Review Article

Open Access

Journal of Foodscience Nutrition and Public Health

jfnp@scientificeminencegroup.com

Effects of Postharvest Application of 3,5,6-Trichloro-2-Pyridyloxyacetic Acid Solutions and Continuous Low Ethylene Exposure on Inhibition of Citrus Calyx Senescence and Loss of Other Quality Parameters

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Citation

Nasiru Alhassan (2022) Effects of Postharvest Application of 3,5,6-Trichloro-2-Pyridyloxyacetic Acid Solutions and Continuous Low Ethylene Exposure on Inhibition of Citrus Calyx Senescence and Loss of Other Quality Parameters. J Food Sci Nutr Public Health 2: 1-16

Publication Dates

Received date: August 07, 2022
Accepted date: September 07, 2022
Published date: September 09, 2022

Abstract

Calyx senescence of citrus fruit after harvest can contribute greatly to deterioration in quality and reduces the market value, as calyx detachment fruit facilitates fungal attacks. In this study, the effects of 3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6-TPA) on delaying calyx disorders of two citrus varieties were investigated. Navel oranges and 'Afourer'mandarins prior to storage were dipped with 3,5,6-TPA concentrations of 0.002, 0.004, 0.008, 0.0016 or 0.0032 mM and submitted to ≤0.001, or 0.1 µL L-1 ethylene concentrations during storage at 20˚C and relative humidity (RH) ≤85%. The 3,5,6-TPA treatments demonstrates a concentration-dependent effect with higher concentrations resulting in the most significant impact of reducing the incidence of calyx senescence and fruit rot incidence. Fruit treated with the auxin also showed lower respiration rate, ethylene production, ethanol accumulation, delayed an increased in TSS or decreased in TA contents. The exposure of fruit to higher ethylene (0.1 and 1.0 µL L-1) in the storage chamber slightly increased calyx changes and therefore decreased fruit quality, although it was better than control fruits. The results provide strong evidence that 3,5,6-TPA treatment can be used to alleviate calyx deterioration and maintain quality in citrus fruit. However, more studies are required to elucidate the regulatory effect of the 3,5,6-TPA auxin involved in the inhibition of calyx senescence of citrus fruit.

Keywords: 3,5,6-TPA; Citrus Fruit; Calyx Senescence; Ethylene Exposure; Quality; Storage

Introduction

Citrus calyx senescence is an important quality parameter during the marketing of the fruits as Consumers usually associate the external appearance with internal quality parameters and thereby mostly influenced their decision to purchase. Auxins can cause the elongation of cells which precedes cell wall degradation. During this process, the cells are insensitive when ethylene is present, hence delaying the separation process, by the presence of auxin [1]. The 2,4-dichlorophenoxy acetic acid auxin (2,4-D) solution has been applied over the years to inhibit calyx alterations and to maintain the quality of citrus fruits [1]. But due to health concerns, the application of 2,4-D on citrus in many countries has been restricted as a consequence of higher toxicity levels. This has prompted a search for a safer and effective substitute to maintain calyx quality and extend storage life [1]. An investigation conducted has demonstrated that detectable 2,4-D residues in food samples were found to be 0.2 mg/kg [2], which is higher toxicity compared with the 0.1 mg/kg required in food substances according to [3]. While other studies show that the residues level of 2,4-D were still detected in birch leaves three years after application [4].

Citrus fruits are categorised as non-climacteric based on their respiration pattern and show little changes in ethylene production and carbon dioxide during fruit storage and maturation [5]. However, during extended storage fruit can senescence and increase respiration rate and ethylene production, hence causing overall fruit quality loss. [6] report that exposure of citrus fruit to low ethylene accelerates degreening of the peel in early-season citrus varieties to achieve uniform yellow colouration. However, the application of a high concentration of ethylene during degreening has accelerated the calyx abscission and browning of citrus fruit [7]. But, [8] found that exposing 'Afourer' mandarins to ethylene at levels lower than 0.1 μL L^{-1} decreased the deterioration of the calyx.

