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Report on the use of prohydrojasmon (Blush™) to enhance fruit color in apples

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Introduction

Apple peel color is an important factor determining apple market acceptance. In general, red apples are preferred, particularly well-colored bright red types. Coloration of red apples is due to a complex phenomenon, in which, among other processes, pigment synthesis (anthocyanins, carotenoids and flavonoids) occurs. Along with the synthesis of these pigments, it is very important that chlorophyll degradation occurs, which also takes place during the final stages of fruit ripening(Grappadelli, 2003).

Anthocyanin biosynthesis is regulated by a complex interaction between internal and external stimuli such as temperature, light, carbohydrates, water stress and plant hormones (Loreti*et al.*, 2008). It has been shown in red-fruited cultivars that anthocyanin synthesis depends primarily on light intensity and quality (Arakawa *et al*., 1985), temperature (Arakawa, 1991) and environmental conditions and management factors in the orchard (Saure, 1990). Ensuring high levels of light penetration in the orchard during harvest would be the first and foremost concern for the apple growers in order to improve fruit coloration. Light levels can be controlled to some extent through orchard management practices; however, it is highly dependent on climatic conditions and growing region, which may not always be favorable for color development.

Apple cultivars have different temperature optima for color formation. Clear days with temperatures of around 25 °C and cool nights (below 15 °C) during the preharvest period (2–3 weeks before harvest) increase anthocyanin concentration in apple peel; and this condition needs to be coupled with high UV availability (Grappadelli, 2003).

A number of cultural techniques have received renewed attention as a management tool to externally control anthocyanin formation and consequently to improve skin coloration, such as summer pruning and thinning to open up the canopy and allow more light to penetrate to otherwise shaded parts (Saure, 1990; Hirst*et al*., 1990), evaporative cooling to reduce fruit temperature andimprove fruit color and reduce sunburn in warmer areas (Evans, 1993; Williams, 1993; Iglesias *et al.*, 2002; Funke and Blanke, 2005), though very expensive; reflective mulches (Mika *et al.*, 2007; Iglesias and Alegre, 2009) to increase light intensity and canopy absorption; and fruit bagging (Arakawa, 1988; Liu *et al*., 2013), which is also labor expensive.

A range of chemical products have been tested for use as direct or indirect color enhancers of apples. Most are applied to delay maturation and retard preharvest fruit abscission or advance fruit maturity.

AVG, an inhibitor of ethylene production, delays fruit abscission and development of watercore (Shafer *et al*.,1995), and indirectly enhances fruit coloration. Delaying maturity prolongs both the harvest season and the fruit's storage life and allows more time on the tree for the fruit todevelop both size and red color. Ethephon works by stimulating the ripening process in the fruit, thereby advancing color development. However, it may shorten the storage life of fruits due to reduction in flesh firmness (Greene *et al*., 1977). Seniphos is a phosphorus–calcium mixture that stimulates anthocyanin accumulation without activation of ethylene production and subsequent ripening (Larrigaudiere and Pinto, 1996).

Recent works have showed the effect of jasmonates (JAs)on promoting color changes of apples. JAs are signal molecules which modulate many physiological processes in plants including stress responses and fruit ripening. Exogenously applied on detached fruit, methyl jasmonate (MJ), a natural derivative of JA, stimulates chlorophyll degradation, β-carotene and anthocyanin accumulation in apple (Saniewski and Czapski, 1983; Perez *et al*., 1993; Rudell and Mattheis 2008), flavonoid accumulation and antioxidant activity in blackberry (Wang *et al*. 2008), and resveratrol biosynthesis in grape (Vezzulli*et al*. 2007).

Reciprocal relationships between JAs and ethylene are not well established. In particular, during fruit ripening, contradictory results have been reported, and the effects of JAs on ethylene seem to strongly depend, besides fruit species and cultivar, upon physiological stage of application (Kondo *et al*. 2007; Ziosi*et al*. 2008).

Although MJ is an effective material for color improvement in apples, its low stability and high price makes it currently unviable for commercial applications. A synthetically produced analogue of MJ, i.e., prohydrojasmon (PHJ), seems to be similarly effective as jasmonic acid in enhancing red color development in apples.

The main goal of this experiment was to determine if preharvest applications of PDJ on apples alters color development and others aspects of fruit quality.

