Research Article

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Postharvest quality of 'Cripps Pink' apple fruit influenced by ethylene antagonists during controlled atmosphere storage with photocatalytic oxidation

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Abstract

BACKGROUND: The present study investigated the efficacy of 1*H*-cyclopropa[*b*]naphthalene (NC) and 1*H*-cyclopropabenzene (BC) with respect to antagonizing ethylene action and maintaining postharvest fruit quality in 'Cripps Pink' apple stored in a controlled atmosphere comprising 3.45 ± 0.45% oxygen and 2.40 ± 0.36% carbon dioxide with photocatalytic oxidation (PCO) at 0 ± 1 °C and 90 ± 5% relative humidity.

RESULTS: The BC, NC, and 1-methylcyclopropene (1-MCP) fumigation treatments delayed the climacteric peaks onset and retarded ethylene production rates compared to control fruit. Treatments with ethylene antagonist also maintained fruit firmness (up to 1.12 times), titratable acidity (up to 1.08 times), malic acid (up to 1.23 times), ascorbic acid (up to 1.12 times) and total phenol levels (up to 1.19 times) higher compared to that in control fruit. The 1-MCP was more efficient in reducing the rates of ethylene production compared to NC and BC, but, in the case of all other fruit quality parameters investigated, the effect of NC and BC treatments were on a par with 1-MCP.

CONCLUSION: The NC and BC have the potential to be used as ethylene antagonists in 'Cripps Pink' apple fruit stored in a controlled atmosphere with PCO. The efficacy of different concentrations of NC and BC in downregulating ethylene action, as well as interactive effects of PCO on the performance of ethylene antagonists, still warrants further investigation.

Keywords: ethylene; fumigation; 1*H*-cyclopropa[*b*]naphthalene; 1*H*-cyclopropabenzene; 1-methylcyclopropene; photocatalytic oxidation

INTRODUCTION

The 'Cripps Pink' apple fruit (*Malus domestica* Borkh.) is relished globally for its tangy-sweet taste, crunchy texture, pleasant flavour, and health benefitting compounds. The apple fruit is rich in dietary fibres, essential bioactive compounds, and minerals, and does not possess free forms of sodium and fats.[1] Apple is classified as a climacteric fruit and postharvest exposure to ethylene can invariantly accelerate the softening, ripening, and senescence process in fruits.[2] Therefore, efficient management of ethylene in the postharvest phase can significantly enhance the storage life at the same time as retaining a marketable fruit quality.[3] Several ethylene management approaches have been investigated by manipulating the storage environment and antagonising ethylene action in the fruit.[3] Methoxy vinyl glycine, 1-aminoethoxy vinyl glycine and amino oxyacetic acid, α-amino isobutyric acid and ethanol are some of the compounds that inhibit the enzymes involved in ethylene biosynthesis.[4] In apple fruit, even after ethylene biosynthesis inhibition, all of the ripening associated changes can be triggered by exposure to even a very low concentration of external ethylene.

A controlled atmosphere (CA) storage environment comprises lower levels of oxygen and higher levels of carbon dioxide compared to the normal atmosphere. At optimum storage temperatures, CA retards the rates of physiological activities in the fruit such as ethylene production and respiration. Furthermore, CA storage also delays senescence and ripening associated changes, consequently extending the fruit storage life.[5] CA storage is widely adopted by different stakeholders of the apple industry for storage, as well for export to distant destinations. On removal from CA

