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Postharvest fruit quality of apple influenced by ethylene antagonist fumigation and ozonized cold storage

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The effects of two new ethylene antagonists namely $1H$-cyclopropabenzene (BC) and $1H$-cyclopropa[b]naphthalene (NC), as well as 1-methylcyclopropene (1-MCP) on ethylene production and fruit quality of Cripps Pink and Granny Smith apple in ozonized cold storage, were investigated. When compared to control, Cripps Pink fruit fumigated with BC and NC exhibited significantly lowest ethylene production and respiration, whilst the Granny Smith fruit treated with 1-MCP exhibited lowest ethylene production followed by NC and BC treatments exhibited lower ethylene production when compared to control. Application of ozone in cold storage maintained higher levels of sugars but elevated ethylene production in both the apple cultivars. No significant interaction was recorded between ethylene antagonists and presence or absence of ozone application in cold storage on the ethylene production, respiration and other fruit quality parameters. In conclusion, results suggest that BC and NC are potential ethylene antagonists in Cripps Pink and Granny Smith apples during the cold storage.

**Keywords:** Ethylene; Ethylene Antagonists; Ozone; $1H$-cyclopropabenzene; $1H$-cyclopropa[b]naphthalene; 1-Methylcyclopropene; Apple; Fruit Quality

**Abbreviations:** BC_1$H$-cyclopropabenzene, NC_1$H$-cyclopropa[b]naphthalene, 1-MCP_1-Methylcyclopropene

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1. Introduction:

A sequence of irreversible events occurs during fruit ripening process which affects their peel and pulp colour, firmness, eating quality, levels of nutrients of the fruit and finally leads to its senescence and deterioration. The phytohormone ethylene invariantly promotes the fruit ripening process in climacteric fruits like apples and affects the postharvest quality and storage life (Tucker, 2012). Apple fruit is very sensitive to external ethylene exposure even at minute concentrations and in turn, produce large amounts of ethylene during the ripening process. Different techniques have been tested to downregulate the ripening process, to extend the storage life and to maintain the postharvest fruit quality by inhibiting ethylene production, action as well as exposure of ethylene (Giovannoni, 2008). The low-temperature storage retards the activities of various enzymes involved in ethylene biosynthesis, metabolic pathways and fruit softening as well as thus delays the ripening-associated physiological changes in the fruit and hence extend the storage life (Gross et al., 2016).

The ethylene antagonists irreversibly block the ethylene receptors at the cellular level and thus inhibit the ethylene action in the fruit (Sisler, 2006). 1-Methylcyclopropene (However, the cold storage cannot effectively downregulate the ethylene levels in the storage environment or the ethylene action in the fruit. 1-MCP) fumigation has been identified as one of the most effective approach ethylene antagonists among the several methods to antagonize impede the ethylene action and retard the internal ethylene production in apple fruit (Watkins, 2006). The commercial encapsulated form of 1-MCP is widely being used by the apple growers around the world. It blocks the ethylene receptors at cellular level irreversibly and thus inhibits the ethylene action in the fruit.
The effects of Application of 1-MCP to the apple fruit, in retarded-retarding the rates of respiration and ethylene production as well as maintaining delayed fruit softening/fruit quality during cold and controlled atmosphere and controlled atmosphere storage have been reported as well as reviewed by several authors (Blankenship and Dole, 2003; Watkins, 2006; Valero et al., 2016; Hackbarth et al., 2017). However, the effectiveness of 1-MCP in inhibiting the ethylene action differed with concentrations, treatment duration, cultivars, storage period and temperatures (Watkins, 2006). The 1-MCP treatment has also been reported to increase the susceptibility to fungal diseases like blue mould (Janisiewicz et al., 2003) as well as aggravated physiological disorders such as flesh browning in some apple cultivars (Watkins, 2006; Saba and Watkins, 2020). Moreover, the 1-MCP at room temperature is an unstable liquid making it difficult to handle (Sisler et al., 2006). Singh et al. (2018) discovered the capacity of two compounds namely $1H$-cyclopropabenzene (BC) and $1H$-cyclopropa[$b$]naphthalene (NC) to antagonize ethylene action in the fruit similarly as to 1-MCP. Previously, Khan (2014) also found the ethylene antagonistic activity of BC and NC in some cultivars of plum, apple and nectarine fruit kept at ambient temperature. The structural properties of these compounds are different from 1-MCP and this allows BC to be as liquid and NC to be as solid at the room temperature. Recently, Tokala et al. (2020) have reported the effectiveness of fumigation treatments with BC and NC in retarding the postharvest ethylene production in the cold stored ‘Cripps Pink’ apple fruit.

