visually on individual fruit (20 fruit per replicate) and scores were given from 0% to 100%. Zero percent represented no red blush while 100% represented fully red apple. For 'Pink Lady', the colour requirement for export markets are set at not less than 40% bright pink blush on a cream-pale green background (Department of Agriculture Western Australia, 2000). Fruit colour was also measured on the fruit surface using a HunterLab ColorFlex 45°/0° Spectrophotometer (HunterLab ColorFlex, Hunter Associates Inc, Reston,

Virginia, U.S.A.) as L*, a* and b*. The data were expressed as Commission Internationale de L'Eclairage (CIE) lightness (L*), chroma (C*) and hue angle (h°).

2.6. Respiration rate

Respiration rate was measured as CO_2 production by the fruit using the method described by (Lalel et al., 2003). In summary, fruit were placed in airtight sealed jars fitted with rubber septums for collecting the headspace gas and 2 mL gas samples were injected into an Infra Red Gas Analyser (Servomex Gas Analyser, Analyser series 1450 Food Package Analyser, Servomex Ltd, East Sussex, England)..

2.7. Internal ethylene concentration (IEC)

Internal ethylene concentration was measured by drawing out 1 ml of internal gas from the core cavity of each apple and injecting the sample into a gas chromatograph (Agilent Technologies, 6890 N Network GC system, Palo Alto, CA, U.S.A.). The detailed procedure is outlined in Whale (2005).

2.8. Analysis of pigments

Skin was removed from fruit using a peeler and any underlying tissue was scraped off. The skin was stored in a freezer (–20°C) until analysis.

2.8.1. Total anthocyanin analysis

Total anthocyanin was determined at 530 nm using an UV/Vis spectrophotometer (Jenway Spectrophotometers Model 6405, Dunmow, Essex, U.K.) according to the method of Bishop and Klein (1975). Concentration of total anthocyanin was calculated using a molar extinction co-efficient of 3.43×10^4 for idaein chloride (Siegelman and Hendricks, 1958).

2.8.2. Chlorophyll analysis

Chlorophyll pigments were extracted using the method outlined by Lancaster et al. (1997). Concentrations of chlorophyll *a*, chlorophyll *b*, and total chlorophyll were calculated using the equations of Maclachlan and Zalik (Holden, 1965).

2.8.3. Flavonoids and phenolic compounds.

Flavonoids and phenolic compounds were extracted by the method outlined by Awad et al. (2000), with some modifications (Whale, 2005). The compounds were quantified using reversed-phase high performance liquid chromatography (RP-HPLC) as outlined in Whale (2005).

2.9. Fruit firmness, acidity, and soluble solids concentrations

Fruit firmness was measured from two peeled sides of each fruit using an electronic pressure tester (Model EPT-1 pressure tester, Lake City Technical Products Inc, Kelwona, BC, Canada) fitted with an 11–mm tip. Total acidity (TA) was determined from the juice of the blended composite of apple slices (17-20 fruit per replication) using a Panasonic juice extractor (Model MJ-66PR, Matsushita Electric Ind. Co. Ltd, Japan). Ten mL of freshly extracted fruit juice were diluted with 20 mL distilled water. Five mL of the above diluted juiced was titrated against 0.1 N NaOH using phenolphthalein as an indicator of the end point by change in colour to pink. Soluble solids concentration (SSC) was determined by measuring the refractive index of juice using an infrared digital refractometer (Atago-Palette PR 101, Atago Co. Ltd, Itabashi-Ku, Tokyo, Japan) at 20°C.

2.10. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Genstat 6, release 6.2 program (Lawes Agricultural Trust, Rothamsted Experimental Station, U.K.). The treatment effects on various parameters were assessed within ANOVA. Least

significant differences (Fisher's protected LSD) were calculated at the 5% level of significance for significant treatment effects within the analysis of variance. Unless otherwise specified, all significant differences in this manuscript are for $P < 0.05$.

3. Results

3.1. Fruit colour and total anthocyanin concentration

At commercial harvest in 2002, AVG alone had significantly delayed development of red blush compared to ethephon alone and AVG followed by ethephon (Fig. 2). Red colour development was least in untreated fruit (47.5%) compared to all other treatments while ethephon treatments with or without AVG enhanced percent red blush. Fruit treated with AVG followed by ethephon showed red blush of 71.1% and was similar to fruit treated with ethephon alone (77.8%).

AVG treatment alone retarded accumulation of total anthocyanin in fruit skin at harvest compared to the ethephon alone treatment (Fig. 2). Ethephon alone significantly increased concentration of total anthocyanin in the fruit skin compared to all other treatments. Ethephon applied to AVG-treated fruit increased total anthocyanin concentration in skin relative to control and AVG alone treatments. However, the inhibitory effect of AVG in the AVG followed by ethephon treatment was still evident, as the treated fruit had lower concentration of total anthocyanin compared to the ethephon alone treatment (Fig. 2).

3.2. Lightness, chroma and hue angle

In 2002, the different AVG and ethephon treatments significantly affected hue angle and lightness measured on both the blush and shaded sides of fruit and chroma (saturation of colour) on the blush side (Table 1).