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storage, the apple fruit exhibits a sudden upsurge in ethylene production under ambient conditions.[6-8] The application of ethylene antagonists such as 1-methylcyclopropene (1-MCP) along with CA storage synergistically retards ethylene production and delays the onset of climacteric peaks. 1-MCP is the commercially popular ethylene antagonist compound used by growers around the world to enhance the storage life of fruits including apples and vegetables.[8] The ethylene antagonistic capacity of 1-MCP differs with treatment duration, concentration, cultivar, and storage temperature.[9] The pure form of 1-MCP is a relatively unstable liquid and vaporizes immediately at room temperature, making it difficult to apply as a treatment other than for fumigation.[10] Presently, several commercial formulations of 1-MCP are available in the market from different companies such as AgroFresh, Hazel®, Logfresh®, etc., for effectively delivering active ingredients (encapsulated, powder and spray forms) during the storage and transit of horticultural crops.[11-13] Singh *et al.*[14] identified the ethylene antagonistic capacity of two compounds, namely 1*H*-cyclopropa[*b*]naphthalene (NC) and 1*H*-cyclopropabenzene (BC), which are structurally different from 1-MCP, although the pure forms are relatively stable at room temperature. These compounds follow a similar mechanism as that of 1-MCP with respect to antagonizing the ethylene action in fruit at the receptor level. Tokala *et al.*[15] reported that fumigation treatments with NC and BC were comparatively more effective than dip formulations in downregulating ethylene production and maintaining the postharvest quality of 'Cripps Pink' apple fruit under low-temperature storage conditions. The ethylene antagonistic capacity of NC and BC was also reported in 'Cripps Pink' and 'Granny Smith' apple fruits in ozonized cold storage[16] and also in controlled atmosphere storage.[17]

Creative Research Technology, SA, Australia developed AiroFresh® technology, which operates via advanced oxidation processes and photocatalytic oxidation (PCO) to oxidize several airborne contaminants and degrade different organic gaseous compounds such as ethylene to form water and carbon dioxide. The instrument functions by the reaction between UVC light and a distinctive substrate coating to destroy the airborne organic particles as they move through the device. AiroFresh® technology also holds international organic certification from The National Association for Sustainable Agriculture Australia (NASAA).[18] Preliminary research studies conducted in South Australia reported that the advanced oxidation processes and PCO occurring in AiroFresh® units, as installed in CA storage, exhibited a high sugar content and fruit firmness in 'Cripps Pink' apples.[18] This technology is also being used by some of the commercial apple fruit storage facilities in Western Australia. No detailed study has reported on the effects of NC, BC, and 1-MCP fumigation treatments on postharvest fruit quality, as well as on the rates of respiration and ethylene production in the 'Cripps Pink' apple fruit stored in CA equipped with PCO. It was hypothesized that the ethylene antagonist treatments and CA conditions with PCO will retard the rates of ethylene production and respiration in 'Cripps Pink' apple fruit after 60 and 150 days of storage. The present study aimed to investigate the effects of NC, BC, and 1-MCP with respect to retarding the ethylene production and maintaining the postharvest fruit quality of 'Cripps Pink' apple in CA storage with PCO.

MATERIALS AND METHODS

Plant materials

The 'Cripps Pink' apple fruit was collected from the apple orchard in Balingup (34°13′S, 116°08′E) on 3 May 2018 at the commercial harvest stage [15.03 ± 0.04% soluble solids content (SSC); 0.82 ± 0.04% titratable acid (TA); 66.76 ± 3.84 N fruit firmness]. At this stage, the amount of ethylene produced by the fruit was negligible and undetectable by gas chromatography. The 'Cripps Pink' apple trees were 23 years old, grafted on M.26 rootstock and trained with a modified central leader system. The spacing of 4.5 × 1 m was maintained with plants oriented in the North–South direction. All the trees received preharvest foliar spray of calcium and after the harvested all the fruit were dipped in an aqueous solution of 'Magnate 750WG' (a.i. 750 g L^{-1} Imazalil) at 0.68 g L^{-1}, 'Stopit' (a.i. 160 g L^{-1} liquid calcium chloride ($CaCl_2$) at 15 mL L^{-1} and DPA (diphenylamine) at 5 mL L^{-1} to protect from postharvest diseases and disorders during storage. After air-drying the fruit properly, they were transferred in an air-conditioned vehicle to Curtin Horticulture Research Laboratory, Perth, packaged in corrugated cardboard boxes. Apple fruit of a relatively uniform size, as well as free from mechanical injuries or pest and disease symptoms, were used for the experiment.

Chemicals

The BC, NC, and 1-MCP chemicals were synthesized in Chemistry Laboratory, Curtin University (Perth, WA, Australia) as described by Davalian *et al.*,[19] Billups and Chow[20] and Fisher and Applequist,[21] respectively.

