Improving the Red Color and Fruit Quality of ‘Kent’ Mango Fruit by Pruning and Preharvest Spraying of Prohydrojasmon or Abscisic Acid

Sudheeran Pradeep Kumar 1, Dalia Maurer 1, Oleg Feygenberg 1, Cliff Love 2 and Noam Alkan 1, *

1 Department of Postharvest Science of Fresh Produce, Agricultural Research Organization (ARO), Volcani Center, P.O. Box 15159, HaMaccabim Road 68, Rishon LeZion 7505101, Israel; pradeep@volcani.agri.gov.il (S.P.K.); daliam@volcani.agri.gov.il (D.M.); fgbolog@volcani.agri.gov.il (O.F.)
2 Extension Service, Ministry of Agriculture and Rural Development, Rishon LeZion 7505101, Israel; cliff1love@gmail.com

* Correspondence: noamal@agri.gov.il; Tel.: +972-3-9683605 or +972-3-9683606; Fax: +972-3-9683220

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Abstract: Pre-harvest application of prohydrojasmon (PDJ) or abscisic acid (ABA) induces the red color in fruits that were exposed to sunlight at the orchard. In this large-scale work, we evaluated the effect of two different pruning techniques of ‘Kent’ mango orchards, one leading to opening the orchard canopy to expose as much fruit as possible to sunlight, while the second pruning leads to square-shaped trees and subsequently reduces the amount of sunlight reaching the fruit. These two pruning methods were combined with preharvest spraying with prohydrojasmon (PDJ) or abscisic acid (ABA) using two different types of sprayers, i.e., regular and air-jet sprayer. Pruning the canopy of the orchards to open and closed trees exposed 80% or 30% of fruits to sunlight, respectively. Both of the application with air-jet and regular sprayers effectively covered the fruit without causing fruit detachment and damage to yield. Both the phytohormones (PDJ and ABA) application treatments induced red blush skin, red intensity, anthocyanin, and flavonoids, particularly in fruit grown outside the tree canopy in both open and closed trees. PDJ and ABA treatments exhibited marginally reduced acidity than the untreated control, while the brix was not affected much by any of the treatments. Besides these, exposure to sunlight and PDJ treatment also reduced postharvest decay and increased chlorophyll degradation and yellowing in comparison to the controls. This study promoted applicative evidence about the positive effects of exposure to sunlight, prohydrojasmon (PDJ), and abscisic acid (ABA) on red color development without compromising the mango fruit’s quality.

Keywords: anthocyanin; blush; prohydrojasmon; abscisic acid; flavonoids; mango; preharvest; red color; fruit quality

1. Introduction

The mango (Mangifera indica L.) is one of the most popular fruits in the world because of its attractive red to yellow color, taste, and nutritional properties [1]. During ripening, most mango cultivars change color from green to yellow. Red-blushed mango skin color plays a vital role in fruit marketability and consumer acceptance [2]. Thus, several breeding programs focus on developing enhanced red-colored cultivars with improved flavor [3].

Several factors influence the accumulation of anthocyanins and carotenoid pigments, including light, temperature, sugars, mineral, nutrition, and the impact of plant hormones. Besides these, orchard management practices such as bagging, pruning, and fertilization can also strongly impact on fruit color pigmentation [4]. Anthocyanins are the most diverse group of plant pigments and derived from...
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secondary metabolites of phenylpropanoids that contribute to the red-colored appearance of mango skin. They are extensively spread in vascular plants where they serve as an inducible sun shield [5]. Anthocyanins have the antioxidant capacity, and are essential phytonutrients in a healthy diet, with antitumor, pro-apoptotic, anti-oxidative, anti-inflammatory, and beneficial effects of human health [6–8]. It is one of the main anthocyanin compounds contributing to the red color of mango fruit skin is cyanidin-3-O-galactoside and methylcyanidin 3-O-β-D-galactopyranoside [9–11]. Prohydrojasmon (PDJ) is an analog of the phytohormone jasmonic acid (JA), which induces blushed coloring of fruit. Prohydrojasmon was selected among JA analogs due to its low environmental load, fast degradation, and low toxicity to animals [12]. Mango fruit at the outer side of the canopy that was exposed to sunlight and pre-harvest application of prohydrojasmon accumulated anthocyanins and reddish skin [11]. Red mango fruits that accumulate anthocyanin and flavonols are known to have an improved cold tolerance and better tolerance to fungal pathogens [13–15].

The role of abscisic acid (ABA) in the development of red skin color during the ripening of non-climacteric fruits is well documented [16–18]. Exogenous application of ABA in vineyard increases levels of the anthocyanins, grape skin color, and grape quality [16,17]. Similarly, the application of ABA also improves color development in strawberries by enhancing the biosynthesis of anthocyanins during storage [18]. Combined application of ABA and Ethrel to Litchi (Litchi chinensis. Sonn) was effective in enhancing both chlorophyll degradation and anthocyanin biosynthesis [19]. The role of (ABA) in enhancing fruit red color development was also reported in apple and peach [20,21].

