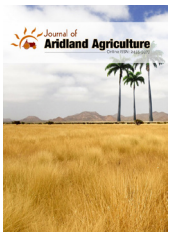
Journal of Aridland Agriculture 2024, 10: 142-149
doi: 10.25081/jaa.2024.v10.8901
https://updatepublishing.com/journal/index.php/jaa

Research Article



ISSN: 2455-9377

Postharvest treatment of navel oranges with fluroxypyr, 2-methyl-4-chlorophenoxyacetic acids, and 2,4-dichlorophenoxyacetic maintains physiological quality during ambient storage

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Received: April 03, 2024
Revised: October 09, 2024
Accepted: October 13, 2024
Published: November 12, 2024

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ABSTRACT

Two batches of Green mature Navel oranges with calyxes attached were obtained from a farmer from Juaben Municipality in the Ashanti region of Ghana and were dipped for 2 minutes with fluroxypyr, and 2-methyl-4-chlorophenoxyacetic acid (MCPA), were assessed against the commercial 2,4-D at 0.1 and 0.2 mmol L^{-1} stored under ambient conditions (24±2 °C and 55-60% RH) for 4 weeks. The results showed that pre-storage dipping with fluroxypyr was the most effective in suppressing endogenous ethylene production and respiration rate, reduced calyx changes, and fruit rot, and retained fruit sensory attributes compared with MCPA 2,4-D, or untreated fruit at the end of storage life of four weeks. The effect of 2,4-D and MCPA on fruit rot, calyx browning, abscission, and fruit internal quality, was similar; which was not significantly different during storage. Dipping fruit with 0.1 mmol L^{-1} fluroxypyr was more effective in maintaining the quality of oranges and could be a substitute for current commercial 2,4-D.

KEYWORDS: Auxins treatment, Calyx changes, Citrus fruit, Quality, Senescence, Storage

INTRODUCTION

Citrus is one of the most important fruits widely produced commercially in over 137 countries around the world (Ismail & Zhang, 2004). Due to its nutritional and antioxidant properties, the citrus industry provides millions of jobs worldwide and enormous benefits to human beings. The cultivation and production levels of citrus have been going up over the years. Citrus is mainly grown by smallholder farmers in the forest regions of Ghana, primarily in the Ashanti, Eastern, and Central Regions, where annual rainfall is over 1000 mm (Akosah *et al.*, 2021). Citrus production is a major sub-sector of commercial tree crops in Ghana, employing over 20,000 people (Akosah *et al.*, 2021), with an annual yield of approximately 743,263 tons (Akpatsu & Jizorkuwie, 2024). Although the production of citrus is increasing, it is estimated that the postharvest losses of fruits in Ghana are between 30% to 50% (Akpatsu & Jizorkuwie, 2024) due to inadequate treatment and storage strategies, making them susceptible to fruit calyx abscission and decay during extended storage period, resulting in postharvest losses (Alhassan *et al.*, 2020). During the abscission process, cell wall materials such as pectin methylesterase (PME), polygalacturonase (PG), β-1,4-glucanase and expansion, pectate lyase (PL) are digested by cell wall degrading enzymes (Roberts *et al.*, 2002). Citrus calyx senescence is mostly seen as a negative quality attribute for the consumers during marketing (Alhassan *et al.*, 2022), and it usually facilitates fungal attacks at the abscission zone of fruit (Cronjé *et al.*, 2005). These losses do not only affect farmers' incomes but also the overall citrus supply chain.

Postharvest application of fruit with plant growth regulators on the fruit has mostly shown inconclusive results (Blake & Stevenson, 1959), although 2,4-D treatment retarded changes associated with ageing in the skin of citrus fruit (Coggins Jr. Lewis, 1965). The 2,4-dichlorophenoxyacetic acid (2,4-D) is an auxin that regulates plant growth and has been commonly used in citrus production since the 1940s as a preharvest application by citrus producers to increase fruit size and improve sugar content and juice acid (Mir & Itoo, 2017). However, in the last three decades, 2,4-D has been employed as a postharvest treatment of auxin to inhibit calyx changes and improve other quality parameters of citrus protracted storage. Although the application of 2,4-D has been effective in ameliorating citrus quality its usage has been limited in citrus-producing countries due to growing human health and environmental issues (Ma *et al.*, 2015). Therefore, there is a need for the citrus industry to find safer alternatives and widely acceptable postharvest treatments as substitutes for 2,4-D to maintain citrus quality.