Fumigation treatments and storage conditions

The ethylene antagonist fumigation was executed for 18 h by placing apple fruit in 60-L hermetically sealable plastic drums at room temperature (20 ± 2 °C and 65 ± 5% relative humidity). The detailed procedure used for the fumigation treatments and control has been reported previously by Tokala.[22] Following 18 h of fumigation treatment, the fruit were arranged on soft board trays in properly labelled corrugated cardboard boxes. The boxes were then transferred to CA storage with AiroFresh® technology at the grower's property in Kirup (33°71′S, 115°90′E), Western Australia. The oxygen and carbon dioxide concentrations in CA storage were 3.45 ± 0.45% and 2.40 ± 0.36%, respectively, with the temperature maintained at 0 ± 1 °C and a relative humidity of 90 ± 5%. The treatments were arranged in two-factor factorial (treatments and CA-PCO storage time), replicated four times with 15 fruit per replication and stored for 60 and 150 days. After completion of respective storage periods, the fruit were transferred back to the laboratory to determine ethylene production and respiration rates, as well as other quality parameters of the fruit. The rates of respiration and ethylene production were determined daily for up to 10–14 days at ambient conditions to determine the climactic patterns of the fruit stored in a CA equipped with PCO. Various quality parameters of the fruit were estimated after the completion of each storage duration.

Determination of respiration and ethylene production rates

Two apple fruit per replication were randomly selected to estimate respiration and ethylene production rates. The procedure to determine respiration and ethylene production rates and details of the instruments used have been reported previously by Tokala.[22] The respiration and ethylene production rates were determined daily until the post-climacteric peaks were observed. The rates of respiration and ethylene production were calculated and expressed as μmol kg^{-1} h^{-1} ethylene and mmol CO_2 kg^{-1} h^{-1}, respectively.

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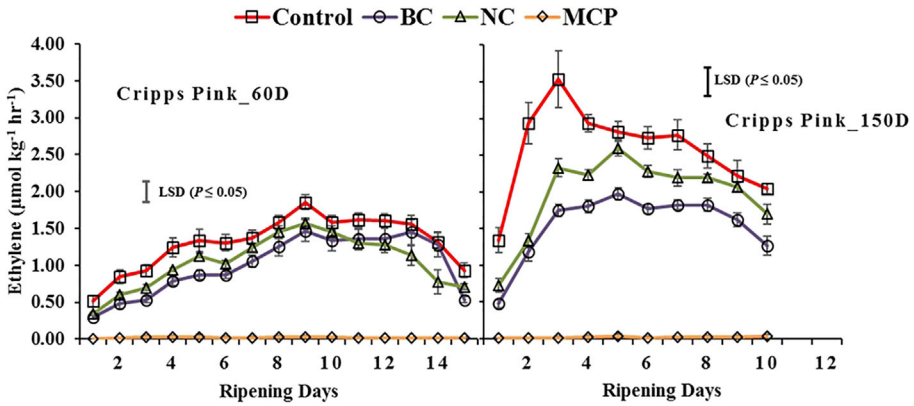

Figure 1. The ethylene production during ripening days (D) affected by 1*H*-cyclopropabenzene (BC), 1*H*-cyclopropa[*b*]naphthalene (NC), and 1-methylcyclopropene (1-MCP) fumigation (T) treatments in 'Cripps Pink' apple fruit stored in CA storage with photocatalytic oxidation for 60 and 150 days. The vertical bars represent SEM values and are not visible when values are smaller than the symbol. $n = 4$ replicates (two fruit per replication). Least significant difference ($P \leq 0.05$) T = 0.07, D = 0.14, TXD = 0.28 for 60 days and T = 0.12, D = 0.19, TXD = 0.39 for 150 days of storage.

Physiological loss of weight (PLW)

The weight of fifteen fruit per replication was recorded using a digital balance before transferring into the respective CA storage room with PCO. The values were recorded as the initial weight. After completion of respective storage durations, the final weight was then measured. The PLW was computed using the following formula and expressed as a percentage:

$$\text{PLW}\,(\%) = \frac{\text{Initial weight}\,(\text{kg}) - \text{Final weight}\,(\text{kg}) \times 100}{\text{Initial weight}\,(\text{kg})}$$

Fruit firmness

The fruit firmness was determined from ten fruit per replication using a texture analyser (TA Plus; Ametek Lloyd Instruments Limited, Bognor Regis, UK). The detailed method of determining the fruit firmness has been reported previously by Tokala.[22] The fruit was punctured at the peeled portion of fruit at the equatorial region on opposite sides with an 11 mm Magnus-Taylor probe. The fruit was punctured with a trigger force of 5 N at a sample depth of 7 mm and a test speed of 100 mm s^{-1}. Nexygen, version 4.6 (Ametek Lloyd Instruments Limited) was used to calculate the fruit firmness in newtons (N).