Treatment with AVG alone increased the hue angle, while ethephon alone reduced the hue angle (change from green to red) on both the shaded and blush sides. Ethephon applied to AVG-treated fruit, decreased the hue angle on the shaded side and was similar to the hue angle of fruit from the ethephon alone treatment. However, on the blush side, although the hue angle was smaller than the AVG alone treatment, ethephon application did not completely overcome the AVG effect (Table 1).

Lightness values following ethephon application alone or in combination with AVG, were significantly reduced on both the blush and shaded sides compared to the AVG alone and control (Table 1). The reduction in lightness values indicates higher concentration of anthocyanin pigments and therefore redder fruit. Lightness on both the shaded and blush sides of control fruit were significantly higher compared to all other treatments.

Chroma measured on the blush side was significantly higher in the ethephon alone treatment compared to all other treatments, indicating higher red colour saturation. Fruit treated with AVG followed by ethephon were intermediate in chroma compared to ethephon alone and AVG alone treatments, and exhibited improved chroma relative to AVG alone treatment (Table 1).

3.3. Fruit colour for export

At commercial harvest, AVG alone significantly reduced the percentage of fruit that met the export criteria. The values (percentage of red blush of more than 40%, ± SE, $_{LSD}$ = 19.5 at $P \leq 0.05$) were 62.5 ± 8.3, 98.8 ± 1.3, 76.2 ± 9.4 and 91.2 ± 2.4 for control, ethephon alone, AVG alone, and AVG followed by ethephon, respectively.

3.4. Chlorophyll concentration in fruit skin

Preharvest applications of AVG and ethephon alone and AVG followed by ethephon, significantly reduced chlorophyll a, chlorophyll b, and total chlorophyll concentrations in skin as compared to control (Fig. 3). Ethephon treatment alone reduced the concentrations of chlorophyll a and total chlorophyll in the fruit skin compared to all other treatments. Fruit treated with AVG alone had higher chlorophyll a concentration compared to AVG followed by ethephon treatment. However, the differences in chlorophyll b and total chlorophyll concentrations in skin of fruit treated with AVG alone or AVG followed by ethephon were not significant. There were no significant differences in chlorophyll b concentrations of fruit treated with ethephon alone, AVG alone or AVG followed by ethephon (Fig 3).

3.5. Flavonoids and phenolic compounds in fruit skin

At commercial harvest, preharvest ethephon treatment alone significantly increased concentration of cyanidin 3-galactoside in fruit skin compared to control and AVG treatments alone or in combination with ethephon. The values ($\mu g \cdot g\ fw^{-1} \pm SE$, LSD = 61.5 at $P \leq 0.05$) were 76.1 ± 12.9, 241.9 ± 40.4, 90.7 ± 15.1, and 152.1 ± 16.9 for control, ethephon alone, AVG alone, and AVG followed by ethephon, respectively. There were no significant differences among the treatments in the concentrations of chlorogenic acid, phloridzin, flavanols, and quercetin glycosides (Whale, 2005).

3.6. Respiration, IEC, SSC, TA and fruit firmness

Fruit treated with preharvest AVG alone had significantly reduced respiration rate compared to fruit treated with ethephon alone or AVG followed by ethephon, while ethephon treatment alone increased respiration rate compared to all other treatments

(Table 3). However, the respiration rates of fruit treated with AVG alone and control fruit were similar. Similarly, there were no significant differences in the respiration rates of fruit treated with AVG followed by ethephon and untreated fruit (Table 3)

Preharvest AVG and ethephon treatments alone or in combination did not significantly affect IEC at commercial harvest in 2002 (Table 3). However, it appears that AVG alone inhibited IEC while ethephon enhanced IEC in the fruit.

At commercial harvest, ethephon and AVG treatments affected only SSC of fruit, while other quality parameters such as firmness and TA were unaffected by the different treatments (Table 3).

Ethephon treatment alone significantly increased SSC of fruit compared to other treatments except for AVG followed by ethephon (Table 3). There were no significant differences in SSC of fruit treated with AVG alone or AVG followed by ethephon.

3.7. Chroma, hue angle and visual colour during fruit maturation following treatment in 2003

Fruit colour parameters including chroma, hue angle, and percent red blush on the fruit surface changed significantly during fruit maturation between treatment application dates (24 March and 7 April) and commercial harvest (26 April) (Fig. 4). Chroma (colour saturation) on the fruit surface significantly decreased during fruit maturation (between time of treatment application and commercial harvest) in control and fruit treated with ethephon (Fig. 4A). Fruit treated with AVG alone or in combination with ethephon showed slight increase in chroma during maturation between 24 March and 7 April and thereafter, decreased until commercial harvest on 26 April. Decrease in chroma after 7 April was more vivid in fruit treated with AVG

followed by ethephon compared to AVG alone. This can be attributed to the follow-up ethephon application on 7 April (Fig. 4A).

