

Methyl Jasmonate Enhances Anthocyanin Accumulation and Modifies Production of Phenolics and Pigments in ‘Fuji’ Apples

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ABSTRACT. Effects of artificial ultraviolet–visible light and methyl jasmonate (MJ) treatment on ‘Fuji’ apple [*Malus sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] fruit peel anthocyanin, phenolic, carotenoid, and chlorophyll production were examined using tristimulus color analysis and reverse-phase high performance liquid chromatography. Anthocyanin synthesis was enhanced by light and MJ treatment. Chlorogenic acid and most cyanidin, quercetin, and phloretin glycosides increased with MJ treatment concentration. Light alone also promoted increased production of most of these compounds. Production of catechin, (–)epicatechin, quercetin, and quercetrin was not enhanced by either light or MJ treatment. Light and MJ enhanced β -carotene and chlorophyll b, synthesis but not xanthophyll or chlorophyll a synthesis. The chlorophyll a/b ratio decreased with MJ dosage. Results suggest MJ may provide a viable means of enhancing apple fruit coloration and other photoprotective mechanisms. Chemical name used: methyl 3-oxo-2-(2-pentenyl)cyclopentane-1-acetate (methyl jasmonate).

A high percentage of red peel color is an important grading criterion for many apple (*Malus sylvestris* var. *domestica*) cultivars including ‘Fuji’. Anthocyanins are the class of compounds responsible for red coloration in apple fruit (Sun and Francis, 1967). Temperature, irradiance, and light quality are environmental factors that impact accumulation of anthocyanins in apple peel (Lancaster, 1992; Saure, 1990). Orchard pruning and training practices influence fruit light exposure and, ultimately, anthocyanin production (Heinicke, 1964, 1966). Irradiation of apples with an ultraviolet (UV)–white light mixture promotes anthocyanin synthesis in the peel (Arakawa et al., 1985).

Mineral nutrition and chemical or growth regulator treatments can alter anthocyanin synthesis. N is important for anthocyanin formation; however, excess N can adversely affect red color development (Saure, 1990). Potassium application can reduce the effects of excess N (Weeks et al., 1958). Seniphos (Phosyn PLC, York, United Kingdom), a mixture containing P, Ca, and N, enhances anthocyanin synthesis (Larrigaudiere et al., 1996) as does application of thiocyanate salts, although with deleterious effects on the foliage (Dustman and Duncan, 1940). Treatment of peel disks with various sugars can lead to increased anthocyanin synthesis (Smock, 1966). Daminozide (Uniroyal Chem. Co., Middlebury, Conn.) and paclobutrazol reputedly promote red coloration while delaying maturation whereas auxins, auxin

analog, and ethylene promote coloration while advancing maturity (Blanpied et al., 1975; Saure, 1990).

Jasmonic acid (JA) and its methyl ester are cyclopentanone-based compounds that modulate many physiological processes in plants including wound responses (Creelman and Mullet, 1997) and possibly fruit ripening (Fan et al., 1998). Methyl jasmonate (MJ) enhances anthocyanin synthesis in soybean seedlings (*Glycine max* Merr.) (Franceschi and Grimes, 1991), *Arabidopsis thaliana* (L.) Heynh. seedlings (Feys et al., 1994), tulip bulbs (*Tulipa gesneriana* L.) (Saniewski et al., 1998a), and peach shoots [*Prunus persica* (L.) Batsch. (Peach Group)] (Saniewski et al., 1998b). MJ also promotes degreening of apple peel (Fan and Mattheis, 1999) as well as β -carotene synthesis (Perez et al., 1993).

Therefore, the objective of this research was to determine if exogenous MJ impacts time-wise accumulation of red color as well as the content of anthocyanins, phenolics, and other pigments in UV-visible light treated ‘Fuji’ apples.

Materials and Methods

FRUIT SOURCE. ‘Fuji’ apple fruit were harvested at 121 (Expt. 1) and 130 d (Expt. 2) after full bloom at the U.S. Department of Agriculture, Agricultural Research Service experimental orchard located near Wenatchee, Wash. Fruit were treated the following day for each experiment.

CHEMICALS. MJ and Tween-20 (polyethylenesorbitan monolaurate) were obtained from Aldrich, Milwaukee, Wis.

MJ TREATMENT. Apples were immersed for 2 min in an emulsion containing MJ at 0, 0.224, 1.12, or 2.24 g·L⁻¹ (Expt. 1) or 0 or 2.24 g·L⁻¹ (Expt. 2) and 0.177% (v/v) Tween-20 in

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deionized water (both experiments). After treatment, apples were placed on pressed paper trays and air-dried for ≈ 15 min at 21 °C.