Many studies have been carried out to find an effective alternative treatment to maintain citrus fruit postharvest quality. In a previous study, Sdiri *et al.* (2013) compared the effectiveness of auxin antagonists-S-ethyl-4-chloro-O-tolyloxythioacetate (MCPA-thioethyl) and fluroxypyr (2-(4-amino-3,5-dichloro-6-fluoropyridin-2yl)oxyacetic acid) with 2,4-D and reported that none of the new auxins exceeded the performance of 2,4-D in delaying citrus fruit senescence. However, other investigations showed that auxins such as 4-amino-3,5,6-trichoropicolinic acid, 3,5,6-trichlo-2-pyridinyloxyacetic acid (3,5,6-TPA, triclopyr) and 4-chlorophenoxyacetic effectively controlled abscission of lemon buttons stored fruit in the presence ethylene (Einset *et al.*, 1981). This effect of 3,5,6-TPA inhibiting calyx abscission agreed with the finding by Carvalho *et al.* (2008) who applied the auxin to several Clementine mandarin cultivars during ethylene degreening and observed a significant reduction of calyx changes without detrimental effect on internal fruit quality.

Despite the positive impact of retaining the calyx quality, 3,5,6-TPA application did not have a significant effect on the fruit quality parameters of oranges (Salvador *et al.*, 2010). Soluble solids content (SSC) increased acid content and reduced the acid level after shelf life relative to initial values recorded at harvest. The reduction in the juice acidity content increased the fruit maturity index whilst the postharvest treatment with auxins on a range of citrus fruit did not affect SSC and acid levels (Sdiri *et al.*, 2013). This effect is consistent with previous reports that the final quality of degreened citrus fruit treated with 2,4-D did not change (Bello *et al.*, 2004). Citrus fruit can accumulate a lot of ethanol during storage depending on the conditions of storage. Sdiri *et al.* (2013) noticed an increase in ethanol content in the juice of auxin-treated citrus fruit during shelf-life but did not appear to influence flavour.

Decay incidence of citrus can occur with extended storage time and could be up to 50% (Abdel-El-Aziz & Mansoor, 2006). Postharvest fruit decay is currently managed by the application of chemical fungicides, such as benzimidazole, sterol inhibitors, and sodium orthophenyl phenate and different mixtures of these compounds (Palou *et al.*, 2008; Talibi *et al.*, 2014). However, the use of these compounds to control fruit rots is also not without health hazards and environmental concerns. Also, continuous application of fungicides could increase fungicide resistance, which would further complicate the control of fruit decay. The increasing demand for high-value fruits based on sustainable, environmentally friendly, green agriculture makes it even more necessary to employ alternatives to maintain citrus fruit quality (Palou *et al.*, 2008). This study compares the efficacy of different concentrations of three auxins (2,4-D, fluroxypyr, and MCPA) to inhibit calyx deterioration and maintain orange fruit quality. Therefore, the objective was to delay the senescence of Navel oranges during storage at an ambient temperature and to extend the storage life with fluroxypyr or MCPA relative to 2,4-D.

MATERIALS AND METHODS

Plant Materials and Experimental Design

Green mature Navel oranges (*Citrus sinensis* (L.) Osbeck) with calyxes were obtained from a farmer from Juaben Municipality in the Ashanti Region of Ghana. The fruits were packaged in wooden crates and transported to Dr. Hilla Limann Technical University Postharvest Laboratory in Wa in the Upper West Region of Ghana for the experiment. Upon arrival, the oranges were sorted based on visual defects, uniformity of weight, and shape. The fruits were sorted and graded into similar colour, shape, size, and appearance (with calyxes attached), and sampled into individual treatment units (n=40 for experiment 1) with each treatment comprised of three replicates in experiment 1. The fruits were dipped for 2 minutes in the following solutions: tap water as control, 0.1 and 0.2 mmol L^{-1} of 2,4-D (amine salt), 2-methyl-4-chlorphenoxyacetic acid (MCPA, Titan Ag. Pty. Ltd.) and fluroxypyr (Starane, Dow Agrosciences Australia Ltd.). The fruits were then packed in cardboard boxes and stored at ambient temperature (24±2 °C) after they were kept in a room for 2 hrs for the water to drain off following dipping in the various auxin solutions.

In experiment 2, orange fruit was obtained from the same location in the Ashanti region of Ghana, and a similar procedure as experimental 1 was followed, but with only 0.1mM auxin treatment concentration. This concentration (0.1 $mMoL^{-1}$) was applied because in experiment 1 it showed promise of inhibiting fruit senescence. Storage temperature and relative humidity (RH) were monitored using TinyTag View 2 loggers, Gemini Data Loggers (UK) Ltd for the entire four 4 weeks of storage. Fruit exterior quality (calyx browning, calyx abscission, and fruit rot) were assessed on day zero (day 0), then subsequently on every 7 days intervals for 4 weeks. The other quality factors (fruit metabolism and sensory attributes) were determined on 0 days and at the end of the storage.