Ethephon alone or in combination with AVG, significantly lowered the hue angles (redder fruit) on the fruit surface (Fig. 4B). Fruit treated with AVG alone had higher hue angles during fruit maturation compared to all other treatments and at commercial harvest (26 April). These fruit were greener compared to control, fruit treated with ethephon alone or in combination with AVG (Fig. 4B). Fruit treated with AVG followed by ethephon had hue angles similar to fruit treated with ethephon alone. This suggests that the inhibitory effect of AVG on colour decreased when AVG-treated fruit were sprayed with ethephon on 7 April.

Between 24 March and 26 April, preharvest ethephon application alone, significantly increased percent red blush from 5% to 36.7%, while AVG alone significantly retarded colour development (Fig. 4C). Fruit treated with AVG followed by ethephon exhibited gradual development of red colour during maturation between 24 March and 7 April and increased significantly thereafter following ethephon application on 7 April. At commercial harvest (26 April), fruit treated with AVG followed by ethephon had developed red blush similar to fruit treated with ethephon alone. In control, colour developed significantly commencing two weeks prior to commercial harvest. However, red colour was significantly less as compared to the ethephon-treated fruit (Fig. 4C).

3.8. Respiration rate, IEC and fruit firmness during maturation

Respiration rate of fruit treated with AVG alone or in combination with ethephon was significantly lower at most sampling dates during fruit maturation compared to control and fruit treated with ethephon alone (Fig. 5A). Ethephon applied to AVG-

treated fruit on 7 April increased the respiration rate on 16 April compared to AVG alone treatment. But its respiration rate was lower compared to fruit treated with ethephon alone and was similar to control. Ethephon treatment alone increased fruit respiration rate during maturation between 24 March and 2 April compared to control and AVG-treated fruit. At commercial harvest fruit treated with ethephon alone had significantly higher respiration rate compared to all other treatments (Fig. 5A).

Ethephon treatment alone significantly enhanced IEC during fruit maturation compared to all other treatments and the effect was observed within three days of treatment application (Fig. 5B). IEC of fruit treated with ethephon alone increased over 17-fold during maturation between 24 March and 2 April followed by a decline between 2 and 7 April. IEC of ethephon-treated fruit then continued to increase until commercial harvest. In contrast, AVG significantly retarded IEC during fruit maturation compared to all other treatments and the effect was evident within three days (27 March) of AVG application. AVG continued to retard IEC until commercial harvest when a non-significant increase in IEC was recorded. IEC of fruit treated with AVG followed by ethephon was similar to AVG alone between 27 March and 7 April. However, IEC significantly increased following the ethephon application on 7 April. At commercial harvest, IEC of fruit treated with AVG followed by ethephon was significantly lower as compared to fruit treated with ethephon alone (Fig. 5B).

Fruit firmness significantly decreased during fruit maturation, between initial application of AVG and ethephon and commercial harvest (Fig. 5C). This decline (27 March–26 April) was greatest in control (by 18 N) followed by ethephon treatment

alone (by 15.9 N) and AVG followed by ethephon (by 12.5 N), while firmness decreased by 9 N in fruit treated with AVG alone (Fig. 5C).

3.9. Fruit colour and total anthocyanin concentration at harvest in 2003

At commercial harvest, ethephon alone and ethephon applied to AVG-treated fruit significantly improved percent red blush on fruit surface compared to control and fruit treated with AVG alone (Fig. 6). Preharvest AVG treatment alone inhibited development of red colour compared to the ethephon treatments. However, colour of fruit treated with AVG alone did not differ from the control. A follow-up application of ethephon on AVG-treated fruit seemed to have overcome the inhibitory effect of AVG on fruit colour, as fruit from this treatment were almost as red as fruit treated with ethephon alone (Fig. 6).

Ethephon treatment alone significantly increased total anthocyanin concentration in fruit skin compared to fruit treated with AVG alone or AVG in combination with ethephon (Fig. 6). There were no significant differences in total anthocyanin concentration between control, AVG alone, or AVG in combination with ethephon. However, AVG followed by ethephon appeared to increase anthocyanin accumulation in skin compared to control and AVG alone treatments.

3.10. Lightness, chroma and hue angle at harvest

At commercial harvest in 2003, only hue angle was significantly affected by the different AVG and ethephon treatments while lightness and chroma were not. The values ($h° \pm SE$, $LSD = 12.9$ at $P \leq 0.05$) for hue angle were 88.5 ± 3.3, 92.1 ± 4.5, 73.5 ± 2.8 and 75.6 ± 2.2 for control, ethephon alone, AVG alone and AVG followed by ethephon, respectively.

3.11. Fruit colour for export at harvest

At commercial harvest in 2003, the percentage of fruit that met the export criteria for colour was significantly higher with ethephon treatments compared to AVG alone and control. The values (percentage red blush of more than 40%, ± SE, LSD = 14.0 at P ≤ 0.05) were 15.0 ± 7.6, 48.3 ± 14.8, 5.0 ± 2.9, and 41.7 ± 14.8 for control, ethephon applied alone, AVG applied alone, and AVG followed by ethephon, respectively.