The objective of this study was to investigate in commercial-scale the efficiency of pruning methods, and pre-harvest spraying of abscisic acid (ABA) and prohydrojasmon (PDJ) in mango orchards. The results were measured by the effects of the practices on the induction of red color development in ‘Kent’ mango fruit skin, fruit quality, and resistance to pathogens. Improving the mango fruit quality would increase the economic value of this product.

2. Materials and Methods

2.1. Plant Material and Storage Conditions

Two mature fully developed 20-year old ‘Kent’ mango orchards at the Kibbutz Ravid, (32°51’03” N 35°27’52” E; elevation +165 m; Mediterranean climate) were pruned in two different manners. The first orchard (open trees) was mechanically pruned—topping and hedging following the harvest at the beginning of August 2017. Additional pruning was undertaken during the second half of November 2017. The trees were manually pruned by selectively removing the internal main apical stem and opening the tree and fruit to sunlight by removing these branches (Figure S1 in supplementary information; 80% of the fruits were exposed to the sunlight by manual evaluation of 8 trees at harvest). The second pruning method (closed trees) was applied only following the previous year’s harvest (August 2017) by a tractor driven mechanical pruning disc that shaped the trees relatively squarely. No internal pruning followed up on these trees (Figure S1 in Supplementary information; 30% of fruits are exposed to the sunlight by manual evaluation of 8 trees at harvest).

During flowering, a weekly application of fungicides were applied against powdery mildew. Nutrient (nitrogen, phosphorus, and potassium) were supplied in the drip irrigation system throughout the irrigation season from spring to late fall. The following fruiting season, orchards were (pre-harvest) sprayed with 0.1% abscisic acid (S-ABA; the commercial product ProTone; Valent BioSciences, Libertyville, IL, USA) or 0.2% prohydrojasmon (PDJ; commercially labeled Blush; Fine Agrochemicals Ltd., Walnut Creek, CA, USA) or combination of products (0.2% ABA + PDJ). The spray of PDJ was applied twice—4 and 2 weeks before harvest, and the ABA was applied just two weeks before harvest. The control trees are non-treated. The spray was conducted with two different types of sprayers, i.e., with ‘Regular Boom Turbo Degania Sprayer’ commonly used in the local industry with radial emitters (1200 L per hectare) and ‘Air-Jet Sprayer’ (OHAD Sprayers, Rishon LeZion, Israel) with cylindrical system emitters (800 L per hectare). Both sprayers delivered full coverage of the fruit. Fruits were subsequently harvested in July 2018.
Each biological treatment consisted of 20–24 trees in one row, with one row separating between treatments. Four replications were conducted for each treatment spread randomly in each orchard (one orchard for closed trees and one orchard for open trees). Two boxes (4 KG, 40 × 25 × 10 cm) of fruit were harvested from the middle trees in each repetition (four repeats in each treatment, 8 cardboard boxes/treatment) in 2018. The fruits from the internal area of the canopy (not subjected to sunlight) were harvested separately than the fruits from the external periphery of the canopy (subjected to sunlight) (8 cardboard boxes/treatment). Measurements of radiation were conducted for fruits from the outer canopy received a considerable amount of sunlight (photon flux of 1500 ± 200 µmol m−2 s−1), while the fruit from inside the canopy received a relatively small amount of sunlight (photon flux of 11.5 ± 6 µmol m−2 s−1), measured at harvest by an HD2302 Photo-Radiometer (Delta-Ohm Srl, Caselle di Selvazzano (PD), Italy). The fruits were transported to Volcani Center, Israel (1.5 h), stored at 12 °C for 21 days, and then transferred to 20 °C for 7 days to mimic commercial practices and shelf life.

2.2. Measurements of Mango Fruit Skin Color

Individual mango fruits, from each treatment, at various time points (at harvest, cold storage, and after shelf life), were evaluated for the percentage of the red color surface area in each fruit. In addition, the fruit red color intensity was assessed on a scale of (0–5; 0-no red color, 1-faint red color, and 5-very intense red color). Data was collected and evaluated from fifty fruits per treatment.

Chlorophyll, anthocyanin, and flavonoid content were also measured by the Multiplex III fluorescence detector (Force A, Orsay, France), which consists of 12 fluorescence signals. The ratios between these signals in different mathematical expressions were interrelated to the fluorescence of major chemical groups, e.g., anthocyanin (FER_RG, the ratio of infra-red emission excited by red or green light), flavonoids (FLAV) and chlorophyll (SER_R). The methodology was followed according to [22,23]. Ten fruits, each from the red side and the green side, were evaluated for each treatment.