Effects of Auxins on Calyx Abscission, Calyx Colour Changes, and Fruit Rot

A visual quality assessment was performed on the external appearance of each fruit during the storage period. Calyx

alterations were assessed every seven (7) days according to the methods of Yongxin *et al.* (2018) and Alhassan *et al.* (2022). The level of browning on the calyxes was scored on a 5-point scale where 1=Green, 2=Slightly yellow, 3=Moderately yellow, 4=Completely yellow, and 5=Brown, and the average score of the fruit in each treatment was calculated as mean averages. A browning score was assigned to a calyx only when it was attached to a fruit. Citrus that had their calyxes detached from the fruit were recorded and expressed as percentages of calyx abscission, based on the number of fruits presented in every treatment. The calyx abscission at the assessment days were done as percentages (%) = (The number of fruits at this level without calyxes)/(Total number of fruits presented in each treatment unit) multiplied by 100%. Rot incidence was quantified by the presence of microbial growth on the citrus fruit every 7 days and the data was expressed in percentage. The rot incidence at the assessment days was done as percentages (%) = (The number of fruits at this point without mycelia)/(Total number of fruits presented in each treatment unit) multiplied by 100%.

Respiration Rate and Ethylene Production of Fruit

Four (4) oranges were randomly selected from each replicate, weighed, and placed in a 2 L airtight glass jar fitted with a septum in the lid. The jars were sealed for two (2) hours at room temperature (24±2 °C), after the sealing time, 1 mL gas sample of the headspace was withdrawn from sealed jars through the septum for analysis. Ethylene concentration in the headspace was measured by using a flame ionization gas chromatograph (Gow-Mac 580, Bridgewater NJ) fitted with a stainless-steel column (2 m × 3.2 mm OD × 2.2 mm ID) packed with Porapak Q (80-100 mesh) (Altech, Sydney), with 110, 90, and 70 °C as the operating temperature of the detector, column, and injector, respectively. The air, nitrogen, and hydrogen were used as carrier and combustion gases set at the flow rates of 300, 60, and 30 mL min^{-1}, respectively. Ethylene production was calculated and expressed as μL C_2H_4 kg^{-1} h^{-1} (Huque *et al.*, 2013).

The same fruits selected for ethylene production were used for the determination of the respiration rate at the end of the experiment The respiration rate of the fruit was measured according to the method of Pristijono *et al.* (2018) with modifications. In brief, a 5 mL gas sample was withdrawn using a syringe from the headspace of fruit weighed, and sealed in the airtight 2 L glass jar at room temperature for three (3) hours. The carbon dioxide (CO_2) levels were measured using an ICA40 series low-volume gas analysis system (International Controlled Atmosphere Ltd., Kent, UK), and the respiration rate of the orange fruit was expressed as mL CO_2 kg^{-1} h^{-1}.

Measurement of Fruit Ethanol Content

The ethanol accumulation in the oranges was evaluated according to the procedure of Kumar *et al.* (2014). In brief, aliquots (10 mL) of orange juice were squeezed manually from three different fruit in each unit into 20 mL vials, and sealed with a crimp top fitted with a 2 mm rubber septum and incubated for 10 minutes in a water bath of 30 °C. A 1 mL sample of gas from the headspace was withdrawn from the vial and injected in a gas chromatograph (Series 580, GOW MAC, Bethlehem, PA, USA) fitted with stainless steel (1.2 m × 3 mm) Porapak® QS 80/100 column and equipped with a flame ionization detector. The operating carrier gas, hydrogen, and air were 30, 19, and 300 mL min^{-1}, respectively. The injector, detector, and column temperatures operated at 164, 163, and 142 °C, respectively. A 10 mL ethanol solution (5 μL L^{-1}) was placed in a 20 mL vial, sealed, and incubated in a 30 °C water bath. The sample was evaluated by the use of a gas chromatograph and used as an internal ethanol standard.

Determination of Fruit Softening

Twenty (20) fruits randomly taken from each treatment unit were selected at the beginning of the experiments, and the same fruits were measured at the end of the experiment. The determination of firmness was performed by using a texture analyser (Lloyd Instrument Ltd., Fareham, UK). The measurement of fruit firmness was conducted according to the method of Cháfer *et al.* (2012) where the maximum force (N) was measured by compressing the fruit at the equatorial zone between two flat surfaces closing together at the rate of 1 mm min^{-1} to a depth of 2 mm, the two readings were taken with the average value recorded as the firmness.