SSC, TA, and SSC:TA

The juice samples extracted from the sector portions of 13 fruit per replication were pooled and used to determine SSC, TA, and SSC:TA. The SSC was determined using the infrared digital refractometer (Atago – Palette PR 101; Atago Co., Tokyo, Japan). The TA was determined by titration method and expressed as a percent of malic acid.

Individual sugars and organic acids

The individual sugars (sucrose, glucose, fructose, and sorbitol) and organic acids (citric acid, tartaric acid, malic acid, succinic acid, and fumaric acid) were detected using reverse-phase high-performance

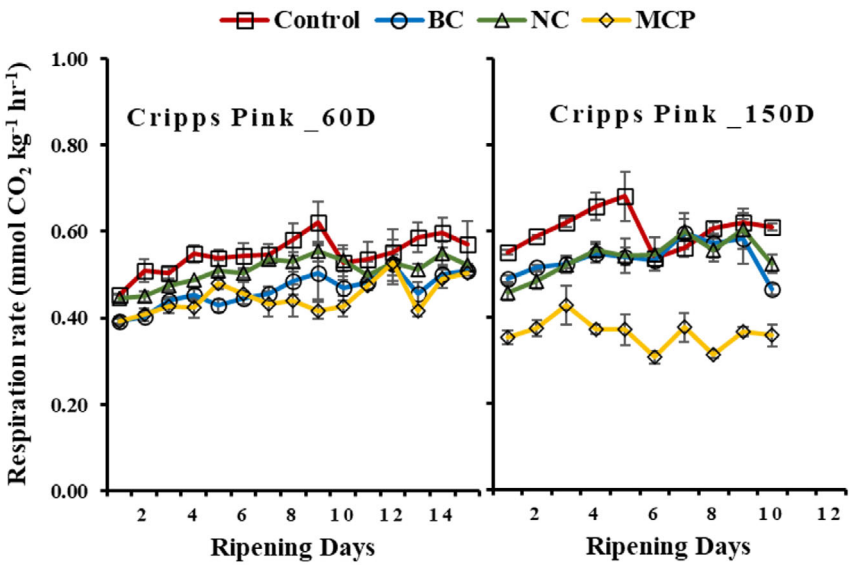

Figure 2. The respiration rate (mmol CO_2 kg^{-1} h^{-1}) affected by 1*H*-cyclopropabenzene (BC), 1*H*-cyclopropa[*b*]naphthalene (NC), and 1-methylcyclopropene (1-MCP) fumigation treatments in 'Cripps Pink' apple fruit stored in CA storage with PCO for 60 and 150 days. The vertical bars represent SEM values and are not visible when values are smaller than the symbol. $n = 4$ replicates (two fruit per replication). Least significant difference ($P \leq 0.05$). T = 0.03, D = 0.06, TXD = non-significant for 60 days and T = 0.03, D = 0.05, TXD = non-significant for 150 days of storage.

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Table 1. Physiological loss of weight (PLW) (%), fruit firmness (N), SSC (%), TA (%), and SSC:TA affected by the 1*H*-cyclopropabenzene (BC), 1*H*-cyclopropa[*b*]naphthalene (NC), and 1-methylcyclopropene (1-MCP) fumigation treatments in 'Cripps Pink' apple fruit stored in CA with PCO for 60 and 150 days