WHOLE-FRUIT ETHYLENE EVOLUTION ANALYSIS. Fruit from each harvest were placed into 19-L plexiglass chambers and C_2H_4 and CO_2 evolution were monitored daily for 7 d. The chambers were purged by a constant flow of Purafil (Purafil Industries, Norcross, Ga.) scrubbed air at 100 mL \cdot min $^{-1}$. Concentrations of CO_2 and C_2H_4 were determined every 24 h using a gas chromatograph (HP-5890 series 2; Hewlett Packard, Palo Alto, Calif.) equipped with a 0.610 m (0.318 cm i.d.) stainless steel column packed with Porapak Q (80/100 mesh) followed in series by a methanizer (John Booker & Co., Austin, Texas) and a flame ionization detector (FID). Flow rates for the N_2 carrier, H_2 , and air were 30, 30, and 300 mL \cdot min $^{-1}$, respectively. Injector, oven, and FID temperatures were 100, 30, and 200 °C, respectively. The methanizer temperature was 290 °C and H_2 at 15 mL \cdot min $^{-1}$ was mixed into the carrier gas through a swept tee upstream of the methanizer inlet.

PEEL COLOR ANALYSIS. Following harvest, the peel of each apple was marked at two sites on the shaded side (not facing the sun on the tree or noncolored side) and color at each site determined using a colorimeter (CR-200; Minolta Corp., Osaka, Japan). Measurements were obtained using the CIE L^* (light to dark), a^* (green to red), b^* (blue to yellow) color space, then a^*b^* values were converted to hue angle (h° , $\tan^{-1} b/a$) (McGuire, 1992). Color measurements were performed before, during, and/or following light treatments.

EXPOSURE TO ARTIFICIAL LIGHT. Following the MJ treatments, fruit were placed ≈ 15 cm (apple surface to lights) under two 2-outlet 1.22-m (length) fluorescent light banks, each containing one 40-W (Sylvania, Versailles, Ky.) cool-white deluxe fluorescent bulb and one 40-W (Phillips, Somerset, N.J.) fluorescent UV lamp. The irradiated area was delimited with aluminum foil. The marked side of each apple faced towards the light source. The intensity of key wavelength ranges were measured at various points within the chamber using a light meter (PMA2100; Solar Light Co., Philadelphia, Pa.) for UV-A and UV-B irradiance and a light meter (LI-250; LI-COR, Inc., Lincoln, Nebr.) for visible light irradiance (400 to 700 nm). The temperature in the light treatment chamber was 25 °C. Apples were exposed to light for 40.3 and 111 h during Expts. 1 and 2, respectively.

PEEL ANALYSIS. Immediately following exposure to artificial light in Expt. 1, peel on the exposed sides of fruit was removed with a fruit peeler, flash frozen in liquid N_2 , and then stored under N_2 gas at -80 °C.

ANTHOCYANIN AND FLAVONOID ANALYSIS. Frozen, crushed peel tissue (0.5 g) was extracted in 2 mL 74 methanol : 25 tetrahydrofuran : 1 HCl (v/v) for 5 h in an ice-water bath covered with aluminum foil. The bath was sonicated for the first and last hour of the extraction period. The extracts were partitioned with 3 mL hexanes. The hexanes phase was discarded and the remaining fraction was centrifuged and filtered.

Pigment composition of the filtered extract was analyzed by reversed-phase high-performance liquid chromatography (HPLC). A 20 μ L sample was injected into a HPLC system (Series 1100; Hewlett Packard) equipped with a 5 mm HP Hypersil ODS (4.0 \times 125 mm) column and a photodiode array (PDA) detector (model 996; Waters, Millford, Mass.). Solvents used for the elution were (A) 1:10:89 H_3PO_4 -methanol-deionized water (v/v) and (B) 1:70:29 H_3PO_4 -methanol-deionized water (v/v). The column temperature was 25 °C and the solvent flow-rate was 1 mL \cdot min $^{-1}$. Solvent A was used for the first 2 min, then a linear gradient of A

plus B was initiated reaching 20% A and 80% B at 34 min. The rate then increased ending at 100% B after 36 min. Chromatograms from 280, 328, 357, and 519 nm were extracted and used for quantification.