Ozone, the tri-atomic form of the oxygen, is a powerful oxidizing agent and well known for its biocidal properties (Kim et al., 1999). Skog and Chu (2001) reported that application of ozone gas in cold storage oxidized the ethylene in the storage environment but did not show any effect on the endogenous ethylene production in apple fruit. Earlier, it has also been reported that the application of ozone maintained postharvest fruit quality as well as extended the storage life by...
reducing the fungal decay (Skog and Chu, 2001). The beneficial effects of the 1-MCP and ozone on extending the storage life and maintaining the quality of different fruit, have been previously reported (Valero et al., 2016; Tokala et al., 2018; Shezi et al., 2020). However, no information is available on the additive and/or synergistic effects of the ethylene antagonists (BC, NC and 1-MCP) fumigation and presence of ozone in the cold storage environment on ethylene production and respiration rate as well as the quality of apple fruit. It was hypothesised that fumigation with ethylene antagonists and ozone application in the cold storage environment would synergistically reduce ethylene action and maintain fruit quality. Therefore, the objective of this investigation was to examine the effect of new ethylene antagonists (BC and NC), 1-MCP as well as fumigation treatment along with cold storage (0 ± 2 °C, 90 ± 5 % RH) with or without ozone gas in retarding ethylene production and respiration rate while maintaining postharvest fruit quality of cold-stored Cripps Pink and Granny Smith apple following 90 and 120 d of storage.

2. Material and methods

2.1. Plant materials and fruit

Apple fruit of Granny Smith (firmness 55.06 ± 3.21 N; SSC 10.94 ± 0.05 %; TA 0.61 ± 0.03 %) and Cripps Pink (firmness 65.95 ± 3.49 N; SSC 13.7 ± 0.08 %; TA 0.74 ± 0.02 %) cultivars was harvested from a commercial orchard at Manjimup, Western Australia (34°13’ S latitude, 116°08’ E longitude) during April and May 2017, respectively. The trees were 11-year old and previously grafted on M.26 rootstock. They were planted in North-South orientation with the spacing of 4.5 × 1 m and received uniform cultivation practices throughout. After the harvest all the fruit were dipped in aqueous solution containing ‘Scholar’ (a.i. 230 g L⁻¹ fludioxonil) @ 2.00 mL L⁻¹, ‘Caltop’ (a.i. 165 g L⁻¹ calcium) @ 7.00 mL L⁻¹, ‘DPA’ (diphenylamine) @ 1.70 mL L⁻¹ to prevent any postharvest diseases or disorders during cold storage. After air-drying the fruit properly, they were
then packed in corrugated cardboard boxes with soft board trays and immediately transported to Curtin Horticulture Research Laboratory, Perth, using the air-conditioned vehicle. The relatively uniform-sized fruit, free from bruises, pests and visible symptoms of diseases were used for the experiment.

2.2. Chemicals

The chemicals used as the treatments in the experiment were all synthesized at Chemistry laboratory, Curtin University. The procedure detailed by Fisher and Applequist (1965) was used to prepare 1-MCP from methallyl chloride, while BC was synthesised from 1,3-cyclohexadiene and NC from naphthene in anhydrous tetrahydrofuran and NC were prepared using following the procedure detailed by Davalian et al. (1980) and Billups and Chow (1973), respectively.

2.3. Fumigation treatments and cold storage

Two independent experiments were conducted using Granny Smith and Cripps Pink apple fruit. The apple fruit were fumigated with ethylene antagonists (BC, NC and 1-MCP) using 60 L hermetically sealable plastic drums at room temperature (20 ± 2 °C and 65 ± 5 % RH). The apple fruit were arranged in each drum and calculated volume of ethylene antagonist solution was dispensed on to Petri-plate with filter paper, placed inside the drum, to produce 1 µM BC (0.09µL.L⁻¹) or 1 µM NC (0.14µL.L⁻¹) or 18 µM 1-MCP (1µL.L⁻¹) vapours and the drums were immediately sealed. Before sealing the plastic drum, 30 g of granular soda lime in a Petri-plate to absorb any excess carbon dioxide (CO₂) produced and a battery-operated portable fan was also placed for uniform distribution of ethylene antagonist vapours. The untreated fruit were considered as a control. The experiment was laid in completely randomised design and each treatment was
replicated for four times with fifteen fruit per replication. On completion of 18 h, the drums were unsealed in an open-air environment and the fruit were then packed in corrugated cardboard boxes with soft board trays. The boxes were duly labelled for the treatments and transferred into the cold storage (0 ± 2 °C, 90 ± 5 % RH) with or without ozone gas (0.1 ± 0.08 µL L⁻¹). The two lots of boxes were stored for 90 d and 120 d in separate cold storages. On the end of the designated storage period, the fruit were taken out for the determination of rates of respiration, ethylene production and other quality parameters.