CA storage period (days)			
Treatment	60	150	Mean (T)
Physiological loss of weight (%)			
Control	2.76 ± 0.22	4.54 ± 0.25	3.65
BC	2.02 ± 0.49	4.38 ± 0.12	3.20
NC	2.33 ± 0.21	4.32 ± 0.17	3.32
1-MCP	2.15 ± 0.35	4.24 ± 0.16	3.20
Mean (D)	2.31 A	4.37 B	
Least significant difference ($P \leq 0.05$)	T = NS	D = 0.45	TXD = NS
Fruit firmness (N)			
Control	53.88 ± 1.08 bc	46.48 ± 0.55 a	50.18 A
BC	54.63 ± 1.56 bcd	52.56 ± 1.01 bc	53.60 B
NC	56.54 ± 0.05 d	51.42 ± 0.19 b	53.98 B
1-MCP	57.27 ± 0.87 d	55.19 ± 0.57 cd	56.23 C
Mean (D)	55.58 B	51.41 A	
Least significant difference ($P \leq 0.05$)	T = 2.10	D = 1.49	TXD = 3.24
Soluble solids content (%)			
Control	16.60 ± 0.06 e	16.43 ± 0.02 d	16.51 B
BC	16.00 ± 0.04 a	16.43 ± 0.02 d	16.21 A
NC	16.23 ± 0.02 bc	16.33 ± 0.02 cd	16.28 A
1-MCP	16.15 ± 0.04 b	16.25 ± 0.04 bc	16.20 A
Mean (D)	16.24 A	16.36 B	
Least significant difference ($P \leq 0.05$)	T = 0.09	D = 0.06	TXD = 0.13
Titratable acidity (%)			
Control	0.65 ± 0.01	0.56 ± 0.01	0.61 A
BC	0.65 ± 0.01	0.59 ± 0.01	0.62 AB
NC	0.65 ± 0.01	0.62 ± 0.01	0.64 BC
1-MCP	0.68 ± 0.00	0.63 ± 0.01	0.66 C
Mean (D)	0.66 B	0.60 A	
Least significant difference ($P \leq 0.05$)	T = 0.02	D = 0.02	TXD = NS
SSC:TA			
Control	25.42 ± 0.24	29.26 ± 0.78	27.34 C
BC	24.51 ± 0.28	27.73 ± 0.46	26.12 B
NC	24.86 ± 0.33	26.23 ± 0.44	25.54 AB
1-MCP	23.63 ± 0.06	25.69 ± 0.44	24.66 A
Mean (D)	24.60 A	27.23 B	
Least significant difference ($P \leq 0.05$)	T = 1.04	D = 0.73	TXD = NS

$n = 4$ replicates [15 fruit (PLW), 10 fruit (fruit firmness), and 13 fruit (SSC, TA, and SSC:TA) per replication]; mean ± SE. Mean separation for significant analysis of variance within the columns and rows were tested by Duncan's multiple range tests at ($P \leq 0.05$). Mean values followed by similar letters are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant.
NS, non-significant; T, treatments; D, CA storage period.

liquid chromatography (RP-HPLC) system (Waters 1525; Waters Corporation, Milford, MA, USA). The specifications of the instruments and detailed procedures were explained previously by Tokala.[22] The levels of individual sugars and organic acids are expressed as g kg^{-1} fresh weight basis.

Total phenols

The total phenols content in fruit pulp samples were determined using the Folin–Ciocalteu reagent method detailed by Robles-Sánchez *et al.*[23] with some modifications as described previously by Tokala.[22] The total phenols were calculated with respect to the gallic acid standard curve and expressed as g gallic acid equivalents (GAE) kg^{-1} fresh weight basis.

Ascorbic acid

The ascorbic acid content in the fruit pulp samples was determined using spectrophotometry. The stepwise procedure to estimate ascorbic acid levels in the fruit pulp samples has been described by Tokala.[22] The calculated ascorbic acid levels were expressed as g kg^{-1} fresh weight basis.

Total antioxidant capacity

The total antioxidant capacity in the fruit pulp samples was estimated using the 2,2-diphenyl-1-picrylhydrazyl assay as outlined by Brand-Williams *et al.*[24] with some modifications described previously by Tokala.[22] The computed levels of total antioxidant capacity expressed as μm kg^{-1} Trolox fresh weight basis.