CAROTENOID AND CHLOROPHYLL ANALYSIS. About 1 g of frozen, crushed peel tissue from each sample was washed repeatedly, in the presence of 56 mg $CaCO_3$, with cold 75 methanol : 25 tetrahydrofuran (v/v), until colorless. The combined washes, on ice, were partitioned with hexanes until the hexanes phase was colorless. The hexanes phase was then dried under a stream of purified N_2 gas at room temperature. Pigments were dissolved in 75 methanol : 25 tetrahydrofuran (v/v) and clarified using centrifugation and filtration before analysis. Light exposure was minimized throughout the entire procedure.

Samples were analyzed immediately following extraction using the same HPLC system described previously. Solvents used for elution were (A) 80:20 methanol-deionized water (v/v), and (B) ethyl acetate. The flow rate was 1.0 mL \cdot min $^{-1}$. Solvent A was for the first 2 min, then solvent B increased linearly and reached 50% at 21 min. This mixture was maintained until the end of the analysis at 33 min. A chromatogram from 446 nm was extracted and used for quantification.

PEAK IDENTIFICATION AND QUANTIFICATION. Specific peaks were identified using spectral and retention comparisons with authentic standards and quantified by response comparison with authentic standards. The wavelengths at which a peak had its greatest response and least interference were used for quantitation. Chlorogenic acid, catechin, (-)epicatechin, phloridzin, rutin, quercetrin, quercetin, β -carotene, xanthophyll, and chlorophyll b were purchased from Sigma (St. Louis). Hyperin, isoquercetin, idaein, kuromanin, and keracyanin were purchased from Indofine (Somerville, N.J.). Reynoutrin and avicularin were purchased from Plantech (Reading, United Kingdom).

SPECTROPHOTOMETRIC CHLOROPHYLL ASSAY. About 0.5 g of frozen, crushed peel tissue was extracted under N_2 with 3 mL cold 80 acetone : 20 deionized water (v/v) in the presence of 56 mg of $CaCO_3$. Samples were sonicated in a covered ice-water bath during extraction. After 2 h, each sample was decanted and centrifuged for clarification. Optical density of the supernatant at 646 and 663 nm was measured using a diode array spectrophotometer (HP 8451A; Hewlett Packard). Chlorophyll a (chl a) and b (chl b) contents were calculated according to Lichtenthaler (1987) for an 80% acetone extraction.

STATISTICAL DESIGN AND ANALYSES. Experiments were conducted using a randomized complete block design with four or two (Expt. 1 or 2, respectively) treatments with 14 single fruit replicates per treatment for the first experiment and 15 fruit per treatment in the second experiment. Regression analyses were performed on data collected from Expt. 1 using SAS statistical analysis software (SAS Inst., Inc., Cary, N.C.). The SE values for replicates in Expt. 2 were used for inter- and intra-treatment comparisons.

Results

Ethylene production and respiration rate of fruit from both harvests did not change during 7 d at 20 °C (data not presented). This indicates that fruit were preclimacteric throughout each test. Exposure to artificial light alone enhanced red coloration (Figs. 1 and 2) and the change in hue angle (Δh°) during light treatment increased with MJ concentration. Results from Expt. 1 showed that this relationship was more quadratic than linear with increas-

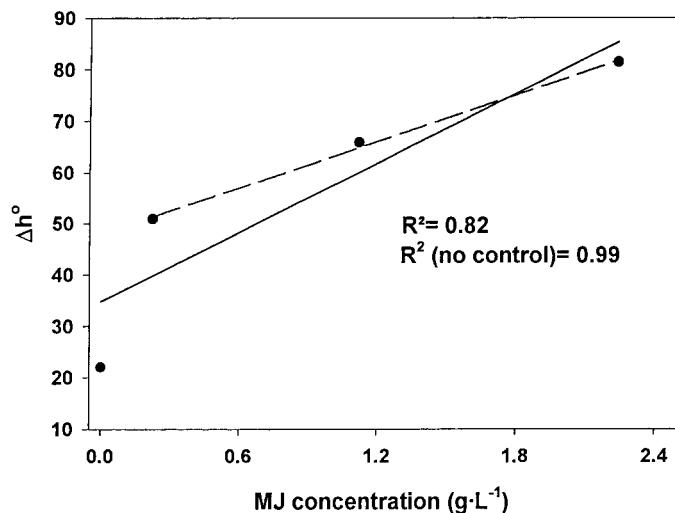


Fig. 1. Change in hue angle (h°) of 'Fuji' apple peel after exposure to visible and UV light (Expt. 1). Apples were treated with methyl jasmonate (MJ) before 40.3 h light exposure. Solid and dashed lines represent regressions with or without control (no MJ), respectively. Regression analyses ($n = 28$; $P \leq 0.05$).

ing MJ concentration (Fig. 1). The cubic trend was the best fit, although it does not provide a realistic model for apple coloration. The rate of red color development increased following an initial lag lasting ≈ 25 h in Expt. 2 (Fig. 2). Red coloration of fruit treated with MJ at $2.24 \text{ g}\cdot\text{L}^{-1}$ increased to a greater degree than that in the controls; however, MJ treatment did not shorten the initial lag period.