The mango skin color (Hue) was measured for ten mango fruits for each treatment using a CR-400/410 Chromometer (Konica Minolta, Osaka, Japan) at two points on the equatorial line of each fruit (20 measurements per treatment). The hue angle (h°) measures color (120 represents green color; 60–70 represents yellow color; 30–40 represents red color).

2.3. Mango Fruit Ripening Parameters and Fruit Decay

Ripening and physiological parameters of mango fruit-firmness, color change (yellowing), total soluble solids (TSS), and percentage of titratable acidity (citric acid equivalents) were determined for the treatments against control at progressive time intervals during the storage period. The color change (yellowing) was measured after cold storage, and further shelf life qualitatively by visual skin color appearance on a relative scale of 1–10 (1 represents no color change, green fruit; 10 represents fruit with the full-color change, yellow fruit; 80 fruits the per treatment). Fruit firmness (Newton) was determined by a hand-held penetrometer (LT-Lutron FG-20 KG, Jakarta, Indonesia) with an 11-mm probe at two points on the equatorial line of each fruit (10 measurements per treatment at each time point). TSS content (percentage) was measured for the fruit pulp juice using Digital Palette refractometer PR-1 (Model DBX-55, Atago, Tokyo, Japan), with 10 measurements per treatment. For determination of total acidity, 1 mL of pulp juice was dissolved in 40 mL double-distilled water and determined as citric acid equivalent mass using an automatic titrator (Titrino Model 719s, Metrohm Ion Analysis Ltd., Herisau, Switzerland). Ten fruits per treatment were measured. The percentage of stem-end rot and Alternaria rot were measured following cold storage and post shelf life in both ‘open and closed’ trees of each box and presented as the average decayed fruit and standard error (80 evaluations per treatment).

2.4. Statistical Analysis

Data presented corresponds to the mean value ± standard error (SE). Multifactorial analysis of variance (ANOVA, Tukey–Kramer HSD test) was performed using JMP (JMP Pro 14 software,
Application of PDJ showed a general decrease in h° color with values of 22.6 ± 3.9 to 24.7 ± 4.1 from outside the tree canopy with both sprayers in comparison to untreated control from the inside of tree canopy with h° value of 125 ± 3.3 to 129.3 ± 1.2 in open and closed trees, respectively (Figure 2C, F).

Thus, in most cases, PDJ significantly reduced the h° color with values of 22.6 ± 3.9 to 24.7 ± 4.1 from outside the tree canopy with both sprayers in comparison to untreated control from the inside of tree canopy with h° value of 125 ± 3.3 to 129.3 ± 1.2 in open and closed trees, respectively (Figure 2C, F).

3. Results

3.1. Evaluation of Mango Fruit Color Parameters

The application of either PDJ or ABA resulted in full coverage of fruit. It did not lead to damage by a detachment of fruit in the orchard. Mango fruits treated with 0.2% PDJ before the harvest with either regular sprayer or airjet sprayer showed significant induction of red-colored fruit surface coverage in comparison to untreated control. Similarly, fruit from the exterior position with direct exposure to sunlight were significantly redder in comparison to fruit from the inner canopy (Figures 1, 2 and S2).

![Representative pictures of boxes from different treatments.](image)

**Figure 1.** Representative pictures of boxes from different treatments. Fruit harvested from orchard pruned to create (A) open trees or (B) closed trees. PDJ-treated (0.2%, 2, and 4 weeks preharvest) with a regular sprayer or air-jet sprayer or untreated control in ‘Kent’ mango fruit after storage and shelf life.

It can be visually observed from the representative picture from each treatment that after shelf life, fruit from the interior canopy position, in the shaded part of the canopy, are green in color, while fruits from the outer canopy were redder (Figure 1). The preharvest treatment with PDJ on fruit from the outer canopy was more effective than on fruit from the inner canopy. A similar trend was seen after cold storage and after shelf life (Figures 1 and 2). Interestingly, during cold storage and shelf life, the fruit acquired a small amount of red color (Figure 2). PDJ significantly induced red color coverage of the fruit skin and the red color intensity mainly in fruit from the outer canopy in comparison to untreated control (Figures 2 and S2). The effect of exposure to the sunlight (a peripheral portion of the tree canopy) in the untreated control on the percentage of red color coverage and on the red color intensity was higher in fruit from the opened trees (Figure 2). In most cases, there was no difference in the induction of red color in ‘Kent’ mango fruit by the spraying methods (Figures 1 and 2).