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Statistical analysis

GenStat, version 14.0 (Lawes Agricultural Trust; Rothamsted Experimental Station, Harpenden, UK) was used to analyze the data recorded. A two-way analysis of variance was performed to evaluate the effects of the ethylene antagonist treatments, CA-PCO storage duration, and their interactions. Duncan's multiple comparison tests were used to compare the treatment means and the results are presented as the mean ± SEM.

RESULTS

Ethylene production and respiration rates

The reduced rates of ethylene climacteric peaks were exhibited in the 'Cripps Pink' apple fruit fumigated with NC, BC, and 1-MCP. The ethylene climacteric peaks were reduced by 11.80%, 16.85%, and 98.31% after 60 days and by 67.13%, 44.29%, and 98.88% after 150 days of CA storage with PCO, in NC, BC, and 1-MCP, respectively, compared to control fruit (Fig. 1). Compared with control fruit, the fruit fumigated with NC, BC, and 1-MCP exhibited a delayed onset of the ethylene climacteric peak by 1.00, 4.00, and 4.50 days after 60 days and by 3.50, 3.50, and 5.50 days after 150 days of CA storage with PCO, respectively (Fig. 1).

The respiratory climacteric peak appeared earlier than the ethylene climacteric peaks in the fruit. Similar to the trend in the ethylene, compared with control fruit, the rates of the respiratory climacteric peak were also reduced by 12.16%, 8.11%, and 32.43% after 150 days of CA storage with PCO in the fruit fumigated with NC, BC, and 1-MCP, respectively (Fig. 2). The respiratory climacteric peak rates were not significantly affected by ethylene antagonist treatments after 60 days of CA storage with PCO. The onset of the respiratory climacteric peak was also delayed by 1.25, 1.00, and 2.25 after 60 days of storage and by 2.75, 3.75, and 1.50 after 150 days of CA storage with PCO in the fruit treated with NC, BC, and 1-MCP compared to the control fruit, respectively (Fig. 2).

PLW and fruit firmness

Treatment with the ethylene antagonists did not significantly affect the PLW values of apple fruit after CA storage with PCO. The PLW increased by 1.89-fold and the fruit firmness decreased by 8.59% with the extension of the storage period in CA with PCO (Table 1). The fruit fumigated with NC, BC, and 1-MCP and stored in CA with PCO exhibited a fruit firmness 1.08, 1.07, and 1.12 times higher than control fruit, respectively (Table 1). Compared with all other treatment combinations, the significantly highest fruit firmness was observed in the fruit treated with 1-MCP (57.27 N), as well as NC (56.54 N), and stored for 60 days in CA with PCO (Table 1).

SSC, TA, and SSC:TA

In comparison with other treatments, the highest TA (0.66%) and the lowest SSC (16.20%) and SSC:TA (24.66) were exhibited in the fruit with 1-MCP fumigation treatment (Table 1). With the extension of the CA storage with PCO duration from 60 to 150 days, the TA decreased significantly and SSC:TA values were increased (Table 1). The control fruit stored for 150 days in CA with PCO had significantly the highest SSC:TA values (29.26) compared to all other treatment combinations (Table 1).

Individual sugars and organic acids

The fruit treated with 1-MCP exhibited significantly highest levels of glucose than all other treatments (1.25 g kg^{-1}). The levels of individual sugars increased with the increase in the storage period from 60 to 150 days in CA with PCO (Table 2). The effect of ethylene antagonist treatments on the levels of fructose, sucrose, and sorbitol in the fruit stored in CA with PCO was not significant ($P \leq 0.05$). The RP-HPLC quantified significant levels of malic acid but the levels of succinic, fumaric, and citric acid in the fruit pulp samples were very low or not quantified. The fruit fumigated with NC, BC, and 1-MCP and stored in CA with PCO exhibited 1.18-, 1.14-, and 1.23-fold more malic acid than control fruit (Table 2). The interaction effect between the duration of CA storage with PCO and ethylene antagonists on the levels of individual sugars was significant, without any specific trend. However, the interaction effect on the levels of malic acid was not significant.