Decreased h° may be attributed partially to increased peel anthocyanin content. Initial h° value for both experiments was 115° and reached values as low as 34° in the first experiment and 23° in the second (data not presented). Accumulation of idaein and keracyanin was promoted by exposure to artificial light

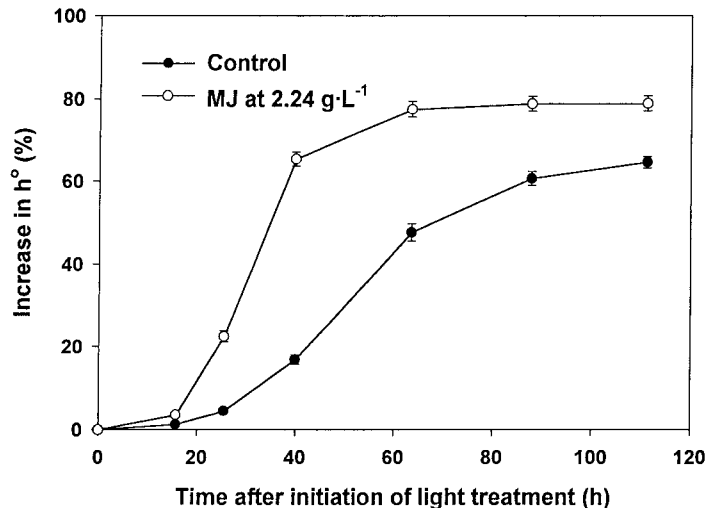


Fig. 2. Effect of light and MJ on red coloration during treatment (Expt. 2). Fruit was exposed to artificial light for 111 h during which multiple peel color readings were taken. Vertical bars represent SE ($n = 30$); where no bars appear, SE was smaller than symbol size.

treatment. Accumulation of idaein (cyanidin 3-galactoside), the major cyanidin glycoside present, increased linearly with MJ dosage (Table 1). Keracyanin (cyanidin 3-rutinoside) and kuromanin (cyanidin 3-glucoside) exhibited similar increases with MJ concentration.

Most quercetin glycosides increased due to artificial light exposure and MJ dosage (Table 1). Hyperin (quercetin 3-O-galactoside), the major quercetin glycoside, increased linearly with MJ concentration. Rutin (quercetin 3-O-rutinoside) and isoquercetin (quercetin 3-O-glucoside) coeluted under these chromatographic conditions. These compounds have similar response factors (RFs), therefore the RF for rutin was used to quantify this

Table 1. Phenolic and pigment constituents of 'Fuji' apple fruit peel (Expt. 1). Fruit were treated with methyl jasmonate (MJ) then exposed to visible and UV light for 40.3 h.

Compound	MJ ($\text{mg}\cdot\text{g}^{-1}$ fresh wt)					Regression analyses		
	Initial	None (control)	at $0.224 \text{ g}\cdot\text{L}^{-1}$	at $1.12 \text{ g}\cdot\text{L}^{-1}$	at $2.24 \text{ g}\cdot\text{L}^{-1}$	Linear	Quadratic	Cubic
Idaein	1.20	54.5	84.8	128	209	*	NS	NS
Kuromanin	ND ^z	ND	ND	ND	0.500	*	NS	NS
Keracyanin ^y	ND	0.767	1.10	1.77	2.77	*	NS	NS
Hyperin	116	675	752	1120	1300	*	NS	NS
Rutin-isoquercetin	31.5	98.0	115	163	183	*	NS	NS
Reynoutrin	77.7	124	109	145	176	*	NS	NS
Avicularin	182	248	228	292	348	*	NS	NS
Quercetrin	159	178	141	164	209	NS	NS	NS
Quercetin	6.70	15.4	8.70	12.7	20.7	NS	NS	NS
Chlorogenic acid	156	322	400	452	568	*	NS	NS
Catechins	16.0	25.0	22.9	20.9	29.4	NS	NS	NS
(-)-Epicatechin	646	742	600	601	616	NS	NS	NS
Phloridzin	105	120	123	162	180	*	NS	NS
Xanthophyll	1.25	1.96	2.40	1.84	2.46	NS	NS	NS
Chlorophyll a ^x	20.8	21.6	24.9	19.1	24.2	NS	NS	NS
Chlorophyll b	7.60	12.7	18.2	16.7	22.5	*	NS	NS
β -Carotene	1.68	2.09	2.35	2.33	3.53	*	NS	NS

^zND = not detected.