Measurement of Brix, Acid, and Brix/Acid Ratio

The total soluble solids (brix) were evaluated according to AOAC (2005) using a hand-held digital refractometer (Atago, Tokyo) at room temperature (24±2 °C), and expressed in °Brix. As for juice acid content three (3) groups of two (2) fruits per treatment were cut in half and squeezed, and total acidity was measured by titrating 5 mL of juice with 0.1 N NaOH to pH 8.2 by an automatic titrator (Mettler Toledo, Switzerland). The data were expressed as citric acid equivalents. The fruit Brix/acid ratio was calculated as the TSS/TA ratio.

Statistical Analysis

The study was conducted in a completely randomized design CRD with three replicates in each treatment unit of the two experiments. The studied quality factors of the oranges were analysed independently from each experiment by applying Analysis of Variance (ANOVA). A two-way ANOVA and the Least Significance Difference (LSD) tests were performed using the SAS software (SAS Version 26, USA). Differences among the means were analysed at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of Auxins Treatment on Calyx Abscission

The attachment of calyx (button) on citrus is an indicator of a good quality of fruit for consumers. The effect of postharvest auxin treatments on retention of the calyx is shown in Table 1, and the results generally showed that calyx detachment was

significantly affected by both concentrations of auxins (0.1 or 0.2 mmol L^{-1}) ($p<0.001$) relative to control fruit. Most of the auxin treatments (0.1 mmol L^{-1} in experiment 1 and 0.1 mmol L^{-1} in experiment 2) inhibited calyx abscission and prolonged fruit storage life of the fruit. Dipping with fluroxypyr demonstrated the greatest delay of calyx abscission, with the higher concentration (0.2 mmol L^{-1}) being significantly more effective than 0.1 mmol L^{-1}. Treatment with 2,4-D (amine salt) was significantly more effective in retarding calyx abscission than MCPA, with the lower concentration (0.1 mmol L^{-1}) more effective than 1 mmol L^{-1}. The results also indicate that dipping the oranges at 0.1 mmol L^{-1} MCPA in both experiments was less effective compared with fluroxypyr or 2,4-D treatments during the four weeks of storage at ambient conditions.

Oranges treated with 2,4-D, fluroxypyr, and MCPA offered beneficial effects of preserving the calyx integrity of the orange fruit. Relating the efficacy of the auxins against 2,4-D treatment, which is the current industry treatment, shows that fluroxypyr produced higher calyx maintenance during storage. It is observed that fluroxypyr was more effective in delaying calyx detachment when treated with 0.2 mmol L^{-1}, whilst 2,4-D was effective at 0.1 mmol L^{-1} concentration. However, also it was noted that fluroxypyr applied with 0.1 mmol L^{-1} was still more effective than 2,4-D treated at 0.1 mmol L^{-1}. It would thus appear that fluroxypyr is a candidate to replace 2,4-D, currently being used by the citrus industry to improve quality (Ma *et al.*, 2015), especially as this auxin offers lower toxicity compared with 2,4-D (EPA, 2012). It has been reported that the acute oral toxicity (LD_{50}) of fluroxypyr in a rat is $\leq$5000 mg kg^{-1}, and it is classed as low toxicity (EPA, 2012), whereas the LD_{50} of 2,4-D in a rat is $\leq$900 mg kg^{-1} (Bus & Hammond, 2007), which is in the category of low toxicity (EPA, 2012). There was no significant difference between oranges dipped with 2,4-D and MCPA before storage, although fruit treated with 2,4-D slightly reduced calyx abscission more than MCPA.

Each value for calyx abscission is the mean of assessments on 3 replicates of 40 fruits taken for 4 weeks. Each value within a column with the same superscript letter is not significantly different.

Effect of Auxins Treatment on Citrus Calyx Browning

There was a significant effect of all auxin treatments (0.1 and 0.2 mmol L^{-1} in experiment 1 and 0.1 mmol L^{-1} in experiment 2 on the rate of calyx browning ($p<0.001$) when compared with control fruit. Fruit treated with fluroxypyr showed the greatest delay in calyx browning, with the higher concentration (0.2 mmol L^{-1}) being significantly more effective compared with 0.1 mmol L^{-1} (Table 2). Fruit dipped with 2,4-D was significantly more effective in delaying calyx browning than those treated with MCPA, with the lower concentration (0.1 mmol L^{-1}) being more effective than the 0.2 mmol L^{-1} treatment. Also, dipping orange fruit at 0.1 mmol L^{-1} MCPA in the two experiments was less effective in retarding calyx browning relative to fluroxypyr during the storage regime at ambient temperature.