Table 2. The levels of glucose (g kg^{-1}), malic acid (g kg^{-1}), total phenols (g GAE kg^{-1}), and ascorbic acid (g kg^{-1}) in the pulp affected by the 1*H*-cyclopropabenzene (BC), 1*H*-cyclopropa[*b*]naphthalene (NC), and 1-methylcyclopropene (1-MCP) fumigation treatments in 'Cripps Pink' apple fruit stored in CA with PCO for 60 and 150 days

	CA storage period (days)		
Treatment	60	150	Mean (T)
Individual sugars			
Glucose (g kg^{-1})			
Control	1.04 ± 0.04 bc	0.79 ± 0.03 a	0.92 A
BC	1.06 ± 0.10 bc	1.08 ± 0.02 bc	1.07 B
NC	0.98 ± 0.06 b	1.20 ± 0.01 c	1.09 B
1-MCP	1.06 ± 0.03 bc	1.45 ± 0.08 days	1.25 C
Mean (D)	1.03 A	1.13 B	
Least significant difference ($P \leq 0.05$)	T = 0.11	D = 0.08	TXD = 0.15
Malic acid (g kg^{-1})			
Control	6.39 ± 0.18	6.41 ± 0.33	6.40 A
BC	7.69 ± 0.15	6.91 ± 0.16	7.30 B
NC	7.56 ± 0.23	7.55 ± 0.13	7.56 BC
1-MCP	8.28 ± 0.20	7.48 ± 0.26	7.88 C
Mean (D)	7.48 B	7.09 A	
Least significant difference ($P \leq 0.05$)	T = 0.45	D = 0.32	TXD = NS
Total phenols (g GAE kg^{-1})			
Control	23.02 ± 1.34 ab	19.18 ± 1.51 a	21.10 A
BC	20.12 ± 0.70 ab	18.99 ± 1.44 a	19.55 A
NC	24.51 ± 1.44 b	19.74 ± 0.83 ab	22.13 AB
1-MCP	30.41 ± 1.64 c	19.74 ± 1.40 ab	25.07 B
Mean (D)	24.51 B	19.41 A	
Least significant difference ($P \leq 0.05$)	T = 3.12	D = 2.21	TXD = 4.41
Ascorbic acid (g kg^{-1})			
Control	11.00 ± 0.17	11.33 ± 0.35	11.16 A
BC	11.91 ± 0.12	11.61 ± 0.24	11.76 AB
NC	12.64 ± 0.68	11.31 ± 0.05	11.98 AB
1-MCP	12.33 ± 0.34	12.62 ± 0.43	12.47 B
Mean (D)	11.97	11.72	
Least significant difference ($P \leq 0.05$)	T = 0.84	D = NS	TXD = NS

$n = 4$ replicates (13 fruit per replication); mean ± SE. Duncan's multiple range tests at ($P \leq 0.05$) was used the test the mean separation for significant analysis of variance within the columns and rows. Mean values followed by similar letters are not significantly different within the columns or rows. Mean values without letters within columns or rows are non-significant.
NS, non-significant; T, treatments; D, storage period.

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Total phenols, ascorbic acid, and total antioxidant capacity

In comparison with all the treatments applied, the fruit fumigated with NC and 1-MCP exhibited higher levels of total phenols (22.13 g GAE kg^{-1} and 25.07 g GAE kg^{-1}, respectively) and ascorbic acid (11.98 g kg^{-1} and 12.47 g kg^{-1}, respectively) after CA storage with PCO (Table 2). The 1-MCP treated fruit stored in CA with PCO for 60 days exhibited significantly highest total phenol levels (30.41 g GAE kg^{-1}) compared to all other treatment combinations (Table 2). With the extension of CA storage with PCO from 60 to 150 days, the total phenols and ascorbic acid levels were lowered (Table 2). NC, BC, and 1-MCP fumigation and period of CA storage with PCO did not significantly affect the total antioxidant capacity compared to the control fruit.

