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Pre-storage Dipping with Low Fluroxypyr Concentration Inhibits Calyx Senescence and Retains the Quality of Citrus Fruits During Continuous Low Ethylene Exposure

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Abstract: This study determined the effects of concentration of fluroxypyr 333 g L^{-1} on calyx senescence and other postharvest qualities of 'Afourer' mandarins and Navel oranges at shelf life in the presence of lower exogenous ethylene. The fruits were dipped in fluroxypyr solutions for 2 minutes at concentrations of 0 (control), 6, 12, or 24 mL L^{-1}, then stored at 20°C and relative humidity of 65-72% for five weeks in the presence of 0.1 ppm ethylene. The tests showed that citrus fruit treated with 6 mL L^{-1} had lower calyx senescence, lower respiration rate, weight loss, and retained firmness level than control fruit and other concentrations. Furthermore, fluroxypyr delayed the increase in TSS and retard the degradation in titratable acidity levels, slowing an increase in the maturity index of both citrus types compared to control. In addition, low ethanol accumulation was observed in the auxin-treated fruits; however, it was slightly higher in 'Afourer' mandarins than Navel oranges, which was expected. Overall, the results demonstrated that the application of fluroxypyr on citrus as a postharvest dipping could delay calyx senescence and maintain fruit quality. However, more studies would seem warranted to understand the mechanism through which the fluroxypyr works to delay postharvest calyx senescence and maintain citrus fruit quality during prolonged storage.

Keywords: Citrus Fruit, Calyx Senescence, Fluroxypyr Treatment, Ethylene Exposure, Quality, Storage

1. Introduction

Calyx senescence of citrus fruit (genus *Citrus*; family *Rutaceae*) is a major external factor influencing other quality parameters and determining storage longevity. Abscission of the calyx usually facilitates fungal attach on the abscission zone of the citrus fruit when stored for long-term, and can lead to fruit decay [1]. 2,4-Dichlorophenoxyacetic acid (2,4-D) is a synthetic plant growth regulator, mostly used in the citrus postharvest industry to prevent calyx senescence and maintain quality. However, the use of 2,4-D is restricted in the citrus industry in many countries due to its negative effects on the environment and food safety concerns. Therefore, searching for safer and more effective preservatives as a substitute to 2,4-D is a common postharvest issue that needs to be solved in citrus-producing countries.

A range of alternative auxins have been investigated for their ability to retain the postharvest quality of citrus fruit. For example, Cronjé et al. [1] evaluated the effects of three agrochemicals such as aminoethoxy vinyl glycine (AVG), 1-naphthylacetic acid (NAA) and 1-methylcyclopropene (1-MCP) on citrus fruits to determine whether they could be a possible alternative to 2,4-D in citrus postharvest handling. The authors, however, found that none of the tested chemicals is an ideal and suitable replacement for 2,4-D. Other researchers have found synthetic auxins, such as 3,5,6-trichloro-2-pyridyloxyacetic acid and 2,4-dichlorophenxoyacetic acid isopropyl ester, applied during ethylene degreening significantly reduce calyx senescence of Clementine cultivars [2]. In addition, plant growth regulators such as S-ethyl-4-chloro-O-tolyloxythioa-cetate (MCPA-thioethyl) have been reported to control calyx senescence and reduce weight loss without altering weight loss the sensory quality [3]. However, these plant growth regulators have

been less effective in preventing calyx senescence as 2,4-D and are broadly accepted in the storage of citrus fruits.

Recently, the effects of a new plant regulator, fluroxypyr was tested during the storage of citrus fruits to determine if it could reduce the rates of calyx senescence in citrus fruits. The fluroxypyr treatment significantly reduced calyx senescence by 2–8 times compared with the control fruit, but there was no significant difference between fluroxypyr and 2,4-D treated fruits [4]. Meanwhile, titratable acidity (TA) in auxin-treated fruit was significantly higher than that of 2,4-D and control, while fluroxypyr has no significant effects on the weight loss, fruit firmness and other internal quality of fruits during long-term storage, suggesting that although fluroxypyr has lower toxicity, it has almost similar effects to 2,4-D [4]. Although the first trial of fluroxypyr on four citrus cultivars reported a reduction of calyx senescence, there is still limited data on the effects of the plant growth regulator, and hence has not yet been registered for postharvest application in any country.