3.12. Chlorophyll concentration in fruit skin at harvest

At commercial harvest in 2003, the different AVG and ethephon treatments had no significant effects on the concentrations of chlorophyll a, b, or total chlorophyll in the fruit skin (data not shown).

3.13. Flavonoids and phenolic compounds in fruit skin at harvest

At commercial harvest, ethephon treatments alone or in combination with AVG significantly increased the concentration of cyanidin 3-galactoside concentration in the fruit skin compared to AVG treatment alone and control. The values (μg·g fw^{-1} ±SE, LSD = 5.6 at P ≤ 0.05) were 9.3 ± 0.9, 21.8 ± 20.7, 7.1 ± 2.5, and 24.6 ± 5.8 for control, ethephon applied alone, AVG applied alone, and AVG followed by ethephon, respectively. There were no significant differences among the treatments in the concentration of chlorogenic acid, phloridzin, flavanols, and quercetin glycosides (data not shown). The exception was for epicatechin (a flavanol) whose concentration was lower for AVG applied alone and AVG followed by ethephon. The values (μg·g fw^{-1} ±SE, LSD = 42 at P ≤ 0.05) were 172.0 ± 13.1, 172.5 ± 19.3, 116.9 ± 15.5 and 141.9 ± 3.1 for control, ethephon alone, AVG alone, and AVG followed by ethephon, respectively.

3.13. Respiration rate, IEC, SSC, TA and fruit firmness

At commercial harvest, respiration rate was significantly lowered by AVG alone compared to control and ethephon alone or in combination with AVG (Table 3). Fruit sprayed with ethephon alone had the highest respiration rate compared to control and AVG treatments. While fruit treated with AVG followed by ethephon had lower respiration rate compared to ethephon alone. There were no significant differences for respiration between control and fruit treated with AVG followed by ethephon and also between AVG alone and AVG followed by ethephon.

AVG treatment alone significantly reduced IEC of fruit compared to ethephon treatments and control (Table 3). Significantly higher IEC was observed in fruit treated with a preharvest application of ethephon alone compared to all other treatments. Fruit treated with AVG followed by ethephon had significantly reduced IEC compared to the ethephon alone treatment, but they had the same IEC as control.

At commercial harvest, the different AVG and ethephon treatments affected SSC and fruit firmness, but TA was unaffected by the different treatments (Table 3). Fruit treated with ethephon alone and AVG followed by ethephon had higher SSC compared to fruit treated with AVG alone and control. There were no significant differences for SSC between the control and fruit sprayed with AVG alone while firmness was highest in fruit treated with AVG alone (Table 3). Fruit treated with AVG followed by ethephon had lower firmness, by 4 N, than those treated with AVG alone. But they were firmer, by 4–5 N, than control fruit and fruit treated with ethephon alone. There were no differences for fruit firmness between control and fruit treated with ethephon alone.

4. Discussion

Preharvest ethephon treatments, with or without a preceding AVG treatment, increased percent red blush (Figs. 2 and 6) and chroma and lowered hue angle and lightness (Table 1) on the fruit surface compared to control and AVG treatment alone at commercial harvest. This improvement in colour can be attributed to increased concentrations of total anthocyanin (Figs. 2 and 6) and cyanidin 3-galactoside and reduced chlorophyll concentrations (Fig. 3) in the skin. On the other hand, AVG treatment alone retarded development of red blush, accumulation of total anthocyanin and cyanidin 3-galactoside and chlorophyll degradation in the skin as compared to ethephon treatments.

AVG has been reported to delay colour development in other apple cultivars such as 'Gala' and 'Jonagold' (Wang and Dilley, 2001), 'McIntosh' (Stover et al., 2003), 'Gala' and 'Pink Lady' (Phan-Thien et al., 2004). Improvement in colour with preharvest ethephon treatments is well documented in many cultivars of apple (Blanpied et al., 1975; Jones, 1979; Larrigaudiere et al., 1996). A combination of AVG followed by ethephon has recently been reported to improve colour in 'Gala' and 'Jonagold' (Wang and Dilley, 2001) and 'McIntosh' apples (Stover et al., 2003).

Ethylene biosynthesis during maturation and ripening (Fig. 5B) appeared to play an important role in the development of colour and anthocyanin accumulation in ethephon and/or AVG-treated fruit. Whilst ethephon treatment alone enhanced IEC of the fruit, AVG treatments alone or in combination with ethephon suppressed it (Fig. 5B and Table 3). The inhibition of ethylene by AVG and enhancement by ethephon was observed on 27 March within three days of applying the treatments (Fig. 5B). Similarly, fruit treated with AVG followed by ethephon had suppressed

IEC during maturation following AVG treatment on 24 March until the follow-up ethephon application on 7 April, after which IEC increased. AVG acts by blocking the activity of ACC synthase, which converts methionine to ACC in the ethylene biosynthetic pathway (Yang and Hoffman, 1984). Suppression of ethylene by AVG has been reported in 'King of 'Pippin' (Bangerth, 1978), 'McIntosh', 'Spartan' and 'Spencer' (Bramlage et al., 1980), 'Gala' and 'Jonagold' (Wang and Dilley, 2001) and 'Redchief Delicious' apples (Silverman Paul et al., 2004).