^yTentative identification.

^xData acquired using spectrophotometric method.

NS,* Nonsignificant or significant fit ($n = 3$; $P \leq 0.05$). Initial values were not included in regression analyses.

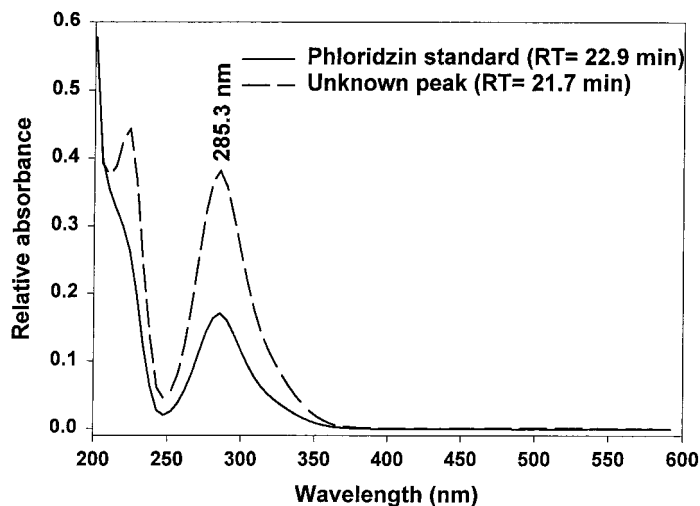


Fig. 3. Comparison of spectra from unknown phloridizin-like peak with that of an authentic phloridizin standard (Expt. 1). RT = retention time.

mixture of components. Rutin–isoquercetin, reynoutrin (quercetin 3-O-xyloside), and avicularin (quercetin 3-O-arabinoside) also increased linearly with MJ concentration. Quercetrin (quercetin 3-O-rhamnoside) concentration was not stimulated by exposure to artificial light or MJ treatment. Quercetin concentration was also unaffected by artificial light exposure or MJ treatment. Chlorogenic acid increased substantially due to light exposure and increased linearly with MJ dosage as did phloridizin (phloretin 5-glucoside). A compound with similar spectra to phloridizin (Fig. 3) but shorter retention time increased with higher rates of MJ. The amounts of monomeric procyanidins, catechin and (–) epicatechin, did not change due to artificial light exposure or with MJ treatment.

Chl a was unstable using the HPLC method. The extraction method for the spectrophotometric chlorophyll assay was faster and less degradation occurred. The amount of chl b determined in both methods were similar. There was no apparent relationship between chl a concentration and treatment (Table 1). Chl b increased sharply due to artificial light exposure and increased linearly with MJ concentration. The chlorophyll a/b (chl a/b) ratio increased slightly due to artificial light exposure and decreased with MJ concentration (Fig. 4). β -Carotene increased due to artificial light exposure and with MJ concentration. There was no relationship between xanthophyll content and exposure to artificial light or MJ treatment.

Discussion

JASMONATES, ANTHOCYANINS, AND ENVIRONMENTAL STRESS. Stimulation of anthocyanin synthesis in ‘Fuji’ apple peel by UV-visible irradiation was enhanced by exogenous MJ. Light exposure is required for anthocyanin production in apple peel (see reviews by Lancaster, 1992; Saure, 1990), and the light requirement was not overcome with MJ treatment. In previous and later experiments, we have found that ‘Fuji’ apple fruit pigments and phenolics reported herein did not change significantly within the relatively short duration of the current experiment when held in the dark (data not presented).

Mechanisms by which MJ enhances anthocyanin synthesis have been suggested to occur via a wound or stress response (Tamari et al., 1995). Physical wounding of apple peel tissue due