This study presents a comparative study assessing the relative efficacy of four fluroxypyr concentrations to inhibit calyx browning and abscission and delay the emergence of postharvest senescence characteristics in oranges and mandarins stored at 20°C for up to four weeks in the presence of lower exogenous ethylene. The objective of this study was to determine the postharvest application of fluroxypyr on the incidence of calyx senescence and internal quality parameters induced by low ethylene exposure on two varieties of citrus fruit relative to control.

2. Materials and Methods

2.1. Plant Materials and Sample Treatments

Navel oranges (*Citrus sinensis* L. Osbeck) were harvested at commercial maturity from a New South Wales Department of Primary Industries (NSW DPI) research farm at Somersby on the NSW Central Coast. After transporting to NSW DPI postharvest laboratories (Ourimbah, NSW), the fruits were sanitized with commercial sodium hypochlorite solution (50 ppm), sorted, sized and randomized. The plant growth regulator, fluroxypyr concentrations of 0, 6, 12, and 24 mL L^{-1} were prepared, and the fruits were dipped in the solutions for 2 minutes each. The zero concentration was only water which was considered a control. The fruit was air-dried and placed in 60 L steel drums as individual treatment units (n = 25 fruits) with three replicates comprising a treatment. The citrus fruits were then stored in steel drums at 20°C and with a relative humidity of 65-72% for five weeks. The drums were connected to humidified air streams with exogenous ethylene of ≤0.001 and 0.1 ppm, flowing at a rate of 2 mL/L. Evaluation of the fruit was done every 7 days during the 5 weeks storage trial. The same process was performed on 'Afourer' mandarins obtained at a commercial matured stage from Plumridge orchards in Victoria, Australia. There were also three replicates in treatment, with each replicate n = 30 fruits.

2.2. Evaluation of Calyx Quality

Seventy-five fruits were visually examined for calyx senescence (browning and abscission) in each treatment. Calyx changes were assessed at seven day intervals according to the method of Alhassan et al. [5] with calyx colour scored on a 5-point scale where; 1 = no browning; 2 = < 25% brown; 3 = 25 - 50% browning; 4 = 50 - 75% browning; and 5 = > 75% browning. A browning score was only assigned to fruit with an intact calyx. For each assessment period, the mean browning score of fruit in each treatment unit was calculated. Calyx which had dropped were counted during each assessment day and were classified as abscised calyx [4], and the data were expressed as the percentage of the change compared to the total number of fruits.

$$\text{Calyx abscission} = \left(\frac{\text{the number of calyx abscissed}}{\text{total number of fruits in the sample}}\right) \times 100\%$$

2.3. Determination of Fruit Weight Loss

Weight loss of fruits was measured according to the previous report by Chaudhary et al. [3] with some modification. First, the weight of each sample was recorded on day zero (0) after treatment by using an electronic analytic weighing scale (Model Kean &Sohn GmbH, D-72336, Germany). Then, weight loss during storage was determined by periodically weighing three groupings of the samples in each treatment. Finally, the percentage weight loss of the fruits was calculated from the initial weight and expressed in percentages.

2.4. Evaluation of Fruit Firmness

Fifteen fruits from each replicate were determined on two opposite sides on the equatorial zone of each fruit with a firmness analyzer (Lloyd Instrument Ltd, Fareham, UK). The compression test was performed at a trigger force of 0.4 N, at a rate of 5 $mm \cdot s^{-1}$, and at a penetration depth of 5 mm. The results were expressed as the average firmness of 45 fruits for each treatment and expressed in Newton (N).

2.5. Determination of Respiration Rate

Five fruits from each treatment unit were sealed in airtight 2-litre glass jars fitted with a septum and kept at 20 ± 1˚C for 3 hours to accumulate respiratory gases. The respiration rate was measured on three containers from treatments. Carbon dioxide (CO_2) concentration in the jar was determined by withdrawing a 1.0 mL gas sample from the headspace and injecting it into gas chromatography (Gow-Mac, Bridgewater, NJ) fitted with two stainless steel columns (60 cm × 1 mm i.d.) connected in series. The operating temperatures for the detector, injector, and gas chromatography column were 110°C, 50°C and 110°C, respectively. The respiration rate of citrus fruits was

calculated according to Li et al. [6].