Material and methods

Three different trials were carried out in 2013 in the Ohio State:

1) The first trial was carried out in a commercial apple orchard located in Carroll OH. The cultivar tested was 'Buckeye Gala' raised on B.9 rootstock. Three treatments were tested: Control (water); 2x 400ppm PDJ (Blush™ 5% - Fine Agrochemicals, Ltd.) and 2x 400ppm PDJ (FAL-1820 10% – experimental formulation containing a proprietary adjuvant package of Fine Agrochemicals, Ltd.). Both products were sprayed approximately 4 and 2 weeks before harvest time (WBH).
2) The second trial was carried out in the experimental apple orchard (Horticultural Research Unit-2) of the Ohio State University at Wooster OH, using the same treatments as specified above. However, the cultivar studied here was 'Fuji'/B.9.
3) In the third trial besides the PDJ it was included aminoethoxyvinylglycine (AVG - Retain™ - ValentBioSciences Corp.). 'Honeycrisp'/B.9 was the cultivar used in this trial and treatments were: Control (water); 2x 400ppm PDJ (Blush™) applied 4 and 2 WBH; 2x 400ppm PDJ (FAL-1820) applied 4 and 2 WBH; 333g/acre AVG applied 2WBH and 400ppm PDJ + 333g/acre AVG applied 2WBH.

Fruits from all treatments were harvested when untreated control fruit reached commercial maturity. Fruit quality evaluation included fruit color, red skin color intensity, ethylene production, fruit weight, flesh firmness and soluble solids concentration. Fruit color was measured by the percent of the apple surface with red color (%). Red color intensity was described by using the Minolta Chromameter CR-200, which measured two color parameters (a* and b* values). Hue angle (arctan b*/a*), a color-intensity indicator for determining color differences in fruit that ripen from green to either yellow or shades of redwas estimated using the formulas provided by McGuire (1992) and reported in degrees (0° = red-purple, 90° = yellow and 180° = bluish-green). Two measurements per fruit were taken on the reddest side of the fruit.

Ethylene production of apples was measured by sealing fruit in 1-L glass jars (one fruit per jar) for a 2-h incubation interval at 20 °C, after which a 1-mL headspace of gas sample was removed using a syringe inserted into the jar through a rubber septum in the jar lid. Ethylene concentration [nL C2H4 g-1 h-1] in the gas samples was analyzed using a Varian CP-3800 (Varian Inc., Palo Alto, CA, USA) capillary gas chromatograph (GC) equipped with a flame ionization detector (FID) set at 250 ºC and a packed column filled with HayeSep R (Varian Inc., Palo Alto, CA, USA) porous polymer packing (80/100 mesh). The oven temperature of the GC was programmed to be initially at 50 ºC held for 5 min then increased to 250 ºC at a rate of 5 ºC.min–1. Helium carrier gas flow on the column was 0.1 mL.min–1. The run time was 1.25 min. Fruit firmness was measured on opposite cheeks on each fruit in the replicate using the penetrometer with a 12 mm tip. The total soluble solids (%Brix) of the juice samples were evaluated using hand refractometer.

All spray applications were made with a backpack sprayer early in the morning under slow drying conditions. No surfactants were added.

Results

Apple peel color is determined first by the ground color of the peel and second by the superimposed red anthocyanin pigmentation (if present). Both formulation of Blush™ increased the percent of visual red color on 'Gala' and 'Fuji' apples (15.4% and 12.3%), and even not being significantly expressed in 'Honeycrisp', fruits tended to develop a redder color (Fig 1 and 2). The use of Retain on Honeycrisp either alone or combined with Blush™ did not show great efficacy regarding color development. Retain itself decreased red color or delayed its development (considering the delay effect of Retain fruits were picked prior reaching maturity); though the addition of Blush™ to the solution seemed to maintain color development (Tab. 1).

Hue angle, which is the most appropriate way of representing changes in color, did not differ between PDJ formulations but greatly differentiated from non-treated fruits and showed the same trend among the cultivars tested. The decline in hue angle demonstrated an increase in red blush development whether fruits had received the two applications of Blush™. Retain maintained the same color intensity as control fruits in 'Honeycrisp'; and to some extent enhanced color whether applied as a combo with Blush™. The promotion of red color development of Blush™ treated fruits could be attributed to the action of jasmonic acid in stimulating anthocyanin biosynthesis (Tab. 1).

No meaningful detectable ethylene production was noticed in 'Fuji' and 'Honeycrisp' PDJ treated fruits, indicating that to some degree of PDJ color change response occurred independently of ethylene. Nevertheless, further investigations are necessary to state that exogenous JA could be or not engaged with the ethylene synthesis (Tab. 3 and 4).

Fruit weight, flesh firmness and soluble solids were not affected by Blush™ applications in all cultivars compared to control treatments. Based on the results presented here, Blush™appeared to enhance red color development without significantly stimulating ripening (Table 2, 3 and 4).

Firmness is an important quality trait and its extent is closely associated to ripening. As previously expected, Retain slowed down ripening in 'Honeycrisp apple' through reducing ethylene production and flesh firmness (Tab. 4).

The combination of Blush™ and Retain™ need to be better studied and evaluated, since Blush™ could be used to promote commercially desirable color changes in apples while minimizing adverse effects of Retain on other fruit qualities attribute.In this trial, fruits were harvested in advance which could have affected the color evolution in Retain treated fruits.