The positive effects of auxin treatment on fruit external quality have demonstrated the suppression of factors that could increase metabolism, leading to a reduction in senescence. A report by Pazmino *et al.* (2011) observed that a reduction in ethylene production and respiration in fruit was associated with a decrease in metabolic due to treatment with auxins that stimulate abscisic acid. In the present study, fluroxypyr was more effective than 2,4-D and MACP as well as control fruit in reducing exogenous ethylene and rate of respiration, and as expected delayed calyx browning of the oranges during storage. This effect has been reported in many studies that the rate of senescence is linked to the level of ethylene present in the storage environment of fresh produce (Wills & Golding, 2015; Yongxin *et al.*, 2018), and therefore, it is speculated that the ability of auxins to suppress ethylene production is key in to delay calyx browning of citrus fruit. This beneficial role of the fluroxypyr (0.2 mmol L^{-1}) treatment concentrations on the calyx browning and the other quality factors of Navel oranges demonstrates that auxins affect aspects of metabolism, thus

Table 1: Impact of dipping with auxins on calyx abscission of Navel oranges grown and stored at room temperature

Treatment	Conc. (mmol L^{-1})	Storage time (weeks)				Mean
		1	2	3	4	
Experiment 1						
Day 0		0				
Control	0	9.1	20.2	26.3	37.2	23.2[a]
2,4-D	0.1	4.0	11.1	16.4	26.5	14.5[d]
Fluroxypyr	0.1	0.0	3.0	10.3	22.4	8.9f
MCPA	0.1	6.1	14.1	22.4	27.5	17.5[c]
2,4-D	0.2	5.1	13.1	20.4	26.5	16.3[c]
Fluroxypyr	0.2	0.0	2.0	7.3	21.5	7.7[g]
MCPA	0.2	7.1	16.2	22.4	31.5	19.3[b]
P-value						0.001
Experiment 2						
Day 0		0				
Control	0	5.8	16.7	22.7	31.9	19.3[a]
2,4-D	0.1	1.7	8.3	15.2	22.7	12.0[c]
Fluroxypyr	0.1	0.8	5.0	11.0	21.0	9.5[d]
MCPA	0.1	4.2	13.3	20.2	25.2	15.7[b]
P-value						0.001

Table 2: Impact of dipping with auxins on calyx browning of Navel oranges grown and stored at room temperature

Treatment	Conc. (mmol L^{-1})	Storage time (weeks)				Mean
		1	2	3	4	
Experiment 1						
Day 0		1 score				
Control	0	2.2	2.6	3.2	3.8	2.95[bc]
2,4-D	0.1	2.0	2.3	2.8	3.3	2.60[d]
Fluroxypyr	0.1	1.7	2.1	2.5	3.1	2.29[e]
MCPA	0.1	2.1	2.4	2.9	3.5	2.73[c]
2,4-D	0.2	2.1	2.4	2.9	3.4	2.66[cd]
Fluroxypyr	0.2	1.6	1.9	2.4	3.0	2.43[f]
MCPA	0.2	2.1	2.5	3.0	3.5	2.78[d]
P-value						0.001
Experiment 2						
Day 0		1 score				
Control	0	1.7	2.4	2.9	3.7	2.68[a]
2,4-D	0.1	1.4	1.9	2.4	3.0	2.18[c]
Fluroxypyr	0.1	1.4	1.7	2.2	2.8	2.03[d]
MCPA	0.1	1.6	2.2	2.6	3.3	2.43[b]
P-value						0.001

resulting in a general reduction in senescence rates, which agreed with the findings by Alhassan *et al*. (2022) where dipping Valencia oranges with auxins prior to storage delayed senescence and maintained fruit quality.

Each value for calyx browning is the mean of assessments on 3 replicates of 40 fruits taken for 4 weeks. Each value within a column with the same superscript letter is not significantly different.

Impact of Auxins Treatment on Citrus Fruit Rot

During storage losses of the fruit through decay were monitored throughout both experiments. This determination was performed every seven days during the storage period of the fruit. There was a significant beneficial effect of all auxin treatments with 0.1 and 0.2 mmol L^{-1} in both experiments ($p<0.001$) compared with control fruit during ambient storage conditions (Table 3). There was also a significant interaction between treatments and storage time. Oranges dipped with fluroxypyr showed the maximum delay in fruit rot or decay, with the higher concentration (0.2 mmol L^{-1}) being more effective relative to 0.1 mmol L^{-1}, probably due to lower toxicity level of the compound. However, the result also shows that dipping at 0.1 mM MCPA in both experiments was less effective than fluroxypyr and 2,4-D treatments. Treatment with 2,4-D was more effective in retarding fruit rot occurrence than MCPA and control, with the lower concentration (0.1 mmol L^{-1}) being more effective than 0.2 mmol L^{-1}. The result of this study is consistent with the findings of Ma *et al*. (2014) whereby fruit rot incidences were significantly reduced by 2,4-D treatment to 11.58% with control fruit reaching 86.37% at the end of storage. The authors inferred that a decrease in ethylene production in 2,4-D-treated oranges could be a result of the retardation of fruit senescence during storage.