2.6. Measurement of Internal Fruit Qualities

Total soluble solids (TSS) were measured from the refractive index of the fruit juice. Six fruits from each treatment (three replicates) were juiced and filtered through two layers of gauze. The TSS contents were determined using a hand-held digital refractometer (Atago Co. Ltd., Tokyo Atago, Japan). The data were expressed as average percentage °Brix. Titratable acidity (TA) content was determined by titrating 5 mL of juice from each replicate with 0.1 N NaOH to pH 8.2 with an automatic titrator (Mettler Toledo, Switzerland). Data were expressed as a percentage of citric acid. The maturity index of the fruits was calculated from the TSS/TA ratio.

Ten (10) mL of juice was transferred into a 20 mL glass vial with crimp-top caps sealed with silicone septa for the ethanol content. The sealed samples were incubated in a water bath of 30°C for 10 minutes before analysis. Ethanol accumulation was determined by headspace analysis using a gas chromatograph (Model 580, Gow-Mac-Bethlehem, PA, USA) equipped with a flame ionization detector and a column (Carbwax, GowMac, USA). The injector was set at 190°C, the column at 68°C, and the detector (FID) at 190°C with gas flow rates of 30, 30 and 300 mL min^{-1} for nitrogen, hydrogen and air, respectively. One (1) mL of gas sample from the headspace was drawn from the vials for the analysis after incubation. The ethanol content of the fruits was expressed as g L^{-1}.

2.7. Statistical Analyses

The experiment was conducted using a completely randomized design (CRD). Data in this experiment were based on three replicates in each treatment and were statistically processed using the SPSS version 24.0 software package (SPSS, Chicago, IL, USA). The two-way analysis of variance (ANOVA) was performed, and differences were considered significant at $p \leq 0.05$.

3. Results and Discussion

3.1. Fruit Calyx Browning and Abscission

The rate of calyx browning of 'Afourer' mandarins and Navel oranges is shown in figure 1 below. There was a significant effect of both fluroxypyr treatment and storage time on calyx browning of both citrus varieties ($p < 0.01$), but these factors also had significant interactions during storage. The results showed that lower fluroxypyr concentrations significantly reduced the rates of calyx browning on the orange fruits compared with control fruits. But the higher concentration of the auxin treatment in the presence of ethylene slightly increased calyx browning of the fruits, but yet contributed to a lower rate of senescent fruit compared with control after the 5 weeks of storage. Pre-storage dipping the citrus with fluroxypyr resulted in beneficial effects during storage for maintaining calyx integrity and a range of internal quality factors. Citrus fruits dipped in 6 mL L^{-1} with ethylene in the storage environment were the most effective in reducing calyx senescence compared to the other concentrations (Figure 1). As shown in the graph, most calyxes of the control fruits were brown and senesced at the end of storage, and the stem ends of some fruits rot around the calyx. However, most of the calyxes of the fruits treated with fluroxypyr were still green and fresh at the storage end. The results showed that the rates of calyx senescence in both mandarin and orange fruits were significantly reduced by about 2 times with fluroxypyr treatments compared with the control. A similar reduction of calyx senescence of Satsuma mandarins, Navel and Valencia oranges by fluroxypyr has been reported during 12 weeks of storage at shelf life [7].