2.4. Determination of ethylene production and respiration rate

After completion of the respective storage duration, two fruit per replication were chosen randomly to determine ethylene production and respiration rate. The observations were recorded until a distinct climacteric peak was achieved in all the treatments and control. The selected fruit were sealed for 1 h in the 1 L glass jar with rubber septum at the top. The 1 mL gas sample was extracted from the headspace and injected into a gas chromatograph (Model 6890N, Agilent Technology, CA, USA) equipped with a stainless-steel column of 2 m long, 3.18 mm internal diameter packed with 80/100 mesh size (Porapak-Q, Supelco, PA, USA) and a flame ionisation detector (FID). The nitrogen (N₂) gas with a flow rate of 20 mL min⁻¹ was used as a carrier gas, and the column, inlet and detector temperatures were maintained at 110 °C, 150 °C and 250 °C, respectively. A gas sample from empty sealed glass jars was injected to test the possibility of the rubber septum to produce ethylene and no ethylene was detected. The headspace gas sample (2 mL) was injected into Infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, East Sussex, UK) to determine rates of respiration as CO₂ production. The concentrations of ethylene production were calculated as µmol kg⁻¹ h⁻¹ and respiration rate as mmol kg⁻¹ h⁻¹ CO₂.

2.5. Physiological loss of weight (PLW)
The fruit were weighed before transferring them into respective cold storage rooms and recorded as initial weight. The final weight was then recorded on completion of the respective storage duration. The PLW was calculated following the below equation and expressed as %.

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

2.6. Fruit firmness

Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK) with 5/16” (8 mm) Magnus-Taylor probe, equipped with a 500 N load cell was used to determine fruit firmness. The peeled portion of the fruit was punctured in two opposite sides at the equatorial region by the probe to 7 mm sample depth with 100 mm s\(^{-1}\) test speed and 5 N trigger force. The fruit firmness in Newton (N), is then calculated by interfaced software Nexxygen\(^{\circledR}\) version 4.6 software installed on the computer.

2.7. SSC, TA and SSC: TA

The pooled juice from the portions cut from thirteen fruit per each replication was used to determine soluble solids concentration (SSC) and titratable acidity (TA). SSC was estimated using a digital refractometer (Atago – Palette PR 101, Atago Co., Tokyo, Japan) and was expressed as %. The diluted juice sample was titrated against 0.01 N sodium hydroxide (NaOH) with 2-3 drops of phenolphthalein indicator till pale pink colour endpoint to estimate TA. The TA was expressed as % malic acid. The SCC and TA values were then used to calculate SSC: TA.

2.8. Individual sugars and organic acids
The levels of individual sugars and organic acids in the pulp samples were estimated by reverse-phase HPLC system (Waters 1525, Milford Corporation, USA). The Dual λ UV absorbance detector (Water 2487, Milford Corporation, USA) at 214 nm was used to estimate individual organic acids, while the individual sugars were estimated by the Refractive Index (RI) detector (Water 2414, Milford Corporation, USA). The detailed description of instruments, procedures to prepare and analyze the samples were previously explained by Tokala (2019). The levels of sugars and organic acids are expressed as g kg\(^{-1}\) fresh weight basis.

2.9. Total phenols

The procedure described by Robles-Sánchez et al. (2009), with few modifications as mentioned earlier by Vithana-Tokala et al. (2018, 2020) was used to determine the levels of total phenols in the fruit pulp samples. The absorbance of the samples prepared was recorded at 750 nm wavelength using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The levels of total phenolic content calculated from the gallic acid standard curve were expressed as g Gallic Acid Equivalent (GAE) kg\(^{-1}\) fresh weight basis.

2.10. Ascorbic acid

The levels of ascorbic acid in the fruit pulp samples were estimated using metaphosphoric acid, following the procedure detailed previously by Vithana-Tokala et al. (2018, 2020). The absorbance of the samples prepared was recorded by a UV/VIS spectrophotometer at 760 nm. The standard L-ascorbic acid curve was used to calculate the levels and expressed as g kg\(^{-1}\) fresh weight basis.