3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6-TPA) is a synthetic auxin which is mostly applied as a pre-harvest treatment to control the incidence of fruit splitting, increase citrus fruit size and prevent fruit drop [9]. However, few studies conducted ascertained the postharvest application of 3,5,6-TPA has been associated with degreening at high ethylene exposure for a short storage duration. The report showed that Clementine fruit dipped with 3,5,6-TPA prior to degreening and held at 2 μL L^{-1} ethylene during seven-day storage at ambient storage demonstrated a delay in calyx changes [10]. While [11] showed that dipping with 3,5,6-TPA before a degreening treatment inhibited calyx senescence of citrus fruit during shelf life.

This current study investigates the efficacy of 3,5,6-TPA auxin on a wider range of concentrations in an atmosphere exposed to different ethylene concentrations within a level of ethylene likely to exist in commercial storage regimes on a wider range of calyx alterations and internal postharvest quality parameters of Navel oranges and 'Afourer' mandarins during shelf life storage.

Materials and Methods

Plant Materials and Experimental Design

This experiment was conducted to evaluate the effects of 3,5,6-TPA on citrus fruit quality. Navel oranges (*Citrus sinensis* L. Osbeck) were harvested at commercial maturity with attached calyxes from a New South Wales Department of Primary Industries (NSW DPI) research farm at Somersby on the NSW Central Coast and waxed 'Afourer' seedless mandarins were obtained from Mildura Fruit Company, Victoria, Australia and transported to the NSW DPI postharvest laboratory in the Central Coast. Upon reaching, the fruits were sanitised with sodium hydrochloride solution (50 μL L^{-1}), sorted, graded for fruit uniformity and randomised into treatment units. Each treatment had three units with each unit consisting of 40 fruits. The fruit were dipped for 2 min in aqueous solutions containing 3,5,6-TPA at concentrations of 0 (control), 0.002, 0.004, 0.008, 0.016 or 0.032 mM. The fruits were air-dried and placed in individual plastic containers. The plastic containers were connected to humidified air streams containing ≤0.001 (ethylene-free), or 0.1 μL L^{-1} ethylene and at 50 mL min^{-1} flow rate. These concentrations were applied because ethylene concentration in the marketing environment of fresh fruits is often ≤1.0 [12]. The fruit was stored at an ambient temperature for 32 days and assessed for fruit decay, calyx browning and abscission every 4 days, while for the internal qualities were evaluated at the end of storage.

Evaluation of calyx changes and fruit decay

Calyx colour was scored on a 5-point scale according to the criteria of [13], where; 1 = no browning; 2 = 1 - 25% brown; 3 = 25 - 50% brown; 4 = 25 - 75% brown; and 5 = greater than 75% brown. The mean of the browning score for the fruit in each treatment unit was calculated during each assessment time, The number of fruit with the detached calyx was counted during the assessment, and the per cent calyx abscission of fruit was

calculated. It should be noted that a browning score was only applied to fruit where the calyx remained attached to the fruit. Fruit decay was also evaluated, and the data were expressed as a percentage of fruit affected in each treatment unit. The limit of consumer acceptability for these characteristics was considered to be when 20% of the fruit in each treatment unit had no calyx attached, the calyx colour score was 2.5, and decay was visible on 20% of the fruit. The data obtained for each of these parameters at each assessment were plotted against the time of storage, and the time to reach each of the above limits was considered as the end of the market life of fruit.

Determination of respiration rate and ethylene production

The rate of respiration of fruit stored was measured by withdrawing 1 mL of gas from the headspace of a 2 L septum-containing glass jar housing three fruits which had been sealed for three hours. The gas samples were then assessed by thermal conductivity gas chromatography as described by [14]. The respiration rate of fruits was then expressed as mL CO_2 kg^{-1} h^{-1}.

The ethylene level in the flow-through airstream was monitored regularly at the drum inlet port by withdrawing 1 mL of the gas sample in a syringe and injecting it into a flame ionisation gas chromatograph (Varian Star CX-3400, Walnut Creek, CA), fitted with a stainless steel column (2 m × 3.2 mm × 2.2 mm i.d.) and packed with Porapak Q (80-100 mesh, Altech Sydney). The operating temperatures for the injectors, column and detector, were 70 °C 100 °C and 150 °C, respectively. The flow rates of, nitrogen, hydrogen and air as the carrier and combustion gases were 15, 20 and 50 mL min^{-1} respectively.