Each value for rot incidence is the mean of assessments on 3 replicates of 40 fruits taken for 4 weeks. Each value within a column with the same superscript letter is not significantly different.

Effect of Auxins Treatment on Fruit Metabolism

Oranges are classified under non-climacteric fruit which by their characteristics do not exhibit significant production of ethylene during postharvest harvest (Burns, 2016). Although non-climacteric fruits do not clearly show increases in ethylene production during ripening, in certain cases, prolonged fruit storage may lead to an increase (Alhassan *et al*., 2020). To determine whether pre-storage dipping with the different auxins was affecting the underlying metabolism of citrus, fruit were assessed for ethylene production and respiration rate every four days. In this study, untreated fruit produced significantly higher ethylene production ($p<0.001$) compared with auxins-treated fruit during storage (Table 4). Treating oranges with 0.1 and 0.2 mmol L^{-1} had lower ethylene production than other treatments with 0.2 mmol L^{-1} being the lowest, while ethylene production in fruit treated with MCPA and 2,4-D had slightly

Table 3: Impact of dipping with auxins on the rot of Navel oranges grown stored at room temperature

Treatment	Conc. (mmol L^{-1})	Storage time (in weeks)				Mean
		1	2	3	4	
Experiment 1						
Day 0		0				
Control	0	11.1	22.2	27.5	38.6	24.9^{b}
2,4-D	0.1	2.0	13.1	17.4	31.5	16.0^{e}
Fluroxypyr	0.1	0.0	5.1	10.3	24.5	10.0^{f}
MCPA	0.1	4.0	14.1	21.3	33.5	18.3^{d}
2,4-D	0.2	4.0	16.2	23.4	30.5	18.5^{d}
Fluroxypyr	0.2	0.0	4.0	8.3	22.4	8.7^{g}
MCPA	0.2	6.1	17.2	24.4	37.6	21.3^{c}
P-value						0.001
Experiment 2						
Day 0		0				
Control	0	5.8	15.0	23.5	31.0	18.8^{b}
2,4-D	0.1	1.7	8.3	16.0	23.5	12.4^{e}
Fluroxypyr	0.1	0.8	3.3	8.5	19.4	8.0f
MCPA	0.1	1.7	11.7	18.5	25.2	14.3^{d}
P-value						0.001

higher ethylene production rates, with no significant difference between 0.1 and 0.2 mmol L^{-1} concentrations, but lower auxin treatment concentration (0.1 mmol L^{-1}) was more effective at the end of storage. Similarly, a previous study reported that ethylene production in citrus decreased when fruits were treated with 2,4-D (Ma *et al*., 2014). However, the greatest suppression of ethylene in fruit was observed on fruit dipped with both fluroxypyr concentrations (0.1 or 0.2 mmol L^{-1}).

There was a significant effect of all the auxin treatments on the respiration rate of the oranges ($p < 0.001$) relative to both experiments. The respiration rate of both control and treated fruit showed a gradual increase in the rate of respiration during the time of storage, but the respiration rate in fluroxypyr-treated fruit was significantly inhibited at 0.1 and 0.2 mmol L^{-1} compared with the control and the other auxins treatment. Overall dipping with 0.2 mmol L^{-1} fluroxypyr was the most effective in reducing respiration rate compared with both 0.1 mmol L^{-1} 2,4-D or MCPA. This reduction in respiration rates retained overall fruit quality for a longer period than untreated fruit and prolonged postharvest life. Treatment with both 0.1 mmol L^{-1} 2,4-D and MCPA significantly delayed the respiration rate of the fruit relative to control, and there was no significant difference between the two auxins on quality. Similarly, a study showed that the respiration rate in 2,4-D-treated fruit was significantly inhibited relative to the control fruit (Ma *et al*., 2014).