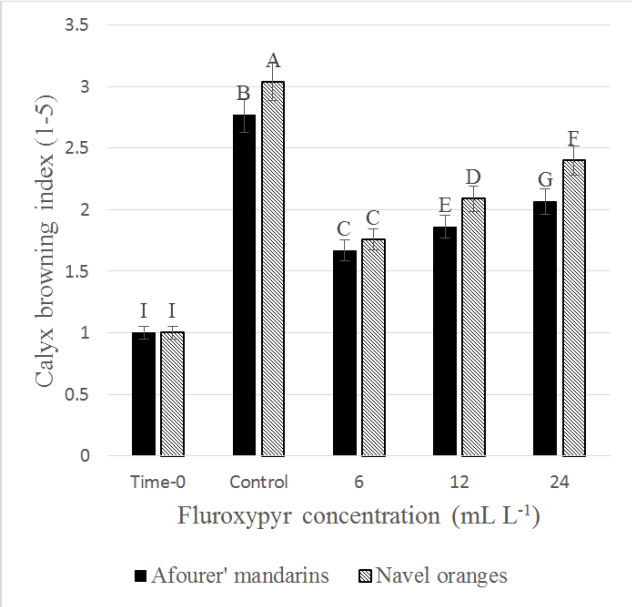

Figure 1. *Postharvest fluroxypyr treatments on calyx browning in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

Similar significance has been shown in the calyx abscission of both citrus fruits after treatment with the same concentrations of the fluroxypyr in this experiment (Figure 2). Calyx abscission of both citrus varieties increased with an increase in the browning index in the different concentrations of the auxins during storage. On the other hand, the rate of calyx abscission was significantly reduced by 6 mL L^{-1} compared to non-treated fruit and the other concentrations in both citrus varieties ($p < 0.01$). This result agreed with Ma et al. [7], where the treatment of four varieties of citrus fruits with fluroxypyr effectively retarded calyx senescence during extended storage. However, fruits dipped in 24 mL L^{-1} concentration slightly induced higher rates of calyx abscission but yet had better retention than control fruits, which means an increase in fluroxypyr concentration may not have the beneficial effects of delaying calyx senescence, as this concentration could be greater than the level the fruits

can metabolize during long-term storage at ambient. But this study has also shown susceptibility of variety where calyx browning and abscission of Navel oranges increased compared to 'Afourer' mandarins. A similar observation was made by Jiménez-Cuesta et al. [8], where cultivar aptitude influences the susceptibility of citrus fruit to calyx abscission and browning during auxin treatment and one-week storage, which is consistent with the report that the susceptibility to postharvest calyx abscission varies among citrus cultivars [2].

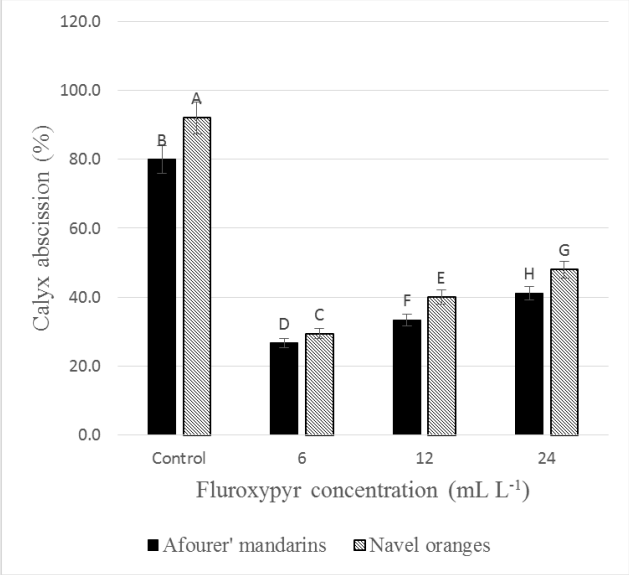

Figure 2. *Postharvest fluroxypyr treatment on calyx abscission in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

3.2. Fruit Weight Loss

There was a significant effect of fluroxypyr and storage time on weight loss of both citrus varieties ($p < 0.05$), while there were no significant interactions between these factors. As shown in Figure 3, the rate of fruit weight loss at the end of storage was significantly reduced ($p < 0.05$) by treatment with 6 and 12 mL L^{-1} relative to the control fruit. At the same time, treatment with 24 mL L^{-1} had little increase in weight loss but lower than untreated fruits. Weight loss in fruits mostly occurs due to transpiration being an important parameter during the postharvest life of citrus fruit. This effect induces peel senescence [9] and negatively affects other quality factors. In the present research, the percentage weight loss observed did alter the commercial quality of the non-treated fruit in both 'Afourer' mandarins and Navel oranges during storage, as most fruits also had their calyxes senesced. Furthermore, the auxin treatments did significantly maintain fruit quality by reducing weight loss, even though some treatments slightly reduced this parameter compared with untreated fruit. This result contradicts the previous finding of Ma et al. [7], which reported no significant effect of fluroxypyr treatment on the weight loss of citrus fruits during long-term storage at 5 or 20°C room temperature.