Evaluation of fruit internal quality

The internal quality of fruit were evaluated according to the method of [13] with little modification. Briefly, two fruit from each treatment unit were juiced using a juicer and filtered through two layers of muslin gauze. The total soluble solids (TSS) of the filtered juice were determined as the refractive index using a portable digital refractometer (Atago, Tokyo) at 20 °C and data was expressed as percentage °Brix. Titratable acidity (TA) 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 the percentage of citric acid equivalents. The fruit maturity index (MI) of the fruits was calculated as the TSS/TA ratio.

For ethanol accumulation of fruit, 10 mL of juice was transferred into a glass vial (20 mL) with crimp-top caps sealed with a silicone septum and incubated in a water bath at 30 °C for 10 min. A 1 mL gas sample was then drawn from the headspace of the vial and analysed for ethanol content by gas chromatography (Model 580, Gow-Mac-Bethlehem, PA, USA) equipped with a flame ionisation detector and a Carbowax column (GowMac, USA). The operating temperatures of the detector, column and injector were 190 °C, 68 °C and 190 °C respectively. The gas flow rates for air, hydrogen and nitrogen were 300, 30 and 30 mL min^{-1} respectively. The ethanol accumulation was then expressed at a g /100 g relative to 5 µl standard ethanol.

Statistical analysis

The experiment was conducted using a completely randomized design (CRD). Data were based on three replicates in each treatment and were statistically processed using Statistical Analysis Software (SAS) (Version 9.4). A two-factor analysis of variance (ANOVA) was performed to determine the effect of the treatments on the quality parameters. Where there was a significant effect between treatments, the least significant difference (LSD) of the treatment means was calculated at P = 0.05 to determine significant differences.

Results

Effect of 3,5,6-TPA on calyx quality and decay incidence

There was a significant effect of 3,5,6-TPA on calyx browning, calyx abscission and decay incidence ($P<0.001$) for all the citrus varieties. The time for fruits to reach a browning score of 2.5 and 20% of fruit to have calyx abscission and exhibit decay increased as the 3,5,6-TPA concentration increased during storage. Fruit dipped at even the lowest 3,5,6-TPA concentrations before storage generally showed a significant delay in abscission, browning and fruit rot when compared with control fruit in this study. In addition 'Afourer' mandarins and Navel oranges exposed to 0.1 ppm ethylene had a shorter time to brown, abscissed, and increased in rot incidence compared with fruit held in ethylene-free air (<0.001 µL L^{-1}) during the storage regime.

The effect of 3,5,6-TPA on fruit internal quality

The treatment with 3,5,6-TPA auxin and storage at 20 °C produced a significant effect on a range of physio-chemical quality parameters of the citrus fruits as shown in Table 1. The respiration rate of both Navel oranges and 'Afourer' mandarins

held at ambient storage with a continuous flow of 0.1 µL L^{-1} ethylene was significantly reduced (P<0.001) by pre-storage dipping with 3,5,6-TPA. Both Afourer' mandarins and Navel oranges treated with higher 3,5,6-TPA concentration and stored in 0.1 µL L^{-1} ethylene atmosphere significantly reduced the respiration rate (P < 0.05) relative to control fruit but there was no significant difference with lower 3,5,6-TPA treatment (0.002 mM) when compared with control fruit at the end of storage.