DISCUSSION

The efficacy of fumigation treatments of two new ethylene antagonists (NC and BC), as well as 1-MCP, in downregulating ethylene action and in maintaining the fruit quality of 'Cripps Pink' apple after CA storage with PCO has been investigated for the first time. CA conditions extend the storage life of apple fruit by reducing the respiration rates and downregulating the biosynthesis of ethylene during storage. However, after being taken out from the CA storage to a normal atmosphere, at ambient temperatures, the apple fruit exhibits a steep rise in ethylene production.[5-8] The apple fruit treated with effective ethylene antagonist compounds downregulates this spike in the production of ethylene.[6] In the present study, compared to control fruit, the onset of the climacteric peak was delayed and the rates of ethylene production at the climacteric peak were reduced in the fruit treated with NC, BC, and 1-MCP, after completion of respective CA storage with PCO (Fig. 1). These changes in the fruit fumigated with ethylene antagonist compounds suggest an effective inhibition of ethylene action in the fruit.[25] 1-MCP hinders the ethylene action by irreversibly binding with the ethylene receptor sites in the fruit, inhibiting the expression of ethylene-responsive genes.[26,27] The proposed mechanism of ethylene action inhibition by BC and NC is similar to that of 1-MCP, even though, structurally, BC and NC are very different from 1-MCP.[14,28] The ring-opening reaction mechanism of cyclopropenes was proposed by Pirrung *et al.*[29] to explain blockade of ethylene action. A copper carbenoid intermediate formed during this reaction irreversibly binds with amino acids of the ethylene receptor protein domain and results in blockade of ethylene action. The NC and BC also react with the copper (I) cofactor of the ETR1 ethylene receptor and act as ethylene antagonists in the fruit.[14-17,28,30] The respiratory climacteric peak was delayed in the fruit treated with ethylene antagonist compared to the control. Similar to other ripening associated physiological changes, the respiration rates in the climacteric fruit are retarded with the action of ethylene antagonists.[9]

Following CA storage with PCO, the fumigation treatment with NC, BC, and 1-MCP maintained significantly higher mean fruit firmness compared to the control fruit (Table 1). The retention of crispiness and fruit firmness during apple fruit storage is an essential quality for marketing.[31] The reduction in fruit firmness during storage occurs primarily as a result of the cell wall hydrolyzing enzyme activity and it is activated by the action of phytohormone ethylene during the ripening process of the fruit.[32,33] Thus, the retention of higher fruit firmness can be associated with the reduction in the ethylene production and/or action of the ethylene antagonists in the fruit.[33,34] When compared with control fruit, the SSC values were lower in the fruit treated with ethylene antagonist (BC, NC, and 1-MCP) fumigation and stored in CA with PCO, but, in contrast, the levels of glucose were highest in the fruit fumigated with 1-MCP (Tables 1 and 2). The accumulation of a specific type of sugars in the apple fruit pulp is not always linked with the perception of ethylene by the fruit and the exact mechanism of ethylene and ethylene antagonists' effects on the levels of sugars in the fruit is not very distinct.[16,17,30,35,36] The levels of malic acid were higher in the fruit treated with BC, NC, and 1-MCP compared to that in control fruit after CA storage with PCO (Table 2). Maintenance of higher malic acid levels in the fruit treated with ethylene antagonist fumigation could be correlated with the reduced rates of ethylene production in fruit.[34] The BC, NC, and 1-MCP fumigated fruit kept in CA storage with PCO retained higher levels of ascorbic acid and total phenols compared to the control (Table 2). In apple fruit, flavanols, ascorbic acid, and phenolics are chief bioactive compounds and are actively involved in the breakdown of the reactive oxidative species produced during the ripening process.[37,38] The retention of high total phenol and ascorbic acid levels in the apple fruit fumigated with ethylene antagonist compounds compared to control fruit signifies the reduced ethylene action, consequently retarding the ripening associated processes in the fruit.[39]

CONCLUSIONS

Ethylene antagonist (NC, BC, and 1-MCP) fumigation treatments effectively retarded the ethylene production rates in 'Cripps Pink' apple fruit stored in CA equipped with PCO. Comparatively, 1-MCP fumigation treatment was more effective in reducing the rates of the climacteric ethylene peaks than NC and BC treatments. The NC fumigation treatment was on a par with 1-MCP in maintaining higher levels of total phenols and ascorbic acid. Therefore, new ethylene antagonist compounds NC and BC have the potential to be used as an ethylene antagonist in 'Cripps Pink' apple fruit without causing any unfavourable effects on the fruit quality during CA storage with PCO. The effects of various concentrations of NC and BC, as well as interactive effects of PCO on the performance of ethylene antagonists in downregulating ethylene production, warrants further investigations.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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