Application of PDJ showed significant differences in Hue in comparison to untreated control. Application of PDJ showed a general decrease in h° color with values of 22.6 ± 3.9 to 24.7 ± 4.1 from outside the tree canopy with both sprayers in comparison to untreated control from the inside of tree canopy with h° value of 125 ± 3.3 to 129.3 ± 1.2 in open and closed trees, respectively (Figure 2C,F). Thus, in most cases, PDJ significantly reduced the h° value and stimulated the development of the red color of mango fruit (Figures 2 and S2).
Thus, in most cases, PDJ significantly reduced the $h^o$ value and stimulated the development of the red color of mango fruit (Figure 2 and Figure S2).

**Figure 2.** Quantification of color parameters of 'Kent' mango fruit. Orchards were treated with prohydrojasmon (PDJ) with different sprayers; the fruit were harvested separately from inside or outside of tree canopy and stored in cold storage (3 weeks at 12°C; CS) and additional shelf life (7 days at 20°C; SL). (A) Red surface area of fruit from open trees (percentage). (B) Red color intensity of fruit skin (index 0–5) from open trees. (C) Hue value for fruit skin from open trees. (D) Red surface area of fruit from closed trees (percentage). (E) Red color intensity of fruit skin (index 0–5) from closed trees. (F) Hue value for fruit skin color from closed trees. Mean ± SE is presented. Columns labeled by different letters are significantly ($p < 0.05$) different within each time point (lower-letter for harvest and capital-letter for shelf life) according to the Tukey–Kramer LSD test. (CS; Cold Storage, and SL; Shelf-life).

### 3.2. Estimation of Chlorophyll, Anthocyanin and, Flavonoids in Fruit Skin

The effect of prohydrojasmon (PDJ) on fruit skin fluorescence was determined for chlorophyll (SER_R), anthocyanin (FER_RG), and flavonoids (FLAV). The chlorophyll (SER_R) contents in control—untreated fruit from inside tree canopy was higher than the chlorophyll level in fruit from the outside of the tree canopy.

Concurrently the levels were higher than the PDJ treated fruit (Figure 3A,D). A similar trend was observed both in open and closed trees. PDJ treatment to fruit from outside the tree canopy showed a significant increase up to 4-fold higher anthocyanin content (Figures 3 and S3). Whereas flavonoids content was only marginally increased by PDJ application (Figure 3).
Figure 3. Effect of pre-harvest treatment with prohydrojasmon (PDJ) on Chlorophyll, Anthocyanin, and Flavonoids quantification by multiplex fluorescence after Shelf-life (SL). (A) Chlorophyll fluorescence from open trees. (B) Anthocyanin fluorescence from open trees. (C) Flavonoids fluorescence from open trees. (D) Chlorophyll fluorescence from closed trees. (E) Anthocyanin fluorescence from closed trees. (F) Flavonoids fluorescence from closed trees. Mean ± SE is presented. Different letters indicate significant differences (p < 0.05), according to the Tukey–Kramer LSD test.

3.3. Fruit Decay

‘Kent’ fruit treated with PDJ showed non-significantly less ‘stem end rot’ caused mainly by Lasiodiplodia theobromae but also by Dothiorella dominicana, D. mangiferae, Neofusicoccum spp., Phomopsis mangiferae, Cytosphaera mangiferae, Alternaria alternata and Colletotrichum gloeosporioides [24] than untreated control after shelf life (Figure 4A) in the open tree network. Similarly, also in the closed tree, there was marginally less ‘stem end rot’ in the PDJ treated fruit in the outer tree canopy in comparison to the control (Figure 4C). The natural ‘Alternaria rot’ percentage was non-significantly decreased in fruit from the outside of tree canopy in comparison to fruit from the inside of tree canopy (Figure 4B,D) and in fruit treated with PDJ in comparison to the untreated control (Figure 4B,D).
Figure 4. Effect of preharvest prohydrojasmon (PDJ) treatments on postharvest disease incidence in ‘Kent’ mango fruit after cold storage (CS) and shelf life (SL). (A) Stem-end rot incidence (percentage) from open trees. (B) Alternaria rot incidence (percentage) from open trees. (C) Stem-end rot incidence (percentage) from closed trees. (D) Alternaria rot incidence (percentage) from closed trees. Mean ± SE is presented.

3.4. Effect of ABA on Mango Fruit Skin Color in Open Trees

The effect of the application of ABA or PDJ or their combination on ‘Kent’ mango fruit (open trees orchards) before harvest with ‘regular sprayer’ were evaluated on fruit skin color. Both ABA and PDJ and their combination induced red-colored on the fruit skin both on internal and peripheral fruit (Figure 5). The induction of red color was visible at harvest and was maintained during cold storage and subsequent shelf life (Figure 5A,B). PDJ enhanced color in fruit from the inside of tree canopy, while ABA-induced better color in fruit from the outside of tree canopy in comparison to untreated control (Figures 5 and 6). The combination of PDJ and ABA did not lead to a higher induction in color in comparison to each of the compounds separately.