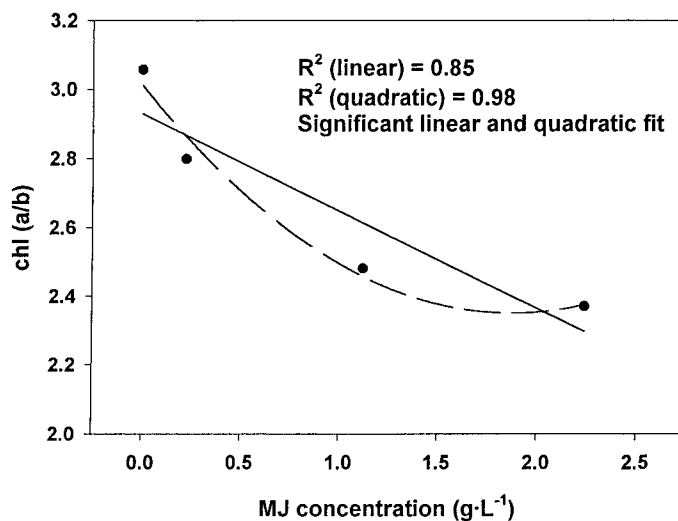


Fig. 4. Relative effect of light and MJ treatment on chlorophyll constituents (Expt. 1). Fruit were treated with artificial light for 40.3 h. Solid and dashed lines represent the linear and quadratic fit, respectively. Regression analyses ($n = 3$; $P \leq 0.05$).

to hail or insect damage during the growing season can lead to a red halo surrounding the affected area. Because jasmonates accumulate in plants or plant organs subjected to stress or wounding (Creelman and Mullet, 1997), apple tissue could be subject to a similar process leading to the direct or indirect stimulation of anthocyanin synthesis. Both of these events have been recorded simultaneously in wounded petunia (*Petunia hybrida* Vilm.) corollas as well as the induction of many genes required for flavonoid biosynthesis (Tamari et al., 1995).

Increased ethylene synthesis resulting from MJ exposure may play a role in stimulation of anthocyanin synthesis. MJ stimulates ethylene synthesis in preclimacteric apple fruit (Fan et al., 1997). This effect increases as the fruit nears the climacteric. However, at the early stage of maturity used in the present experiment it is unlikely that ethylene production induced by exogenous MJ had much of an effect during the short period of this test. Only a very slight reduction in red coloration was observed in ‘Fuji’ apple fruit treated with MJ at $1.12 \text{ g}\cdot\text{L}^{-1}$ following a 12-h treatment with $10 \text{ mg}\cdot\text{L}^{-1}$ 1-methylcyclopropene (1-MCP), an ethylene action inhibitor, when compared to fruit treated with either MJ or exogenous ethylene exposure alone (data not presented). In light of this preliminary evidence, ethylene may have a limited additive effect on anthocyanin synthesis in immature ‘Fuji’ apple fruit.

Chilling stress can lead to increased anthocyanin accumulation in seedlings of maize (*Zea mays* L.), cabbage [*Brassica oleracea* L. (Capita Group)], and sorghum (*Sorghum bicolor* Moench.) (Christie et al., 1994; Rabino and Mancinelli, 1986; Shichijo et al., 1993) as well as *Arabidopsis thaliana* (Leyva et al., 1995) and petunia floral tissue (Shvarts et al., 1997). Low night temperatures lead to more red color accumulation in ‘McIntosh’ apple fruit peel (Creasy, 1968; Uota, 1952). Creasy (1968) also reported anthocyanin accumulation stops during periods of warm weather. Low night temperatures increase phenylalanine ammonia lyase (PAL) activity (Faragher, 1983). This enzyme catalyzes the initial step in anthocyanin synthesis (Lancaster, 1992). Low temperatures can promote anthocyanin accumulation (a red blush) on the light exposed side of the typically green-colored ‘Granny Smith’ apple (Reay, 1999). Similarly, anthocyanin production is greatly enhanced using artificial light with MJ treatment in

'Granny Smith' (data not presented). Furthermore, anthocyanin accumulation occurs in apple and peach shoots during cold acclimation (Leng et al., 2000), the latter of which reportedly increases in anthocyanin content with MJ exposure as already mentioned.

Anthocyanin accumulation in apple fruit requires light suggesting that temperature effects are additive to those stimulated by light (Mol et al., 1996). This additive effect may result from increased enzyme activity including PAL (Faragher, 1983) and/or increased transcription of genes for other enzymes involved in anthocyanin synthesis, such as chalcone synthase (Shvarts et al. (1997). Increased irradiance and/or duration alone could be sufficient to trigger a stress response. Anthocyanins may fill a gap in the absorption of light energy between 500 to 600 nm in light-stressed apple fruit (Merzlyak and Chivkunova, 2000). Enhanced jasmonate and ethylene synthesis is promoted by UV-B generation of reactive oxygen species in wild-type, *jar1*, and *etr1-1* (ethylene insensitive mutant) *Arabidopsis thaliana* suggesting that signaling pathways including these compounds are required for UV-B defense (Mackerness et al., 1999). That light is necessary to stimulate anthocyanin accumulation in MJ-treated apple fruits suggests that the MJ effect is additive to the effects of light alone.