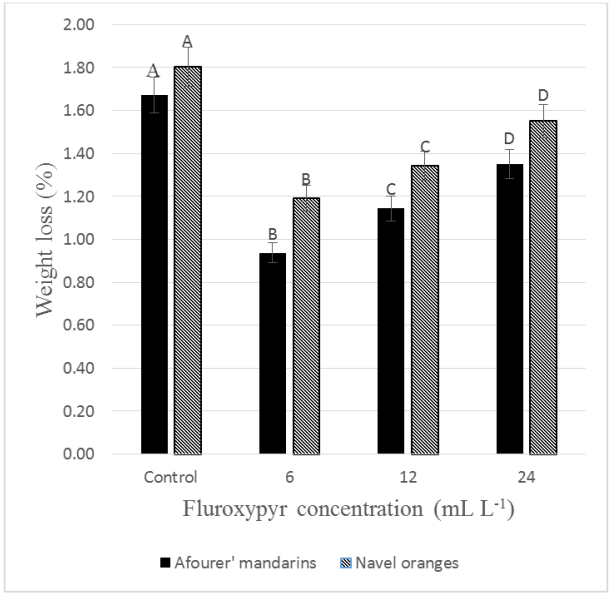

Figure 3. *Postharvest fluroxypyr on weight loss in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

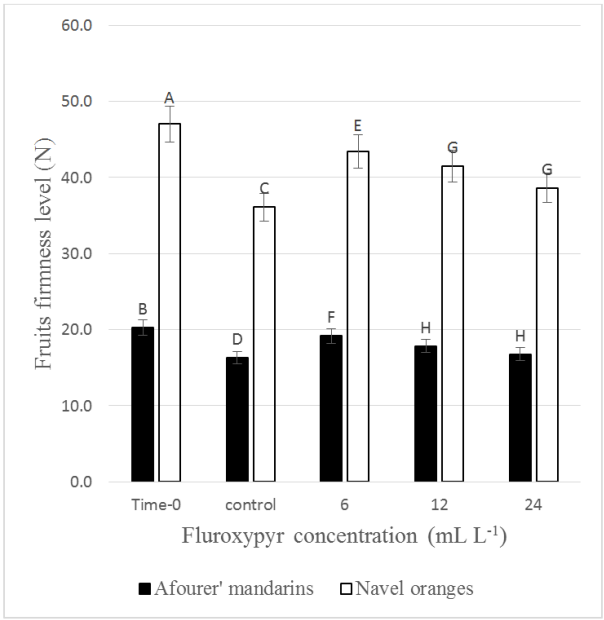

Figure 4. *Postharvest fluroxypyr dipping on firmness in Afourer mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

3.3. Fruit Firmness Level

The influence of fluroxypyr and storage time on fruit firmness and quality was significant ($p < 0.05$). Fruit firmness was continuously the highest after the treatment with 6 mL L^{-1} during storage, and it was significantly higher than that of the control fruit. But the increase in treatment concentrations had little influence on retention of fruit firmness, as indicated in figure 4. However, fluroxypyr-

treated fruits had higher firmness than control fruit on both 'Afourer' mandarins. Again, treatment 6 and 12 mL L^{-1} with low ethylene exposure significantly reduced the decline of fruit weight loss and effectively retained fruit firmness during storage. Increased auxin concentrations slightly reduced fruit firmness but retained higher firmness than untreated fruit. This result is at variance with previous findings of Ma et al. [7], which observed no significant effects of fluroxypyr treatment on fruit firmness of Satsuma mandarins, Newhall navel oranges and Olinda Valencia oranges during long-term storage at 5 or 20°C for 12 weeks. This investigation demonstrated that treated 'Afourer' mandarins were less firm relative to Navel oranges at the end of storage, considering the physiology of the two varieties of citrus.