2.11. Total antioxidant capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay detailed by Brand-Williams et al. (1995) and procedure explained previously by Vithana et al. (2018) and Tokala et al. (2020) was used to determine...
the total antioxidant capacity in the fruit pulp samples. The absorbance of the samples was recorded at 515 nm by a UV/VIS spectrophotometer. The levels of total antioxidant capacity were calculated with reference to the standard curve of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (97 %)) and expressed as µM kg⁻¹ Trolox fresh weight basis.

2.12. Statistical analysis

The data recorded were analysed by using the GenStat software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK). The data were analysed by two-way analysis of variance (ANOVA) with ethylene antagonist treatments and cold storage type as two factors. The least significant difference (LSD) with 5 % error probability was determined by the F-test. The treatment means were compared using Duncan multiple comparison tests. The results were presented as means ± standard errors (SE) of the means. The data of 90 d and 120 d of storage were analysed separately.

3. Results

3.1. Ethylene production and respiration rates

The BC, NC or 1-MCP fumigation have reduced the rates of ethylene production when compared to the control fruit in Cripps Pink and Granny Smith apple following 90 and 120 d of cold storage (Figure 1 and 2). In comparison with the control, the Cripps Pink apple fumigated with BC, NC and 1-MCP reduced the rates of ethylene climacteric peak by 83 %, 88 % and 40 % in 90 d stored and by 89 %, 90 % and 70 % in 120 d stored fruit, respectively (Figure 1 and 2). When compared to control fruit, Granny Smith apple fumigated with BC, NC and 1-MCP also expressed the reduced rates of ethylene climacteric peaks by 26 %, 70 % and 99 % following 90 d and by 14 %, 72 % and 99 % following 120 d of cold storage, respectively (Figure 1 and 2). The Cripps Pink...
apple fruit stored in ozonised cold storage exhibited 29 % and 21 % higher rates of ethylene 
climacteric peaks when compared to cold storage without ozone, following 90 and 120 d of 
storage, respectively (Figure 1 and 2). Likewise, the Granny Smith apple stored in cold storage 
with ozone also exhibited 21 % and 11 % higher rates of ethylene climacteric peaks when 
compared to non-ozonised cold storage, following 90 and 120 d of storage, respectively (Figure 1 
and 2). The onset of ethylene climacteric peak was delayed by 8, 7 and 9 d following 90 d and by 
4, 5 and 4 d following 120 d in the Cripps Pink apple fruit fumigated with BC, NC and 1-MCP 
when compared to control fruit, respectively (Figure 1 and 2). The ethylene climacteric peak onset 
in the Granny Smith fruit fumigated with BC, NC and 1-MCP was delayed by 4, 5 and 8 d for 90 
d of storage and by 2, 3 and 5 d for 120 d of storage (Figure 1 and 2). There was no significant 
effect of ozone application on the ethylene climacteric peak onset in Cripps Pink and Granny Smith 
apple fruit during 90 and 120 d of cold storage.

When compared to the control fruit, the Cripps Pink apple fumigated with BC, NC and 1-MCP 
exhibited reduced rates of the respiratory climacteric peak by 15 %, 20 % and 5 % following 90 d 
and by 22 %, 30 % and 15 % following 120 d of cold storage, respectively (Figure 3). Whilst, in 
the Granny Smith apple, the rates of respiratory climacteric peaks were reduced in the fruit 
fumigated with BC, NC and 1-MCP by 19 %, 9 % and 29 % in 90 d cold storage and by 14 %, 27 
% and 34 % in 120 d cold storage, respectively in comparison with control (Figure 3). When 
compared to the control fruit the onset of respiratory climacteric peaks was delayed by 6, 6 and 5 
d following 90 d storage and by 3, 3 and 4 d following 120 d storage, in the Cripps Pink fruit 
fumigated with BC, NC and 1-MCP, respectively (Figure 3). The respiratory climacteric peak 
onset in the Granny Smith fruit fumigated with BC, NC and 1-MCP was delayed by 4, 5 and 2 d 
for 90 d of storage when compared with control, but not significantly affected for 120 d of storage
Figure 3). The respiratory climacteric peak rates and onset were not significantly affected by ozone application in Cripps Pink and Granny Smith apple fruit during 90 and 120 d cold storage. The interaction effect between ethylene antagonist treatments and application of ozone on the rates and onset of ethylene and respiration climacteric peak was not significant.