	Time (in days) to defined loss of quality						Quality at the end of storage															
							Respiration rate (mL CO_2 kg^{-1} h^{-1})			Ethanol (g /100 g)			TSS (°Brix)			TA (% citric acid)			TSS: TA		
3,5,6-TPA conc. (mM)	Calyx abscission (20% loss)		Calyx browning (2.5 scores)		Decay incidence (20%)																	
Ethylene (µL L^{-1})	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.1		0.0	0.1		0.0	0.1		0.0	0.1		0.0	0.1	
	01		01		01		01			01			01			01			01		
Afourer' mandarin																						
Control	4.7^{e}	4.0^{e}	10.3^{d}	8.0^{e}	11.6^{e}	8.0^{e}	15.3	17.4^{a}		3.8	4.7		14.2^{a}	14.5^{a}		0.60^{e}	0.55^{d}		23.7^{a}	26.4^{a}	
0.002	7.9^{d}	6.5^{d}	11.9^{d}	9.2^{de}	13.3^{e}	10.7^{d}	12.5	15.7^{ab}		2.2	4.3		14.0^{a}	14.1^{ab}		0.72^{bc}	0.64^{c}		19.4^{b}	22.0^{b}	
0.004	11.8^{c}	10.3^{c}	16.0^{c}	11.0^{d}	16.0^{cd}	12.0^{d}	11.9	13.6^{bc}		1.7	4.1		13.9^{a}	14.0^{abc}		0.74^{ab}	0.66^{bc}		18.8^{b}	21.2^{b}	
0.008	13.2^{c}	12.0^{c}	17.8^{c}	15.6^{c}	18.7^{bc}	16.0^{c}	10.6	12.9^{c}		1.6	2.6		13.7^{ab}	13.8^{bc}		0.75^{ab}	0.68^{ac}		18.3^{bc}	20.3^{bc}	
0.016	21.3^{b}	17.9^{b}	24.8^{b}	22.7^{b}	21.3^{b}	19.7^{b}	10.0	11.3^{cd}		1.5	2.4		13.4^{b}	13.5^{cd}		0.76^{ab}	0.71^{ab}		17.6^{bc}	19.0^{cd}	
0.032	31.8^{a}	26.7^{a}	32.0^{a}	28.7^{a}	27.7^{a}	22.7^{a}	9.1	10.0^{d}		1.3	2.0		12.8^{b}	13.1^{d}		0.85^{a}	0.76^{a}		15.1^{c}	17.2^{d}	
P-value	*0.0*	*0.0*	*0.0*	*0.0*	*0.01*	*0.0*	*0.40*	*0.0*		*0.14*	*0.15*		*0.0*	*0.0*		*0.05*	*0.0*		*0.0*	*0.0*	
	01	*01*	*01*	*01*		*01*		*01*					*01*	*01*			*01*		*01*	*01*	
Navel orange																						
Control	5.6^{d}	3.9^{f}	11.6^{e}	8.0^{e}	20.0^{d}	19.3^{d}	26.9	28.4^{a}		1.5^{a}	1.6^{a}		12.3^{a}	12.6^{a}		1.18^{d}	1.14^{d}		10.5^{a}	11.1^{a}	
0.002	8.7^{cd}	7.0^{e}	13.3^{e}	10.7^{d}	22.7^{cd}	21.3^{cd}	22.8	23.7^{ab}		1.2^{ab}	1.5^{ab}		12.1^{ab}	12.3^{ab}		1.22^{cd}	1.18^{cd}		9.9^{ab}	10.4^{b}	
0.004	11.5^{c}	10.1^{d}	16.0^{cd}	12.0^{d}	25.3^{bcd}	24.0^{bc}	20.0	22.1^{bc}		1.1^{abc}	1.4^{ab}		11.8^{ab}	12.0^{bc}		1.25^{bcd}	1.21^{bc}		9.4^{bc}	9.9^{bc}	
0.008	15.7^{b}	11.8^{c}	18.7^{bc}	16.0^{c}	28.0^{abc}	26.7^{ab}	19.1	21.0^{bc}		1.0^{bc}	1.3^{abc}		11.7^{bc}	11.9^{bc}		1.28^{abc}	1.24^{bc}		9.1^{c}	9.6^{cd}	
0.016	17.8^{b}	15.6^{b}	21.3^{b}	19.7^{b}	29.3^{ab}	28.0^{ab}	16.3	20.8^{bc}		0.9^{bc}	1.2^{bc}		11.6^{bc}	11.8^{c}		1.31^{ab}	1.27^{ab}		8.8^{cd}	9.3^{e}	
0.032	28.6^{a}	26.4^{a}	27.7^{a}	22.7^{a}	32.0^{a}	29.3^{a}	16.2	16.3^{c}		0.7^{c}	1.0^{c}		11.4^{c}	11.6^{c}		1.36^{a}	1.32^{a}		8.4^{d}	8.8^{e}	
P-value	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.0*	*0.30*	*0.05*		*0.05*	*0.05*		*0.05*	*0.01*		*0.01*	*0.01*		*0.0*	*0.0*	
	01	*01*	*01*	*01*	*01*	*01*													*01*	*01*	