Conclusion

Present results show that exogenous JAs (Blush™), applied 2 and 4WBH enhanced red blush development in apples without negatively interfering with ripening and some fruit quality traits. Differences in efficacy were observed between varieties. Combination of Blush and Retain was superior to Retain-alone treatment for color development; JAs appeared to be effective in counteracting Retain's negative effect on color development.

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Tables

Table 1: Percentage of red color (of the total fruit surface) and color intensity (hue angle) in 'Buckeye Gala', 'Fuji' and 'Honeycrisp' cultivars at harvest. Different letters above bars represent significant differences among treatments at $P < 0.05$ level within cultivars.

Treatment	'Buckeye Gala'		'Fuji'		'Honeycrisp'	
	Colour (%)	Hue Angle	Colour (%)	Hue Angle	Colour (%)	Hue Angle
Control	69.2 b	46.5 a	67.7 b	46.5 a	56.8 a	52.8 a
Blush	83.8 a	34.0 b	80.8 a	38.9 b	65.5 a	35.5 b
FAL-1820	85.3 a	33.2 b	79.2 a	36.0 b	63.0 a	36.8 b
Retain	-	-	-	-	46.7 b	57.1 a
Retain + Blush	-	-	-	-	57.9 a	43.6 ab

*Table 2:*Fruit weight (g), flesh firmness (lbs) and soluble solids (^{o}Brix) in 'Buckeye Gala' cultivar at harvest. Different letters above bars represent significant differences among treatments at $P < 0.05$ level within cultivars.

Treatment	Fruit Weight (g)	Firmness (lbs)	Soluble Solids (^{o}Brix)
Control	184.3^{ns}	18.7^{ns}	13.1^{ns}
Blush	183.1	18.4	12.8
FAL-1820	179.5	18.7	13.0

ns: non-significative

Table 3: Ethylene production (ppm), fruit weight (g), flesh firmness (lbs) and soluble solids (°Brix) in 'Fuji' cultivar at harvest. Different letters above bars represent significant differences among treatments at $P < 0.05$ level within cultivars.

Treatment	C_2H_4 (ppm)	Fruit Weight (g)	Firmness (lbs)	Soluble Solids (°Brix)
Control	34.5 ^{ns}	178.9 ^{ns}	17.3 ^{ns}	16.2 ^{ns}
Blush	31.9	184.3	17.1	15.7
FAL-1820	32.7	180.2	17.0	16.1

ns: non-significative

Table 4: Ethylene production (ppm), fruit weight (g), flesh firmness (lbs) and soluble solids (°Brix) in 'Honeycrisp' cultivar at harvest. Different letters above bars represent significant differences among treatments at $P < 0.05$ level within cultivars.

Treatment	C_2H_4 (ppm)	Fruit Weight (g)	Firmness (lbs)	Soluble Solids (°Brix)
Control	16.7 a	174.6 ^{ns}	15.5 a	12.7 ^{ns}
Blush	14.5 a	169.4	15.4 a	12.4
FAL-1820	15.3 a	178.0	15.2 a	12.6
Retain	5.2 b	170.9	16.9 b	12.5
Retain + Blush	8.9 b	172.2	16.1 ab	12.4

ns: non-significative

Figures

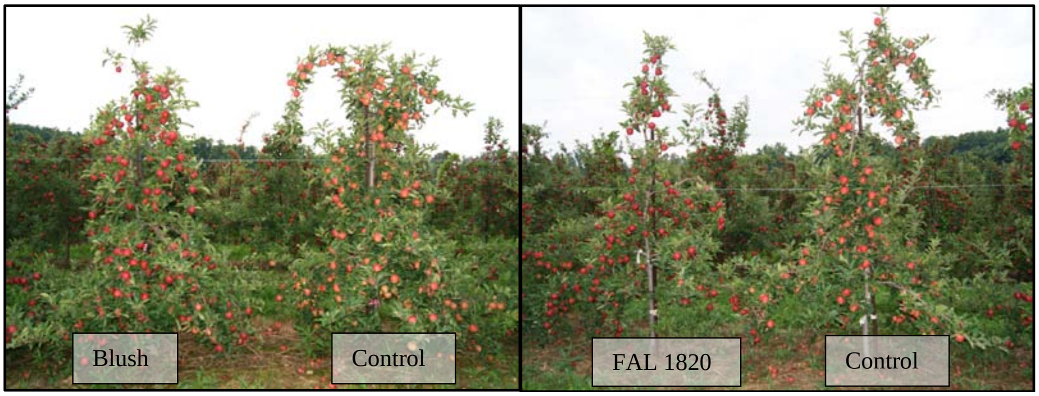

Figure 1:Tree appearance of ‘Buckeye Gala’fruits one week before commercial harvest under different treatments: untreated control, Blush 2x application, and FAL 1820 2x application.

Figure 2: Color appearance of the sun-exposed side of the fruit at the commercial harvest under different treatments: untreated control, Blush 2x application, and FAL 1820 2x application.