There was a significant effect of auxin treatments on ethanol accumulation in fruit compared with untreated fruit ($p<0.001$) at the end of storage. Fruit treated with fluroxypyr has been shown to have provided the greatest reduction in ethanol content of the oranges than control fruit, with both 0.1 and 0.2 mmol L^{-1} concentrations being effective. Treatment with both 2,4-D concentrations at 0.1 and 0.2 mmol L^{-1} and for both locations and MCPA (0.1 mmol L^{-1}) as well lowered the level of ethanol accumulated by fruit during the storage regime, with the untreated fruit accumulating significantly higher ethanol

Table 4: Impact of dipping with auxins on the metabolism of Navel oranges stored at room temperature

Treatment	Conc. (mmol L^{-1})	Ethylene production ($\mu L\ C_2H_4\ kg^{-1}h^{-1}$)	Respiration rate ($mL\ CO_2\ kg^{-1}h^{-1}$)	Ethanol content (g 100 mL^{-1})
Experiment 1				
Day 0		0.4^{e}	12.9^{e}	2.4^{e}
Control	0	6.1^{a}	26.0^{ab}	5.1^{bc}
2,4-D	0.1	2.9^{c}	22.4^{c}	4.0^{d}
Fluroxypyr	0.1	1.4^{d}	16.8^{d}	2.9^{e}
MCPA	0.1	3.3^{c}	22.8^{c}	4.3^{d}
2,4-D	0.2	4.4^{bc}	23.2^{c}	4.5^{d}
Fluroxypyr	0.2	0.5^{e}	13.8^{e}	3.1^{e}
MCPA	0.2	5.8^{ab}	24.0^{bc}	4.7^{cd}
P-value	0.001	0.001	0.001	0.001
Experiment 2				
Day 0		0.2^{c}	5.4^{d}	0.1^{d}
Control	0	1.2^{a}	13.3^{b}	2.2^{b}
2,4-D	0.1	0.8^{b}	10.4^{c}	0.7^{cd}
Fluroxypyr	0.1	0.3^{c}	6.7^{d}	0.1^{d}
MCPA	0.1	9.0^{b}	10.8^{c}	0.9^{c}
P-value	0.001	0.001	0.001	0.001

in both experiments. No significant difference was observed between both concentrations (0.1 and 0.2 mmol L^{-1}) of 2,4-D and MCPA resulting in a similar but smaller reduction in ethanol production in the fruit, which is in agreement with previous studies where no significant effect of 2,4-D on the accumulation of ethanol in Navelina oranges or Clemenule mandarins at the end of storage (Sdiri *et al.*, 2013). The effects of the treatment on ethanol content in this study are consistent with the levels of ethylene and respiration rate in the experiments, with the greatest reduction of ethanol found in oranges dipped at 0.2 mmol L^{-1} prior to storage at room temperature.

Each value for ethylene production, respiration rate, and ethanol content is the mean of assessments on 3 replicates of 40 fruits taken at the end of storage. Each value within a column with the same superscript letter is not significantly different.

Effect of Auxins on Internal Quality

A study has shown that a decrease in fruit firmness is a result of cell wall degradation, as a consequent coordinated action of cell wall-modifying enzymes of citrus (Pathak & Sanwal, 1998). In both experiments, the auxins-treated fruits showed softening significantly lower than untreated citrus fruit at the end of the storage ($p<0.001$). Fruit dipped with fluroxypyr showed the highest fruit firmness in both experiments, but there was no significant difference between the two treatment concentrations (0.1 and 0.2 mmol L^{-1}). There was no significant difference between 0.1 mmol L^{-1} 2,4-D and both concentrations of fluroxypyr although 0.2 mmol L^{-1} of the latter showed the highest firmness. Greater fruit firmness after harvest is an indicator that such fruit will be more resistant to fungi infection (Garcia *et al.*, 2016). However, dipping with MCPA showed no significant effect on softening of the orange fruit at either treatment concentration from either location, which agreed with the findings by El-Otmani and Coggins Jr. (1991) that 2,4-D has little effect on fruit rind firmness and weight loss during storage. The effects of this treatment on the softening of the oranges decreased with a decrease in rots and calyx senescence, which demonstrates that cell wall degradation may have a greater influence on calyx abscission and fruit decay incidence (Baldwin, 2004).