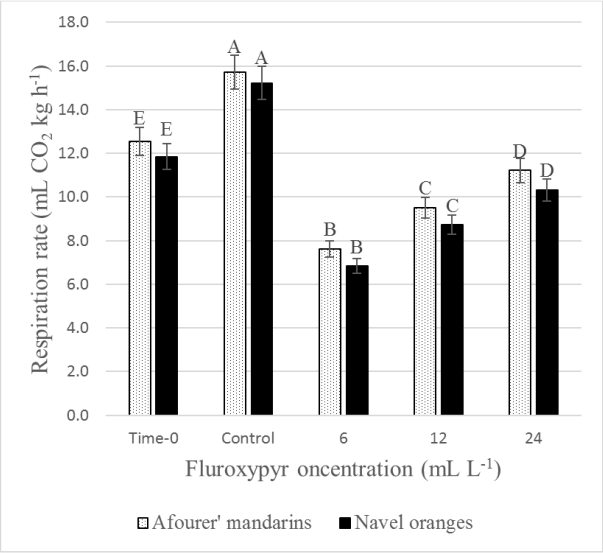

Figure 5. *Effect of postharvest fluroxypyr treatment on respiration rate in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

3.4. Respiration Rate of Fruits

There was a significant effect of fluroxypyr and storage time on the fruit respiration rate of both citrus varieties ($p < 0.05$). Afourer' mandarins treated with fluroxypyr showed a significantly low respiration rate compared with control fruits at the end of the storage period. It was the same in Navel orange fruits. The previous report suggests that Satsuma mandarins and Navel oranges were treated with fluroxypyr and stored at 5 or 20°C for 12 weeks, and an additional 1 week of shelf life showed significantly lower respiration than control fruit [7]. In this experiment, the respiration rate increased in untreated fruits compared with the auxin-treated fruit in storage (Figure 5). This metabolic process is related to the advancement of fruit maturity and might directly influence the other quality parameters in this experiment. Citrus fruits dipped in fluroxypyr concentrations of 6 or 12 mL L^{-1} showed a reduction in respiration rates in storage. But increased concentration (24 mL L^{-1}) of the treatment slightly increased the respiration rates of the fruits at the end of storage. It was lower than untreated fruit during the 5 weeks of storage at 20°C and 65-72% relative humidity. Raghavan et al. [10] reported that high auxins concentration could stimulate cell division and elongation while inhibiting plant growth, adversely affecting fruit quality.

3.5. Ethanol Accumulation in Fruit

The result showed that pre-storage dipping with fluroxypyr and storage time significantly affects the ethanol content of fruit ($p < 0.05$). Citrus fruit accumulates a lot of ethanol during storage depending on the cultivars and storage conditions, and large amounts over the threshold cause off-flavours [11, 12]. Moreover, several studies attributed the development of off-flavours in mandarins largely to induction of ethanol-fermentation metabolism and accumulation of high ethanol [13-17]. In the present study, the ethanol content in juice increased during shelf-life in both varieties compared with the ethanol values recorded at the start of the experiment. Nevertheless, this increase was lower in fluroxypyr-treated fruits than in control fruit in all cases. When exposed to ethylene for long during storage, Ethanol content increased in fruits. However, treatment with the auxin before exposure to ethylene in storage significantly affected ethanol accumulation in fruit. In general, citrus fruits applied with 6 and 12 mL L^{-1} showed low ethanol content in juice than the control fruit. However, dipping fruit in 24 mL L^{-1} concentration increased this parameter but was better than the control. This result indicates that 'Afourer' mandarin accumulates more ethanol than Navel oranges treated with similar concentrations of fluroxypyr and storage conditions, which was expected. It is known that mandarins are more perishable than other citrus fruit and especially suffer from the rapid decline in sensory acceptability after harvest [18]. Therefore, they could not be held at shelf-life temperatures for long periods.