Ethylene is crucial for regulating colour development in apple (Saure, 1990; Lancaster, 1992). Therefore poor colour development with AVG alone and enhanced colour with ethephon treatments with or without AVG are attributed to the action of ethylene in regulating anthocyanin biosynthesis. Improved colour development in fruit treated with AVG followed by ethephon can be ascribed to the ethylene released from ethephon, which although is considered transient, promotes anthocyanin biosynthesis without triggering autocatalytic ethylene. This has also been suggested in 'Gala' and 'Jonagold' apples treated with AVG followed by ethephon (Wang and Dilley, 2001). It is also likely that, ethylene released from ethephon stimulated the activity of phenylalanine ammonia-lyase (PAL), a key enzyme in the anthocyanin biosynthetic pathway (Faragher and Brohier, 1984; Lister et al., 1996). Ethephon has been previously reported to increase the activity of PAL in the skin of 'Starking Delicious' (Larrigaudiere et al., 1996) and 'Fuji' apples (Li et al., 2002). Additionally, ethylene may have also triggered the expression of anthocyanin biosynthetic genes, while AVG may have inhibited or delayed their expression (Awad and de Jager, 2002). It has been suggested that the anthocyanin biosynthetic pathway can be triggered and enhanced by a brief exposure to ethylene (Wang and Dilley, 2001; Awad and de Jager, 2002). When the anthocyanin biosynthetic pathway is

activated by ethylene, the pathway continues, and the ethylene released from ethephon dissipates.

Although the two locations, Mullalyup and Carmel cannot be compared statistically, in general colour development was better at Mullalyup in 2002 compared to Carmel in 2003. This is most likely due to lower mean day and night temperatures at Mullalyup (Fig. 1A) compared to warmer night temperatures at Carmel (Fig. 1B) prior to harvest. Colour development in 'Pink Lady' apples throughout Western Australia during 2002–03 was poor due to warm temperatures prior to harvest (Department of Agriculture Western Australia, 2003). Although the optimum temperature for colour development varies for different apple cultivars, in general low temperatures promote red colour development (Blankenship, 1987) by enhancing anthocyanin biosynthesis (Creasy, 1968).

The different AVG and ethephon treatments did not affect the concentrations of flavonoids such as quercetin glycosides, catechin, epicatechin, chlorogenic acid and phloridzin in the skin (data not shown). Since neither ethylene-enhancing ethephon nor ethylene-inhibiting AVG treatments had any significant effects on the concentrations of flavonoids apart from the anthocyanin group, it may be deduced that ethylene does not regulate the accumulation of catechin, epicatechin, phloridzin and quercetin glycosides. Similarly, flavanol and quercetin glycoside concentrations in 'Jonagold' apples were unaffected by AVG and ethephon treatments (Awad and de Jager, 2002). This also implies that although the anthocyanins, quercetin glycosides and flavanols are formed from the same biosynthetic pathway, they may be independently regulated and physically separated at the cellular level (Awad and de Jager, 2002).

Ethephon treatments enhanced chlorophyll degradation in the skin, while, AVG retarded its degradation (Fig.3). Ethylene released from ethephon may have increased activities of chlorophyllase and chlorophyll degrading peroxidase enzymes resulting in the accumulation of chlorophyllide a, and reduction in chlorophyll a concentration. Similarly, Yamauchi et al. (1997) reported increased chlorophyll degradation in 'Wase Satsuma' mandarin following ethylene treatments and ascribed it to the formation of chlorophyllide a by chlorophyllase enzyme, while AVG treatments have been reported to reduce the loss of chlorophyll in 'Bartlett' pears (Clayton et al., 2000).

In both years, AVG suppressed respiration rate, while ethephon treatment alone enhanced respiration rate (Tables 2 and 3). Ethephon applied to AVG-treated fruit did not overcome the inhibitory effect of AVG on respiration as these fruit showed reduced respiration compared to fruit treated with ethephon alone. Reduced respiration with AVG application may be due to reduced ethylene biosynthesis and this is confirmed by Bangerth (1978) in 'King of Pippin' and 'Golden Delicious' apples.

The delayed fruit ripening property of AVG is also observed in the retention of fruit firmness in AVG-treated fruit which is again attributed to reduced ethylene biosynthesis and respiration. Fruit treated with AVG followed by ethephon were firmer compared to ethephon alone (Fig. 5C and Table 3). This implies that AVG continued to slow down the ripening process even in the presence of ethephon, while increased ethylene concentration in ethephon-treated fruit may have led to reduced firmness. Retention of firmness with AVG treatments have also been reported in 'King of Pippin' and 'Golden Delicious' (Bangerth, 1978), 'Gala' and 'Jonagold'

(Wang and Dilley, 2001), 'Gala' and 'Pink Lady' apples (Phan-Thien et al., 2004). Increased SSC with ethephon treatments (Tables 2 and 3) is related to enhanced ripening initiated by ethylene, while reduced SSC in AVG treated fruit is attributed to retardation of ripening.