Hue color parameters in the treated fruit from outside of tree canopy showed a general decrease in $h^\circ$ color value to (20.4 ± 1.1 to 24.4 ± 4.0), while the untreated control from the inside of tree canopy had $h^\circ$ color value of (125.9 ± 3.3 to 91.3 ± 3.0) (Figure 5C). PDJ, ABA, and their combination significantly reduced the $h^\circ$ value and stimulating the development of redder color in mango fruit (Figures 5, 6 and S4).
Figure 5. Quantification of color parameters of ‘Kent’ mango fruit. ‘Kent’ mango orchard with open trees were treated with prohydrojasmon (PDJ), or Abscisic acid (ABA) and their combination, the fruit were harvested separately from inside or outside the tree canopy and stored for three weeks in 12 °C cold storage (CS) and additional shelf life (SL) at 20 °C. (A) Red surface area (percentage of fruit). (B) Red color intensity (index 0–5). (C) Hue value for fruit skin. Values are mean ± SE. Columns labeled by different letters are significantly (P < 0.05) different within each time point by the Tukey–Kramer LSD test. (lower-letters for harvest and capital-letters for shelf life).

Figure 6. Representative pictures of ‘Kent’ mango fruit from treatments with prohydrojasmon (PDJ), or Abscisic acid (ABA) and their combination on an orchard with open trees, after storage and shelf life. Fruit harvested from (A) Inside the canopy, and (B) Outside the canopy.

3.5. Evaluation of Chlorophyll, Anthocyanin and Flavonoids in Fruit Skin

The effect of PDJ, ABA, and their combination on fruit skin color was evaluated for chlorophyll (SER_R), anthocyanin (FER_RG), and flavonoids (FLAV) in open trees. The chlorophyll contents in
untreated fruit were higher than the treatments with PDJ and ABA (Figure S5A). The chlorophyll levels from inside tree canopy were higher than outside tree canopy, which was higher than the treatments with PDJ and ABA (Figure S5A). Whereas anthocyanin and flavonoids were higher in fruits from outside the tree canopy, and ABA or PDJ treatments had higher anthocyanin content and flavonoids content than untreated control (Figure S5B,C).

3.6. Evaluation of Physiological Parameters and Fruit Decay

Preharvest application of PDJ and ABA in the orchard had a relatively minor effect on fruit ripening after cold storage or after shelf life. The treated fruit with PDJ and ABA were softer after cold storage in comparison to untreated controls, while no significant differences in firmness were noticed between the treatments after shelf life, whereas fruit from outside tree canopy were firmer than fruit from inside the tree canopy (Table S1). Similarly, no differences were detected between the treatments in TSS, and a minor reduction in total acidity was noticed in response to ABA (Table S1). Interestingly, the treated fruit showed a constant increase in color change from green to yellow. This difference in yellowing was intensified after shelf-life storage (Figure S5F), which is the desired quality trait for mango fruit. Additionally, fruit from an outside tree canopy had a decreasing percentage of stem-end rot in comparison to fruit from inside the tree canopy (Figure S5D).

4. Discussion

Previous studies have shown that the red color is an essential factor in market apple acceptance [25,26]. Similarly, the red mango is desirable and could be marketed at an increased value. As grown in Israeli orchards, ‘Kent’ mango fruit is relatively green and with minimal red tint [11]. Therefore, the goal of this study was to induce the desirable red color in the ‘Kent’ cultivar on a commercially implemented and applicable scale.

In our previous study, we had shown that mango fruit (cv. Shelly), which developed on the peripheral portion of the tree canopy, is subjected to sunlight, developed an enhanced red color, while fruit that developed at the internal portion of tree canopy remains green [14]. Also, the application of phytohormones could affect the phenylpropanoid pathway and lead to induced resistance to control postharvest decay of fruits and vegetables [27]. For example, preharvest application of S-ABA increases levels of the anthocyanins, and red color skin in grape, strawberries, Litchi, apple and peach [16,17,20,21]; while, preharvest application of PDJ induced red skin and improved mango fruit quality in ‘Kent’, ‘Shelly’, and ‘Maya’ [11]. The current study examines preharvest application with PDJ, ABA, and their combination and the effect of different commercial sprayers in two different pruning technique’s on mango fruit color and quality.

We demonstrate that the application of PDJ or ABA significantly increases the red color surface coverage and red intensity of fruit skin, leading to more appealing ‘Kent’ mango. Fruit’s from outside tree canopy showed a 2-fold increase of anthocyanin in comparison to the inner side of the tree canopy (Figure 3). Application of PDJ and ABA further increased the anthocyanins and flavonoid levels in peripheral fruit that were subjected to sunlight in comparison to fruit from the internal tree canopy. However, both PDJ and ABA did not increase flavonoid levels. These results indicate that fruit exposure to sunlight induces the whole phenylpropanoid pathway [28], while both ABA and PDJ probably induce mainly the anthocyanin synthesis part of the pathway.