In many mid- to late-ripening red or blush cultivars of apple fruit, the majority of anthocyanin accumulation occurs during the final stages of maturation which, because of the typical climate where they are grown, usually coincides with colder temperatures. In 'Golden Delicious' apples, endogenous JA and MJ concentrations increase a few weeks before the climacteric (Fan et al., 1998), a period that coincides with changes in fruit color. An increase in the incidence of sun scald, a disorder caused in part by high light exposure, occurs after initiation of an increase in red coloration before the climacteric in 'Fuji' apple fruit (Preston Andrews, personal communication), suggesting increased sensitivity to light during this period. With reference to increased light and chilling stress, intracellular transport of jasmonates may mediate signals from the membrane or chloroplasts to other cellular components and visa versa (Creelman and Mullet, 1997).

Upon exposure to artificial light, the maximum rate of anthocyanin synthesis in apple fruit is preceded by an induction period or lag phase (Dong et al., 1995; Faragher and Chalmers, 1977; Saure, 1990; Siegelman and Hendricks, 1958). During this period, relatively little anthocyanin accumulation occurs. The length of the induction period for 'Fuji' apples was ≈ 16 h (Fig. 2) which is consistent with reports for other cultivars.

In the present investigation, the length of the lag phase was not changed by MJ treatment even though the rate of anthocyanin synthesis and the final anthocyanin content was higher in MJ-treated fruit. Enhanced anthocyanin accumulation may result from MJ stimulation of enzyme activity within this pathway or increased substrate availability for this pathway. Increased anthocyanin synthesis without MJ treatment may result partially from an increase in the endogenous jasmonate content promoted by light stress. Taken as a whole, the evidence suggests the effect of MJ on anthocyanin synthesis may be additive to other stimulative and enhancing effects such as light and temperature.

EFFECTS OF MJ-LIGHT TREATMENT ON COMPOSITION OF PIGMENTS AND OTHER PHENOLIC COMPOUNDS. All of the cyanidin glycosides in 'Fuji' apple peel have been identified previously in apple fruit (Gómez-Cordovés et al., 1996; Lancaster, 1992). While Gómez-Cordovés et al. (1996) identified cyanidin 3-arabinoside as the second most abundant cyanidin glycoside in

'Starking Delicious' apples, our results tentatively indicate the compound may be keracyanin. Idaein was the principal compound contributing to red color in 'Fuji' peel, a result consistent with other reports (Dong et al., 1995; Sun and Francis, 1967; Timberlake and Bridle, 1971).

All of the quercetin glycosides identified in 'Fuji' have been detected previously in apple peel (Dick et al., 1987; Oleszek et al., 1988). However, the present study appears to be the first report of quercetin in apple fruit. While it is possible quercetin may result from hydrolysis of one or more quercetin glycosides during the extraction process, repeated analyses of a single sample did not result in a noticeable increase in quercetin or decrease in quercetin glycosides.

Increased light exposure results in a higher peel content of quercetin glycosides (Awad et al., 2000; Lancaster et al., 2000), and our results indicate most of the quercetin glycosides, including hyperin, increased with MJ concentration as well. Quercetrin did not increase with light or MJ treatment, possibly due to a difference in metabolic origin or regulation of UDP-rhamnose synthesis. Increased quercetin glycoside synthesis may be expected considering the structural and metabolic similarities between the quercetin and cyanidin glycosides. These classes of compounds are synthesized by combination of the specific aglycon and UDP-mono or disaccharide catalyzed by a flavonoid glycosyltransferase (Lister et al., 1997). The aglyconic portion of these compounds are synthesized from the same intermediates as cyanidin which occurs two metabolic steps downstream from the precursor (dihydroquercetin) to quercetin.