This study was conducted to investigate the effects of the different auxins during storage at ambient temperature, and the results (Table 5). The result shows that the brix content was significantly lower than the control ($p<0.001$) in fruit from the two growing locations, with the greatest reduction occurring in fruit treated with fluroxypyr with no significant difference between the 0.1 or 0.2 mmol L^{-1} treatments. Dipping with 0.1 mmol L^{-1} 2,4-D and MCPA (in both experiments) was equally effective in delaying and increasing in brix of fruit. There was no significant effect of 2,4-D and MCPA applied at 0.2 mmol L^{-1} on the juice of the fruit. Similarly, it was reported that 2,4-D treatment before storage has little effect on extractable TSS (El-Otmani & Coggins Jr., 1991). The data shows that the acid level was significantly higher in orange fruit with dipped to auxins ($p<0.001$) relative to control fruit. The acid level varied among auxin treatments, with a significantly higher acid content observed in fruit from both growing locations for fluroxypyr (0.1 or 0.2 mmol L^{-1}). 2,4-D dipping produced significantly higher TA at both treatment concentrations. Increased fluroxypyr concentrations greatly reduced brix content and maintained higher TA, but decreasing concentrations of MCPA and 2,4-D maintained a higher TA and lower brix content at the end of storage. The result is however in contrast with the findings of El-Otmani and Coggins Jr. (1991) that 2,4-D treatment has little effect on the TA of citrus fruit.

The TSS/TA ratio is an important factor that relates to the maturity index (Alhassan *et al.*, 2022), and other quality characteristics of citrus fruit (Barros *et al.*, 2012). In this investigation, the Brix/acid ratio of fruit was significantly reduced relative to the control fruit for fluroxypyr at both 0.1 and 0.2 mmol L^{-1} concentrations. The brix/acid ratio was also significantly lower than both 2,4-D concentrations but was not

Table 5: Impact of dipping with auxins on internal quality of Navel oranges stored at room temperature

Treatment	Conc. (mmol L^{-1})	Firmness (N)	Brix (°B)	Acid (% citric acid)	Brix/acid ratio
Experiment 1					
Day 0		31.1^{a}	8.7^{d}	1.38^{a}	6.3^{e}
Control	0	24.9^{cde}	11.4^{ab}	0.89^{d}	12.8^{a}
2,4-D	0.1	27.4^{abc}	10.2^{c}	1.12^{b}	9.1^{d}
Fluroxypyr	0.1	29.0^{ab}	9.1^{d}	1.30^{a}	7.0g
MCPA	0.1	25.7^{cde}	10.4^{c}	1.00^{cd}	10.4^{c}
2,4-D	0.2	27.1^{bcd}	10.9^{b}	1.04^{c}	10.5^{c}
Fluroxypyr	0.2	29.2^{a}	9.0^{d}	1.34^{a}	6.7^{e}
MCPA	0.2	26.3^{cde}	11.2^{b}	0.91^{cd}	12.3^{b}
P-value	0.001	0.01	0.001	0.001	0.001
Experiment 2					
Day 0		32.0^{a}	8.9^{d}	1.32^{a}	6.7^{c}
Control	0	26.8^{cd}	11.5^{b}	0.95^{c}	17.7^{a}
2,4-D	0.1	28.5^{b}	10.4^{c}	1.02^{b}	10.2^{b}
Fluroxypyr	0.1	30.9^{a}	9.3^{d}	1.31^{a}	7.9^{c}
MCPA	0.1	27.3^{bcd}	10.6^{c}	1.09^{b}	9.7^{b}
P-value	0.001	0.01	0.001	0.01	0.001

Note: Each value for fruit firmness, Brix, and acid content is the mean of assessments on 3 replicates of 40 fruits taken at the end of storage. Each value within a column with the same superscript letter is not significantly different.

as effective as dipping fruit with fluroxypyr. The change in brix/acid ratio is shown to be due mainly to a higher acid in auxin-treated fruit rather than a lower level of brix, which suggests that fluroxypyr is a potential postharvest treatment for maintaining orange quality during ambient storage (24±2 °C) conditions.

CONCLUSION

This study showed that the pre-dipping application of fluroxypyr, MCPA, or 2,4-D, followed by storage at ambient temperature preserved orange fruit quality compared to untreated. Treatment with fluroxypyr offered the highest internal and external fruit qualities of the oranges by delaying senescence with a greater effect. There was no significant difference between MCPA or 2,4-D applied prior to storage on fruit quality, although both treatments maintained external quality relative to control. However, the application of both MCPA and 2,4-D did not significantly affect the Brix or acid contents at the end of storage. Overall, the fluroxypyr application resulted in improved fruit quality by inhibiting calyx browning and abscission, and also reducing rot incidence, through suppression of ethylene production and respiration rate, and could be a potential alternative to 2,4-D currently used in the citrus industry. However, more studies are required to assess the effect of the application of fluroxypyr, followed by storage at lower temperatures (such as 10 °C) to determine its mode of action on the quality of a wider variety of citrus fruit.

ACKNOWLEDGEMENT

The authors thank the Government of Ghana (GoG) for providing Annual Research and Book allowance through the Ghana Tertiary Education Commission (GTEC), which was used in funding this work.

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