Treatments of PDJ or ABA did not affect the majority of the ripening parameters, such as TSS and total acidity (Table S1). In apple, methyl jasmonate enhanced the synthesis of anthocyanin in fruit skin without affecting fruit quality [29]. Similarly, the preharvest application of abscisic acid (ABA) promotes a significantly increased concentration of anthocyanins in the pericarp of litchi fruit without major effects on postharvest quality [30].

Color change from green to yellow is a desired characteristic for mango fruit. PDJ and ABA had a significant effect on chlorophyll degradation and color change from green to yellow in comparison to the non-treated control. Similarly, the application of PDJ or ABA reduced chlorophyll content after
storage, which indicates that both PDJ and ABA induce chlorophyll degradation. Indeed, in our previous work, we observed that PDJ induce chlorophyll degradation [11], and it is well documented that ABA acts together with ethylene and leads to chlorophyll degradation [31].

Peripheral fruits that were exposed to sunlight had less postharvest decay (Figures 4 and S5) as was previously observed [14,15]. Mango fruit that were exposed to sunlight accumulated antifungal flavonols and anthocyanins, which increased their resistance to various fungal pathogens [14,15]. Also, preharvest PDJ application to mango fruit had a minor effect in reducing postharvest decay after storage and shelf life. Similarly, in our previous manuscript, the preharvest application of PDJ reduced postharvest decay [11]. It has also been reported that exogenous MeJA applications enhance postharvest disease resistance in fruit, reducing fungal attacks, allowing a longer and better postharvest life [32,33]. Therefore, the accumulation of anthocyanin and red color in the skin of mango fruit is correlated to increased fruit resistance to postharvest fungal pathogens and chilling.

In this work, we also checked the effect of commercial spray with either regular or air-jet sprayer. The application of either PDJ or ABA resulted in full coverage of fruit, and the application before harvest did not lead to damage by a detachment of fruit. Whereas, the color induction and the fruit quality were similar in air-jet and in regular sprayers. The air-jet sprayer uses a higher volume of air with smaller droplets of aerosols [34]. Thus, a lower amount of phytohormones was applied and lead to a similar effect of induction of red color (Figure 1) and reduction of decay (Figure 4).

Another aspect of this work included the effect of pruning practice to enhance fruit quality in modern agriculture. Eighty percent of the fruits in the opened trees pruning (late fall period, manually pruned) were exposed to sunlight, while only 30% of the fruits in the trees that were automatically pruned (previous fruiting season) were exposed to sunlight. Therefore, in the manually pruned orchard, 50% more fruit developed red color in comparison to the closed orchard. Additionally, the PDJ and ABA had much better activity in fruits that were directly exposed to sunlight, especially in the opened orchard. Those fruit’s developed better red color and had increased chlorophyll degradation and yellowing (Figures 1 and 3).

5. Conclusions

The exogenous application of phytohormones as PDJ or ABA in combination with exposure to sunlight enhanced the fruit red color development probable by induction of phenylpropanoid pathway in mango fruit, leading to increased anthocyanin and chlorophyll degradation that lead to a more appealing fruit with a minor effect on fruit ripening. Furthermore, exposure to light and pre-harvest application of PDJ reduced postharvest decay, which controls fruit loss. Thus, pruning regimes that expose ‘Kent’ cultivar fruit to sunlight and preharvest treatments with phytohormones and their derivatives can increase the production of secondary metabolites such as flavonols and anthocyanins and other antioxidants molecules, enhancing the fruit quality and postharvest life, and fruit health properties.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/7/944/s1, Table S1. Preharvest application of prohydrojasmon (PDJ) or Abscisic acid (ABA) on ripening parameters. Figure S1. Representative picture of pruning results. Figure S2. Detailed statistics of the effect of PDJ applied with different sprayers on color parameters in ‘Kent’ mango fruit presented in Figure 2. Figure S3. Detailed statistics of the effect of PDJ on chlorophyll, or anthocyanin, or flavonoids in ‘Kent’ mango fruit grown inside or outside the tree canopy, and presented in Figure 3. Figure S4. Detailed statistics of the effect of PDJ or ABA or their combination on color parameters in ‘Kent’ mango fruit grown inside or outside the tree canopy, and presented in Figure 5. Figure S5. Effect of preharvest treatments of prohydrojasmon (PDJ) or Abscisic acid (ABA) and their combination of color parameters and postharvest disease incidence in ‘Kent’ mango fruit.