3.2. PLW and fruit firmness

The PLW in the Cripps Pink apple was reduced by 33 %, 19 % and 27 % following 90 d and by 7 %, 13 % and 14 % following 120 d cold storage in the fruit fumigated with BC, NC and 1-MCP, respectively, when compared to control (Table 1). Similarly, in Granny Smith apple fruit, in comparison with control the fumigation treatment with BC, NC and 1-MCP has reduced PLW by 22 %, 6 % and 44 % in 90 d cold storage and by 7 %, 14 % and 17 % in 120 d cold storage, respectively (Table 1). The application of ozone in the cold storage has reduced the PLW in Cripps Pink apple by 23 % and by 28 % as well as in Granny Smith apple by 17 % and 4 %, following 90 and 120 d of storage, respectively (Table 1). When compared to the control the fruit firmness in the Cripps Pink apple fumigated with BC, NC and 1-MCP were maintained 1.01, 1.03 and 1.02 times higher following 90 d of storage and by 1.07, 1.05 and 1.07 times higher following 120 d of storage, respectively (Table 1). In case of Granny Smith apple, when compared to control, the fumigation treatment with BC, NC and 1-MCP have maintained the fruit firmness 1.16, 1.12 and 1.21 times higher in 90 d stored and by 1.09, 1.17 and 1.28 times higher in 120 d stored, respectively (Table 1). The fruit firmness in Cripps Pink apple fruit was not significantly affected by the application of ozone gas following 90 and 120 d of cold storage, while the Granny Smith apple fruit stored in ozonised cold storage showed comparatively lower firmness values for 90 d cold storage (Table 1). The interaction effect between ethylene antagonist treatments and application of ozone on the PLW and fruit firmness was not significant.
3.3. SSC, TA and SSC: TA

The Cripps Pink apple fruit fumigated NC and 1-MCP exhibited significantly lowest SSC values when compared to other treatments following 90 d (14.69 % and 14.58 %, respectively) and 120 d (14.46 % and 14.38 %, respectively) of cold storage (Supplementary material, Appendix 1, Table 1). Whilst in case of the Granny Smith apples, when compared to all other treatments the fruit fumigated with 1-MCP exhibited significantly highest values of SSC following 90 d (12.81 %) and 120 d (12.65 %) of cold storage (Supplementary material, Appendix 1, Table 1). The apple fruit stored in the ozonised cold storage exhibited comparatively higher values following 90 d but had lower SSC values following 120 d in both Cripps Pink and Granny Smith fruit (Supplementary material, Appendix 1, Table 1). The interaction effect between ethylene antagonist treatments and cold storage type on SSC did not exhibit any specific trend in Cripps Pink and Granny Smith apple fruit. There was no significant effect of ethylene antagonists or ozone application on the TA values of Cripps Pink and Granny Smith apple fruit following 90 and 120 d of cold storage. The ethylene antagonist treatments did not show any significant effect on the SSC: TA values of Cripps Pink and Granny Smith apple fruit. The effect of ozone application in cold storage as well as the interaction effect between ethylene antagonists and ozone application on the SSC: TA values of Cripps Pink and Granny Smith apple fruit did not follow any specific trend (Supplementary material, Appendix 1, Table 1).

3.4. Individual sugars and organic acids

The Cripps Pink and Granny Smith apple fruit fumigated with 1-MCP exhibited significantly higher levels of glucose but lower sucrose and sorbitol levels when compared to all other treatments following 90 d and 120 d of cold storage (Figure 4). The Cripps Pink apple fruit stored in the cold storage with ozone gas exhibited comparatively higher levels of glucose (4.96 folds),
fructose (1.07 folds), sorbitol (1.01 folds) and lower sucrose (1.09 folds), following 90 d of storage (Figure 4). Whilst, following 120 d of cold storage all the individual sugars studied were higher in the Cripps Pink fruit stored in ozonised cold storage (Figure 4). Similarly, the levels of glucose (1.10 folds), fructose (1.04 folds) and sucrose (1.01 folds) were higher in the Granny Smith apple fruit cold stored with ozone following 90 d of storage (Figure 4). Whereas, the levels of sorbitol were comparatively lower in the Granny Smith apple fruit cold stored with ozone following 90 d (1.15 folds) and 120 d (1.14 folds) of storage (Figure 4). Overall, the Cripps Pink and Granny Smith apple fruit stored in ozonised cold storage had higher levels of total sugars when compared to the fruit stored in cold storage without ozone.