In conclusion, a preharvest AVG spray (four–five weeks before harvest) followed by ethephon (two–three weeks later) effectively improves colour and maintains quality of fruit at commercial harvest. Therefore this could be an effective cultural practice for 'Pink Lady' apples especially when seasonal fluctuations in temperature result in poor colour development and market rejection of fruit.

Acknowledgments

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Table 1. Effects of preharvest applications of AVG and ethephon on lightness, chroma, and hue angle for the blush and shaded sides of fruit at harvest in 2002. Any two means within a column followed by different letters are significantly different using LSD at $P < 0.05$.

Treatment	Lightness (L*)		Chroma (C*)		Hue angle (h°)	
	Shaded	Blush	Shaded	Blush	Shaded	Blush
Control	37.3 [a]	26.7 [a]	15.6 [a]	17.4 [c]	90.0 [a]	38.4 [a]
AVG	34.3 [b]	24.5 [b]	15.1 [a]	17.0 [c]	80.7 [b]	34.5 [ab]
Ethephon	32.5 [c]	21.5 [c]	15.7 [a]	20.1 [a]	61.6 [c]	22.8 [c]
AVG + Ethephon	31.7 [c]	22.0 [c]	14.9 [a]	18.8 [b]	65.1 [c]	26.8 [bc]

Table 2. Effects of preharvest applications of AVG and ethephon on respiration rate, internal ethylene concentration, SSC, TA and fruit firmness at harvest in 2002. Any two means within a column followed by different letters are significantly different using LSD at $P < 0.05$.

Treatment	Respiration rate (mmol $CO_2 \cdot kg^{-1} \cdot h^{-1}$)	Internal ethylene ($nL \cdot L^{-1}$)	SSC (%)	TA (% malic acid)	Fruit firmness (N)
Control	0.52 [bc]	235.4 [a]	14.4 [c]	0.85 [a]	92.5 [a]
AVG	0.50 [c]	151.1 [a]	14.7 [bc]	0.86 [a]	91.7 [a]
Ethephon	0.71 [a]	392.1 [a]	15.7 [a]	0.92 [a]	93.7 [a]
AVG + Ethephon	0.61 [ab]	334.0 [a]	15.3 [ab]	0.90 [a]	91.7 [a]

Table 3. Effects of preharvest applications of AVG and ethephon on respiration rate, internal ethylene concentration, SSC, TA and fruit firmness at harvest in 2003. Any two means within a column followed by different letters are significantly different using LSD at $P < 0.05$.

Treatment	Respiration (mmol $CO_2 \cdot kg^{-1} \cdot h^{-1}$)	Internal ethylene ($nL \cdot L^{-1}$)	SSC (%)	TA (% malic acid)	Fruit firmness (N)
Control	0.51 [b]	61.1 [b]	13.3 [b]	0.78 [a]	83.1 [c]
AVG	0.43 [c]	17.3 [c]	13.1 [b]	0.80 [a]	92.4 [a]
Ethephon	0.65 [a]	499.1 [a]	14.1 [a]	0.76 [a]	82.9 [c]
AVG + Ethephon	0.47 [bc]	124.8 [b]	13.9 [a]	0.75 [a]	88.9 [b]

Caption to figures

Fig. 1. Average day and night temperatures during fruit maturation recorded at Mullalyup in 2002 (A) and at Carmel in 2003 (B) using Tinytag*Plus* Gemini Data loggers.

Fig. 2. Effects of preharvest applications of AVG and ethephon on total anthocyanin concentration (TAC) in fruit skin and visual colour at harvest in 2002. Vertical bars represent standard error of means.

Fig. 3. Effects of preharvest applications of AVG and ethephon on the concentrations of chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and total chlorophyll in fruit skin at harvest in 2002. Vertical bars represent standard error of means.

Fig. 4. Changes in chroma (A), hue angle (B) and visual colour (C) with preharvest applications of AVG and ethephon during fruit maturation. Vertical bars represent standard error of means. Arrows show treatment application dates.

Fig. 5. Changes in respiration (A), internal ethylene concentration (B) and fruit firmness (C) with preharvest applications of AVG and ethephon during fruit maturation. Vertical bars represent standard error of means. Arrows show treatment application dates.

Fig. 6. Effects of preharvest applications of AVG and ethephon on total anthocyanin concentration (TAC) in fruit skin and visual colour at harvest in 2003. Vertical bars represent standard error of means.