The effects of light and MJ treatment on catechin, (-) epicatechin, phloridzin, and chlorogenic acid in our study varied. The monomeric procyanidins catechin and (-) epicatechin, the former a minor and the latter a major constituent of apple peel (Lancaster et al., 2000; Prabha and Patwardhan, 1985), did not change in response to light or MJ treatment. Lack of a response to light is consistent with previous reports (Awad et al., 2000; Lancaster et al., 2000). Lack of MJ effect on the catechin-epicatechin content is notable as polyphenoloxidase (PPO), an enzyme that catalyzes oxidation of these compounds and is promoted by stress, is activated by MJ (Masia et al., 1998). Moreover, these compounds are synthesized indirectly, as with quercetin and cyanidin, from dihydroquercetin (Lancaster, 1992). This may indicate that availability of the UDP-saccharide moiety for synthesis of certain quercetin and cyanidin glycosides may be enhanced by MJ. Sugar treatment of fruit and floral structures can lead to enhanced anthocyanin synthesis (Smock, 1966; Weiss, 2000) and certain sugars may be involved in a complex anthocyanin synthesis signaling scheme in grape (*Vitis vinifera* L.) (Vitrac et al., 2000). Jasmonates could also modulate the genes or proteins directly responsible for synthesis of quercetin and cyanidin glycosides. The procyanidin dimers B2 and B5 are major constituents of apple peel (Lancaster et al., 2000), although we did not identify them in this study. A buildup of these products may evince up-regulation of this pathway that could not be detected by just measuring the monomeric precursors.

Chlorogenic acid and phloridzin were two other major phenolic components detected in this study. Both chlorogenic acid and phloridzin increased with light exposure and MJ concentration, while the unknown constituent with a phloridzin-like spectra increased with MJ concentration. Phloretin xyloglucoside (Oleszek et al., 1988) or phloretin xylogalactoside (Burda et al., 1990) elute before phloridzin on a C-18 column as with our unknown compound. Lancaster et al. (2000) reported an increase

in chlorogenic acid with artificial UV-B exposure whereas Awad et al. (2000) noted chlorogenic acid content is similar in peel from the exposed and shaded sides of the fruit. Phloridzin content reportedly differs little between the exposed and shaded side of the fruit (Awad et al., 2000). Chlorogenic acid is also a substrate for PPO in apple fruit (Prabha and Patwardhan, 1985); however, phenolic acids, including chlorogenic acid are derived from a different branch in the phenolic synthesis pathway. Phloridzin is a dihydrochalcone glucoside product of chalcone or, possibly, caffeic acid (Lancaster, 1992). Regardless of its origins, phloridzin and the compound with the related spectra accumulate with a pattern similar to the flavonoid glycosides, again suggesting accumulation of specific UDP-saccharides or the glycosylation of the chromophore may be affected by MJ exposure.

Chlorophyll and carotenoid contents were also affected by light and MJ treatment. Methyl jasmonate stimulates chlorophyll degradation, either alone (Emery and Reed, 1996; Perez et al., 1993) or in concert with ethylene (Hung and Koa, 1996). Cuello (1997) suggested that chl a degrades more quickly than chl b in barley (*Hordeum vulgare* L.) following MJ treatment and the chl a/b ratio in 'Golden Delicious' apple peel decreases with increasing MJ exposure (Perez et al., 1993). Light treatment was not used by Perez et al. (1993). While this aspect mirrors our results, these authors also report a decrease in chlorophylls a and b as well as xanthophyll. Our results show a significant chl b increase and a relatively stable chl a and xanthophyll content. Lack of chlorophyll loss may result from increased chlorophyll synthesis due to the light treatment in our study (Table 1). Other factors that may result in lack of chlorophyll reduction could be low ethylene synthesis by these preclimacteric fruit, or the relatively short duration of these experiments when compared to Perez et al. (1993) (10 d). Xanthophyll content may closely match that of chlorophyll a because of a more direct or different role in photoprotection.

β -Carotene increased with light and increasing MJ exposure in our test in agreement with previous reports (Perez et al., 1993; Saniewski and Czapski, 1983) although Perez et al. (1993) did not use a light treatment. Peel from 'Fuji' apple fruit, treated with MJ but kept in the dark for a similar length of time, did not have increased β -carotene content (data not presented).

In conclusion, treatment of 'Fuji' apples with MJ followed by UV-visible light treatment promotes cyanidin and quercetin glycoside (with the exception of quercetrin), chlorogenic acid, phloridzin, and β -carotene synthesis in apple fruit. While glycoside accumulation increases, closely related chromophoric moieties and monomeric procyanidins do not increase, suggesting that MJ may regulate formation of UDP-saccharides. The lag in anthocyanin synthesis induction by UV-visible light was not affected by MJ treatment indicating that induction of this pathway is not induced by MJ. Instead, MJ may have an "additive" effect on anthocyanin synthesis similar to that suggested for temperature. It is unclear whether MJ had an effect on chlorophyll degradation because of the possibility of simultaneous synthesis and degradation. However, the chl a/b ratio decreased similar to other reports. MJ enhanced β -carotene but not xanthophyll synthesis. Mechanisms by which MJ augments these diverse pathways need further elucidation. Results indicate that MJ may be a useful tool for stimulation of red coloration in commercial apple fruit.

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