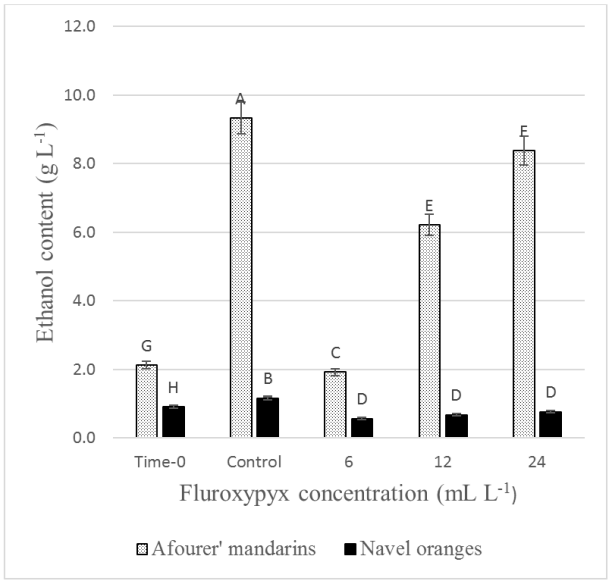

Figure 6. *Postharvest fluroxypyr on ethanol content in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65.72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

3.6. Total Soluble Solids, Titratable Acidity and Maturity Index

It was observed that fluroxypyr did significantly affect the internal qualities of 'Afourer' mandarins and Navel oranges ($p < 0.05$), but there was no effect on storage time. There was also a significant interaction between fluroxypyr treatment and storage time on this quality parameter. For example, the TSS content increased in both citrus varieties as storage time prolonged but was more observed in 'Afourer' mandarins than Navel oranges. Furthermore, lower TSS content was observed at 6 mL L^{-1} fluroxypyr treated fruits, while in the other concentrations, the TSS increased but was less than in non-treated fruit as indicated in figure 7. This result is in contrast to the finding of Ma et al. [7], who observed no difference in TSS in the juice of Satsuma mandarin, Newhall navel orange, and Olinda Valencia orange whether the fruits were treated with fluroxypyr or 2,4-D during storage at 5 or 20°C, suggesting that fluroxypyr has almost similar effects to 2,4-D.

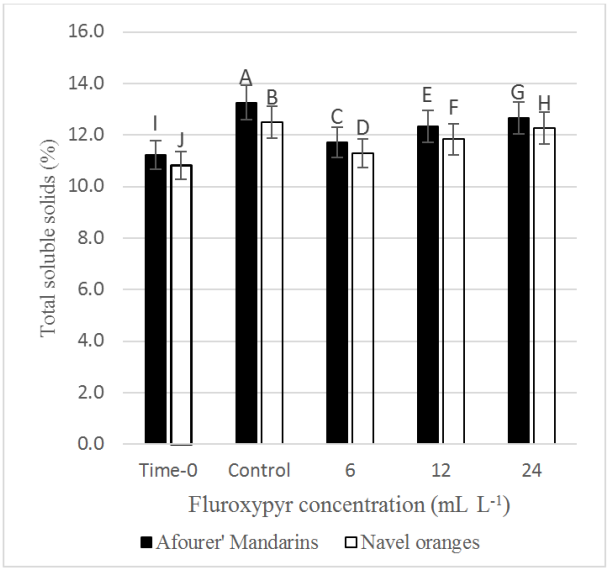

Figure 7. *Postharvest fluroxypyr treatment on TSS content in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

The titratable acidity (TA) level in all treatment concentrations was significantly lower than that of non-treated citrus fruits, while TA in 6 mL L^{-1} treated fruit was the highest among all treatments (Figure 8). The effects of 12 and 24 mL L^{-1} concentrations on TA contents of both citrus varieties were relatively low after storage. But, it appears that control fruits had a quicker degradation of the organic acids during storage, leading to an increase in the TSS contents in fruits (Figure 7). However, the previously reported study did not find a significant difference in the TA level of fluroxypyr treatment on four varieties of citrus fruits during storage [7]. Moreover, there were significant effects of fluroxypyr-treatments and storage time on the maturity index ($p < 0.05$) of 'Afourer' mandarins and Navel oranges. Due to the higher acidity, the TSS/TA ratio in both citrus types was lower in fruit dipped at 6 mL L^{-1} of fluroxypyr as shown in figure 9. While the 24 mL L^{-1} concentration increased the TSS/TA ratio, it was still less than non-treated fruits, delaying the fruit maturity index during the storage of both citrus varieties and maintaining quality.