Fig. 1. (Whale et al)

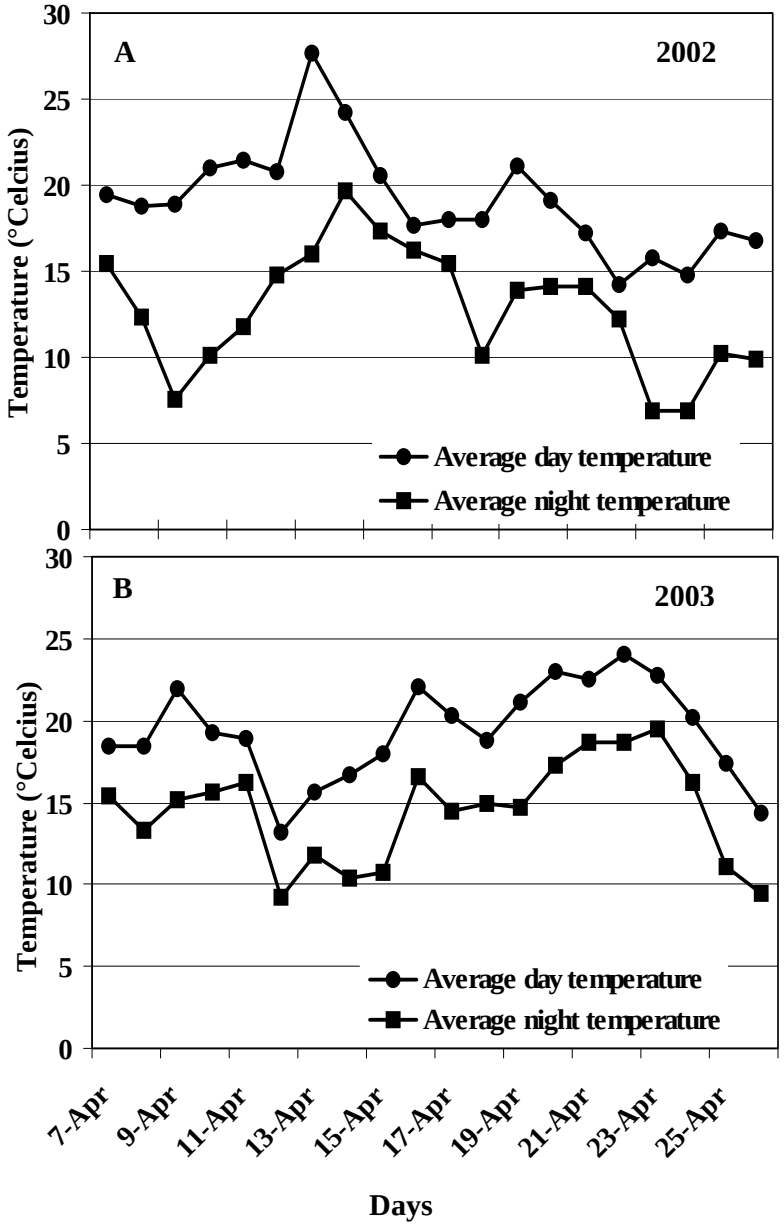


Fig. 2 (Whale et al)

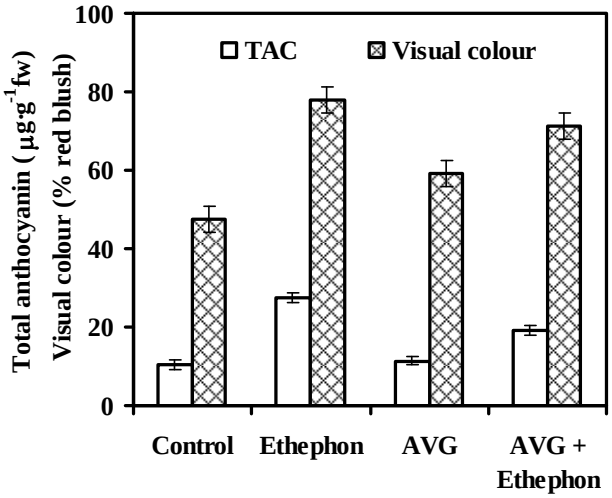


Fig. 3. (Whale et al)

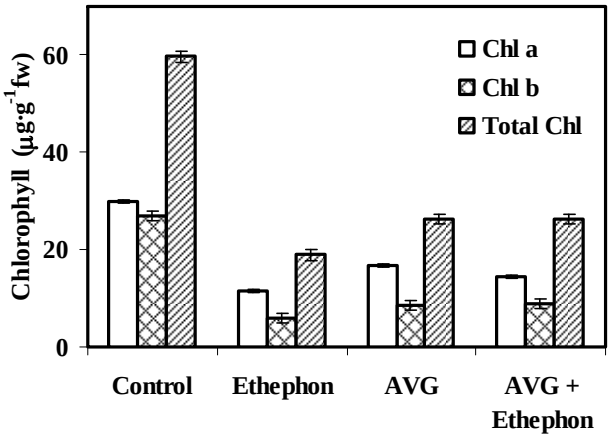


Fig 4. (Whale et al)