For each fruit, values for quality factors within a column with the same superscript letter are not significantly different at P = 0.05. Each value is the mean of assessments on 3 treatment units of 45 fruits.

Table 1: Effects of 3,5,6-trichloro-2-pyridyloxyacetic acid (TPA) and low ethylene exposure on visual and internal quality factors of 'Afourer' mandarins and Navel oranges during storage for 32-days at 20°C

The effect of pre-storage dipping with 3,5,6-TPA and storage with 0.1 μL L^{-1} ethylene atmosphere on ethanol accumulation varied between the two citrus types at the end of storage. The analysis shows there was no significant effect of 3,5,6-TPA on ethanol content of 'Afourer' mandarins, but there was a significant reduction of ethanol accumulation for Navel oranges ($P<0.05$) during storage. Pre-storage dipping with 3,5,6-TPA significantly and storage in low ethylene environment significantly affected the total soluble solids (TSS) and titratable acidity (TA) content in all the citrus fruit with increased concentration resulting in a decline in TSS and an increase in TA over control fruits. The effects of these changes led to a significant increase in the TSS/TA ratio. The contents of TSS, TA and TSS/TA ratio of fruit dipped with the higher 3,5,6-TPA concentrations were similar to values obtained on day zero of the experiment demonstrating that 3,5,6-TPA had delayed the changes in these quality parameters during the storage period.

Discussion

Citrus calyx browning and abscission and fruit decay are common physiological changes that are accompanied by senescence. The occurrence of these disorders is mainly a result of increased ethylene synthesis in fruits after harvest. By dipping citrus fruit with 3,5,6-TPA prior to storage, it has been found that senescence was reduced in the citrus fruit, delayed the emergence of fruit decay and retained internal quality on the citrus fruits investigated would appear to have potential as a commercial treatment. As an auxin 3,5,6-TPA, it would appear to act in a similar way relative to the current commercial 2,4-D treatment on citrus fruit. Although 2,4-D auxin inhibits calyx browning and abscission by maintaining its vitality and thus reduces fruit decay [15], finding a desirable substitute is important as far as health perspective and environment is a concern [1] as residues remain longer after application. A study [16] has shown that the detection limits of 0.05 ng/L for triclopyr, and 0.5 ng/L for 2,4-D, which may account for lower calyx senescence in 3,5,6-TPA treatments. The 3,5,6-TPA which often contain 1 gram of the active ingredient per tablet, are readily soluble during application [17] and uptake into wheat and barley leaves were virtually complete 12 h after treatment [18]. But another investigation by [19] indicates residues of 2,4-D were still detected in samples of birch leaves for up three years post-application.

Overall, the result of this study shows that all the auxins concentrations applied were efficacious in retaining calyx quality and inhibited fruit decay incidence compared with control fruits, but increased 3,5,6-TPA concentrations were more effective in maintaining fruit this external quality. This finding is consistent with the findings of [10], who found that the treatment of 'Clementine' mandarins with 3,5,6-TPA during degreening treatment and storage inhibited calyx changes. Furthermore, early studies by [10] demonstrate that an increase of 3,5,6-TPA treatment concentration without exogenous ethylene in the storage atmosphere delayed calyx senescence and extended 'Afourer' mandarins and Navel and Valencia oranges prolong storage. The beneficial effect of 3,5,6-TPA, therefore, is delaying calyx deterioration and rot development and could limit the reliance on 2,4-D auxin and fungicides treatments to control physiological disorders of citrus fruits during long-term storage.