Author Contributions: S.P.K. conducted experiments, analyzed the data, and prepared the manuscript; D.M. conducted the experiments and analyzed the data; O.F. conducted the experiments and analyzed the data; C.L. designed the experiments and analyzed the data, and prepared the manuscript; N.A. coordinated the experiments, data analysis, and the manuscript preparation. All authors have read and agreed to the published version of the manuscript.
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References

1. Alkan, N.; Kumar, A. Post-harvest storage management of mango fruit. In Achieving Sustainable Cultivation of Mangoes; Sauco, V.G., Lu, P., Eds.; Burleigh Dodds Science Publishing Limited: Cambridge, UK, 2018; pp. 377–393.

2. Medlicott, A.; Bhogal, M.; Reynolds, S. Changes in peel pigmentation during ripening of mango fruit (Mangifera indica var. Tommy Atkins). Ann. Appl. Biol. 1986, 101, 651–656. [CrossRef]

3. Kuhn, D.N.; Bally, I.S.; Dillon, N.L.; Innes, D.; Groh, A.M.; Rahaman, J.; Ophir, R.; Cohen, Y.; Sherman, A. Genetic map of mango: A tool for mango breeding. Front. Plant Sci. 2017, 8, 577. [CrossRef] [PubMed]

4. Muengkaew, R.; Chaiprasart, P.; Warrington, I. Changing of physiochemical properties and color development of mango fruit sprayed methyl Jasmonate. Sci. Hortic. 2016, 198, 70–77. [CrossRef]

5. Grotewold, E. The genetics and biochemistry of floral pigments. Annu. Rev. Plant Biol. 2006, 57, 761–780. [CrossRef] [PubMed]

6. Buer, C.S.; Imin, N.; Djordjevic, M.A. Flavonoids: New roles for old molecules. J. Integr. Plant Biol. 2010, 51, 98–111. [CrossRef]

7. de Pascual-Teresa, S.; Moreno, D.A.; García-Viguera, C. Flavanols and anthocyanins in cardiovascular health: A review of current evidence. Int. J. Mol. Sci. 2010, 11, 1679–1703. [CrossRef]

8. Spencer, J.P. The impact of fruit flavonoids on memory and cognition. Br. J. Nutr. 2010, 104, S40–S47. [CrossRef]

9. Berardini, N.; Fezer, R.; Conrad, J.; Beifuss, U.; Carle, R.; Schieber, A. Screening of mango (Mangifera indica L.) cultivars for their contents of flavonol O-and xanthone C-glycosides, anthocyanins, and pectin. J. Agric. Food Chem. 2005, 51, 1563–1570. [CrossRef]

10. López-Cobo, A.; Verardo, V.; Diaz-de-Cerio, E.; Segura-Carretero, A.; Fernández-Gutiérrez, A.; Gómez-Caravaca, A.M. Use of HPLC-and GC-QTOF to determine hydrophilic and lipophilic phenols in mango fruit (Mangifera indica L.) and its by-products. Food Res. Int. 2017, 100, 423–434.

11. Sudheeran, P.K.; Love, C.; Feygenberg, O.; Maurer, D.; Ovadia, R.; Oren-Shamir, M.; Alkan, N. Induction of red skin and improvement of fruit quality in ‘Kent’, ‘Shelly’ and ‘Maya’ mangoes by preharvest spraying of prohydrojasmon at the orchard. Postharvest Biol. Technol. 2019, 149, 18–26. [CrossRef]

12. Koshiyama, M.; Watanabe, H.; Mitomi, M.; Imamura, K. Development of a new plant growth regulator, prohydrojasmon. Regul. Plant Growth Dev. (Jpn.) 2006, 41, 24–33.

13. Sudheeran, P.; Feygenberg, O.; Maurer, D.; Alkan, N. Improved cold tolerance of mango fruit with enhanced anthocyanin and flavonoid contents. Molecules 2018, 21, 1832. [CrossRef]

14. Sivankalyani, V.; Feygenberg, O.; Diskin, S.; Wright, B.; Alkan, N. Increased anthocyanin and flavonoids in mango peel are associated with cold and pathogen resistance. Postharvest Biol. Technol. 2016, 111, 132–139. [CrossRef]

15. Sudheeran, P.K.; Ovadia, R.; Galsarker, O.; Maoz, I.; Sela, N.; Maurer, D.; Feygenberg, O.; Oren Shamir, M.; Alkan, N. Glycosylated flavonoids: Fruit’s concealed antifungal arsenal. New Phytol. 2020, 221, 1788–1798. [CrossRef]

16. Cantín, C.M.; Fidelibus, M.W.; Crisosto, C.H. Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of ‘Crimson Seedless’ grapes. Postharvest Biol. Technol. 2007, 41, 237–241. [CrossRef]

17. Sandhu, A.K.; Gray, D.J.; Lu, J.; Gu, L. Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (Vitis rotundifolia) skins. Food Chem. 2011, 121, 982–988. [CrossRef]