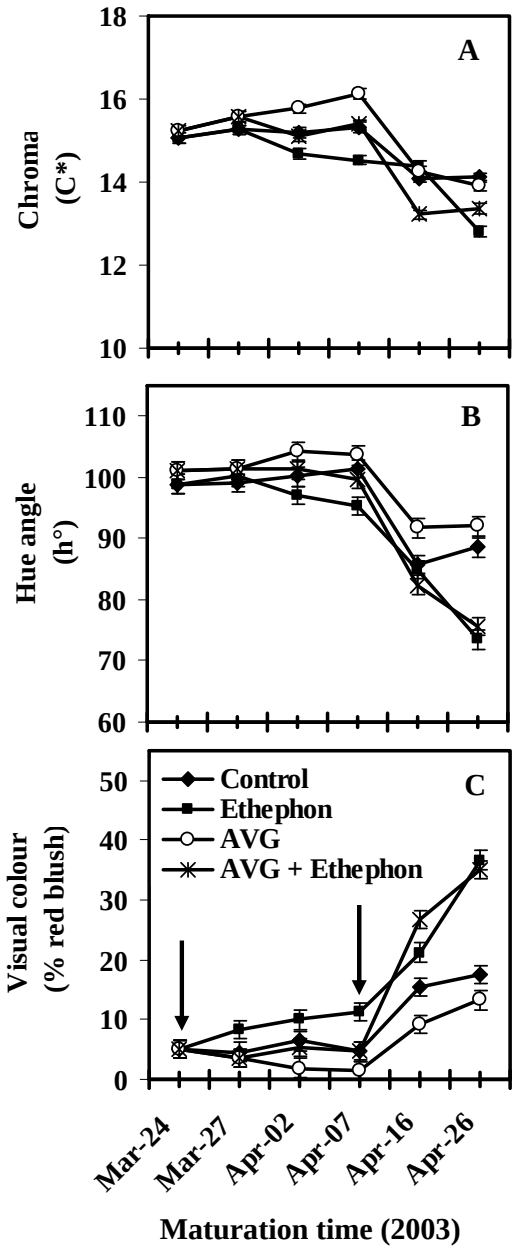


Fig 5. (Whale et al)

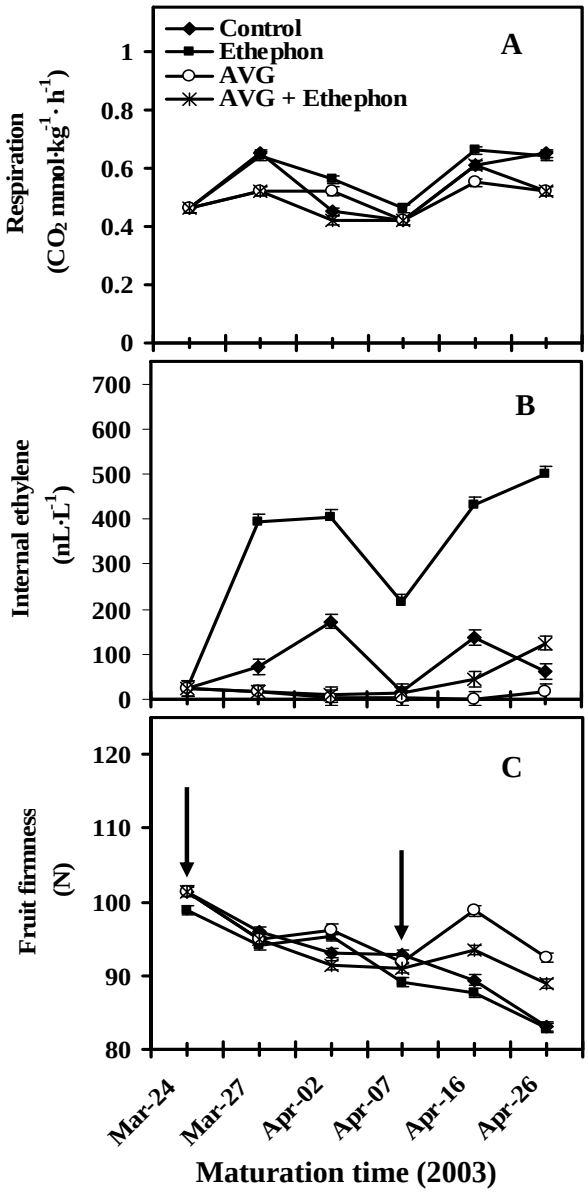
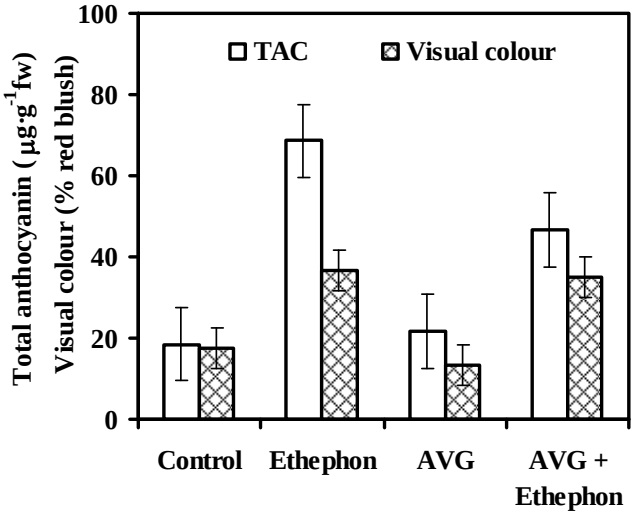


Fig. 6 (Whale et al)



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