The respiration rate of fruit is a significant indicator of physiological and biochemical changes, and it increases as fruit condition deteriorates. The result shows that 3,5,6-TPA inhibited respiration rates of the citrus even in an ethylene atmosphere compared to control fruits. The combined treatment of ≤0.001 μL L^{-1} ethylene with increased auxin concentrations reduced fruits' respiration, while an increase in respiration rate was observed at 0.1 μL L^{-1} ethylene atmosphere although lower than in control fruit. Navel oranges had higher respiration than 'Afourer' mandarins with ethylene. However, [20] showed that mandarins are generally more susceptible to ethylene exposure than other citrus varieties. The respiration rate of fruits dipped at higher 3,5,6-TPA concentration with ethylene in the storage environment was significantly low although fruit exposed to ≤0.001 μL L^{-1} ethylene was lower. This beneficial effect suggests the auxin could be suppressing ethylene action and therefore mitigates the general metabolism of the fruit during storage. Moreover, the ability of 3,5,6-TPA to inhibit senescence of the citrus fruits which could be induced by the presence of 0.1 μL L^{-1} ethylene in the storage chamber is further encouraging as this level of ethylene is often encountered during the storage and marketing of all fruit and vegetables [12].

Concerning physio-chemical quality, studies have demonstrated that citrus fruit accumulates a large amount of ethanol. The increase depends on the fruit type and treatment conditions and the accumulation of ethanol leads to the development of off-flavour [21,22]. In the present study, increased concentrations of 3,5,6-TPA auxin significantly lower ethanol accumulation in the juice of all citrus fruits although no significant difference in ethanol level was detected in 'Afourer' mandarins, but [10] found an increased 3,5,6-TPA concentration cause an increase in ethanol content in Oronules and Clemenpons mandarin

stored for 7 days at 20 °C, although no significant difference was found in Oronules mandarins, and might be due to exposure of ethylene, either at short or long-term storage. As would be expected, ethanol accumulations were not particularly high in Navel oranges in all treatments compared to 'Afourer' mandarins. Several studies demonstrated a large amount of ethanol reached in mandarin varieties at the end of storage [22, 23].

Retaining internal quality on the three citrus fruits investigated appears to have potential as a commercial treatment. Among the treatments, increasing 3,5,6-TPA concentration with or without ethylene in the storage environment maintained higher content of TSS at the end of storage, which is consistent with the finding by [10] of soluble solids content (SSC) of mandarins. This increase is probably due to an increase in sugars. Meanwhile, degradation of TA was slowed, indicating that 3,5,6-TPA might act as a replacement of natural auxins to prevent fruit from maturing, thus extending citrus fruit quality. Ma and Chen (2003) reported that a higher TA might be used as the carbon source in the tricarboxylic acid cycles in the respiration process to prolong the storage life of fruit. The elevation of TSS and delay in TA degradation reflected levels of increase in TSS/TA ratio reached at the end of storage with control fruit recording the highest. However, [10] found a lower SSC/TA ratio in control fruit compared with 3,5,6-TPA treatments of mandarins during 7-day storage.

Conclusion

Pre-storage dipping of citrus fruits with 3,5,6-TPA auxin has been exhibited to delay calyx senescence and fruit decay incidence and maintained good quality. The efficacy of the auxin was concentration-dependent with increased concentration shown to be more effective in retaining postharvest quality parameters. These positive results shown in 3,5,6-TPA treatments suggest that the auxin could potentially be used to delay calyx senescence and maintain the internal quality parameters of citrus fruits. But further studies would seem warranted to elucidate the mechanism through which the auxin works to maintain the postharvest calyx quality of citrus fruit during large scale storage.

Acknowledgement

The author the University of Newcastle, Australia, and the NSW Department of Primary Industries (NSW DPI), Ourimbah, Australia. This project was also supported and a contributed of Horticulture Innovation Australia –'Citrus Postharvest Science Program' (CT19003).

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

Conflict of interest

No conflict of interest in regards to the publication of this of paper.

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