18. Kondo, S.; Uthaibutra, J.; Gemma, H. Comparison of 1-aminocyclopropane-1-carboxylic acid, abscisic acid and anthocyanin content of some apple [Malus pumila] cultivars during fruit growth and maturation. J. Jpn. Soc. Hortic. Sci. (Jpn.) 1991, 60, 505–511. [CrossRef]
19. Zhang, M.; Leng, P.; Zhang, G.; Li, X. Cloning and functional analysis of 9-cis-epoxycarotenoid dioxygenase (NCED) genes encoding a key enzyme during abscisic acid biosynthesis from peach and grapefruits. *J. Plant Physiol.* 2009, 161, 1241–1252. [CrossRef]

20. Whale, S.; Singh, Z.; Behboudian, M.; Janes, J.; Dhaliwal, S. Fruit quality in ‘Cripp’s Pink’ apple, especially colour, as affected by preharvest sprays of aminoethoxyvinlyglycine and ethephon. *Sci. Hortic.* 2008, 111, 342–351. [CrossRef]

21. Wang, X.; Yin, W.; Wu, J.; Chai, L.; Yi, H. Effects of exogenous abscisic acid on the expression of citrus fruit ripening-related genes and fruit ripening. *Sci. Hortic.* 2008, 111, 342–351. [CrossRef]

22. Ghozlen, N.B.; Cerovic, Z.G.; Germain, C.; Toutain, S.; Latouche, G. Non-destructive optical monitoring of grape maturation by proximal sensing. *Sensors* 2010, 11, 10040–10068. [CrossRef] [PubMed]

23. Bahar, A.; Kaplunov, T.; Zutahy, Y.; Daus, A.; Lurie, S.; Lichter, A. Auto-fluorescence for analysis of ripening in Thompson Seedless and colour in Crimson Seedless table grapes. *Aust. J. Grape Wine Res.* 2012, 11, 353–359. [CrossRef]

24. Galsurker, O.; Diskin, S.; Maurer, D.; Feygenberg, O.; Alkan, N. Fruit stem-end rot. *Horticulturae* 2018, 1, 50. [CrossRef]

25. Dar, J.A.; Wani, A.A.; Ahmed, M.; Nazir, R.; Zargar, S.M.; Javid, K. Peel color in apple (*Malus domestica* Borkh.): An economic quality parameter in fruit market. *Sci. Hortic.* 2019, 244, 50–60. [CrossRef]

26. Silvestri, C.; Cirilli, M.; Zecchini, M.; Muleo, R.; Ruggieri, A. Consumer acceptance of the new red-fleshed apple variety. *J. Food Prod. Mark.* 2018, 24, 1–21. [CrossRef]

27. Romanazzi, G.; Sanzani, S.M.; Bi, Y.; Tian, S.; Martínez, P.G.; Alkan, N. Induced resistance to control postharvest decay of fruit and vegetables. *Postharvest Biol. Technol.* 2016, 122, 82–94. [CrossRef]

28. Brunetti, C.; Guidi, L.; Sebastiani, F.; Tattini, M. Isoprenoids and phenylpropanoids are key components of the antioxidant defense system of plants facing severe excess light stress. *Environ. Exp. Bot.* 2015, 119, 54–62. [CrossRef]

29. Kondo, S.; Tsukada, N.; Niimi, Y.; Seto, H. Interactions between jasmonates and abscisic acid in apple fruit, and stimulative effect of jasmonates on anthocyanin accumulation. *J. Jpn. Soc. Hortic. Sci.* 2001, 71, 546–552. [CrossRef]

30. Singh, S.P.; Saini, M.K.; Singh, J.; Pongener, A.; Sidhu, G.S. Preharvest application of abscisic acid promotes anthocyanins accumulation in pericarp of litchi fruit without adversely affecting postharvest quality. *Postharvest Biol. Technol.* 2014, 96, 14–22. [CrossRef]

31. Asad, M.A.U.; Zakari, S.A.; Zhao, Q.; Zhou, L.; Ye, Y.; Cheng, F. Abiotic stresses intervene with ABA signaling to induce destructive metabolic pathways leading to death: Premature leaf senescence in plants. *Int. J. Mol. Sci.* 2019, 21, 256. [CrossRef]

32. Ghasemnezhad, M.; Javaherdashhti, M. Effect of methyl jasmonate treatment on antioxidant capacity, internal quality and postharvest life of raspberry fruit. *Casp. J. Environ. Sci.* 2008, 1, 73–78.

33. Osorio, G.T.; Oliveira, B.S.; Di Piero, R.M. Effect of fumigants on blue and gray molds of apple fruit. *Trop. Plant Pathol.* 2013, 31, 63–67.

34. Furness, G.O.; Pinczewski, W.V. A comparison of the spray distribution obtained from sprayers with converging and diverging airjets with low volume air-assisted spraying on citrus and grapevines. *J. Agric. Eng. Res.* 1985, 31, 291–310. [CrossRef]

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