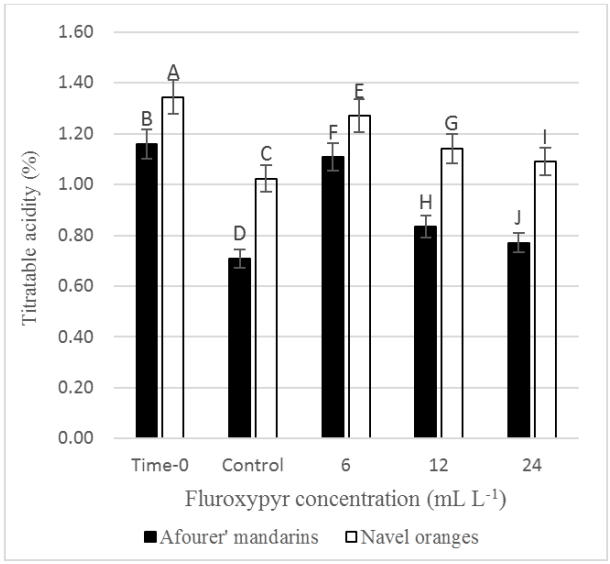

Figure 8. *Postharvest fluroxypyr on TA in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

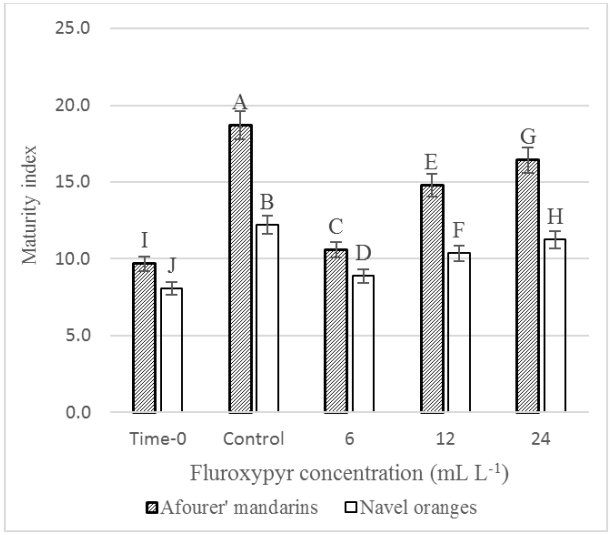

Figure 9. *Postharvest fluroxypyr on maturity index in 'Afourer' mandarins and Navel oranges during exposure to (2 mL/L of ethylene and air, 20°C, 65-72% RH) for 5 weeks. Treatments applied: control fruit (0), 6, 12 and 24 mL L^{-1}. Means with different letters are significantly different according to Fishers' LSD test ($p \leq 0.05$).*

4. Conclusions

This study demonstrated that postharvest application of fluroxypyr auxins delayed calyx senescence of 'Afourer'

mandarins and Navel oranges. Furthermore, it showed that lower concentrations applied were more beneficial in maintaining the fruit quality factors than control and higher concentrations applied. This auxin treatment also reduced both citrus varieties' respiration rate, weight loss, and declined firmness. Pre-storage dipping with fluroxypyr maintained the overall internal qualities and delayed an increase in fruit maturity index after 5 weeks of storage. The potential benefit of fluroxypyr is delaying fruit maturity level, which is linked closely with the delay in the degradation of TA and suppression of increased TSS, which is generally associated with increased senescence of fruits. This result is one of the first reports on the effects of fluroxypyr treatment on reducing calyx changes and maintaining quality in mandarins and oranges. Although approval of fluroxypyr in food has not yet been granted, it is an effective auxin in controlling physiological disorders and improving the fruit quality of various citrus in long-term storage. This study highlights the importance of considering the 3,5,6-TPA concentration on external and internal quality of two varieties of citrus fruit. But, there is need for further study of the auxin on other citrus varieties to fully understand the mechanism that controls calyx senescence following dipping with fluroxypyr prior to shelf-life storage.

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Ethics Statement

The author declares that this research is the original work and has not been previously published or is not under consideration for publication elsewhere.

Conflicts of Interest

The author declares no conflict of interest regarding the publication of this work.

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