The Cripps Pink apple fruit fumigated with NC exhibited highest levels of malic acid when compared to all other treatments, following 90 d (5.51 g kg\(^{-1}\)) and 120 d (6.65 g kg\(^{-1}\)) (Supplementary material, Appendix 1, Table 2). Whilst, in the case of Granny Smith apple fruit, there was no significant effect of ethylene antagonist treatment on the levels of malic acid following 90 and 120 d of cold storage. The fruit fumigated with 1-MCP exhibited the highest levels of succinic acid in both the cultivars studied. The succinic acid levels were 1.15 and 1.16 folds higher in Cripps Pink and 1.39 and 1.21 folds higher in Granny Smith apple fruit, when compared to the control fruit, following 90 and 120 d of storage, respectively (Supplementary material, Appendix 1, Table 2). The interaction effect between ethylene antagonists and application of ozone on the levels of individual sugars and organic acids did not follow any definite trend in both the cultivars studied.

3.5. Total phenols, ascorbic acid and total antioxidant capacity

The levels of total phenols were recorded highest in the Cripps Pink apple fruit fumigated with NC following 90 d (33.12 g GAE kg\(^{-1}\)) and 120 d (40.61 g GAE kg\(^{-1}\)) cold storage when compared to
all other treatments (Table 2). Whilst, in the Granny Smith apple fruit, there was no significant
effect of ethylene antagonists on the levels of total phenols. The Cripps Pink and Granny Smith
apple fruit stored in cold storage with ozone maintained higher levels of total phenols following
90 d but again the levels of total phenols were lower following 120 d when compared to fruit stored
in the cold storage without ozone (Table 2). The effect of ethylene antagonists or ozone on the
levels of ascorbic did not follow any significant trend in Cripps Pink and Granny Smith apples.
The Cripps Pink and Granny Smith apple fruit fumigated with 1-MCP exhibited higher levels total
antioxidant capacity following 90 d and 120 d of cold storage when compared to all other
treatments (Table 2). The Cripps Pink apple stored in ozonised cold storage exhibited higher levels
of total antioxidant capacity following 90 d (11.31 µM kg$^{-1}$ Trolox) and 120 d (11.62 µM kg$^{-1}$
Trolox) when compared to the fruit stored in the cold storage without ozone (Table 2). Contrarily,
the levels of total antioxidant capacity were comparatively lower in the Granny Smith apple fruit
stored in ozonised cold storage in 90 d (10.99 µM kg$^{-1}$ Trolox) and 120 d (12.78 µM kg$^{-1}$ Trolox)
than fruit stored in cold storage without ozone (Table 2). The interaction effect between ethylene
antagonists and ozone application on the levels of total phenols, ascorbic acid and total antioxidant
capacity was not significant or did not follow any definite trend in cold-stored Granny Smith and
Cripps Pink apple fruit.

4. Discussions

The effects of new ethylene antagonists (BC and NC) and 1-MCP fumigation, as well as ozone
application during storage on the rates of respiration and ethylene production as well as on various
fruit quality parameters, have been investigated for the first time in the cold stored Cripps Pink
and Granny Smith apples. The 1-MCP fumigation antagonises the ethylene action in the fruit as
well as retard ethylene production, by irreversibly binding to ethylene receptors in the fruit
Pirrung et al. (2008) explained ethylene antagonistic action of 1-MCP by proposing a cyclopropene ring-opening reaction mechanism to form a copper carbenoid intermediate. The intermediate formed to blocks the ethylene action by irreversibly reacting with amino acids of the protein domain of ethylene receptor. The structure of BC and NC is different from 1-MCP, but the mechanism of ethylene action inhibition in the fruit is similar to that of 1-MCP. The BC and NC compounds react with copper (I) cofactor situated with the ETR1 ethylene receptor and antagonize the ethylene action (Singh et al., 2018). The two new ethylene antagonistic compounds (BC and NC) are structurally different from 1-MCP but their proposed mode of action is similar to that of 1-MCP in binding the ethylene receptors and antagonising ethylene action in the fruit (Singh et al., 2018). The rates of respiration and ethylene production were retarded, and the onset of the climacteric peaks was delayed in the Cripps Pink and Granny Smith apple fumigated with BC, NC and 1-MCP, when compared to the control fruit (Figure 1, 2 and 3). This suggests that all the ethylene antagonist treatments could successfully block ethylene receptors and hinder ethylene action in fruits (Sisler, 2006). Ozone gas is well known for its broad-spectrum biocidal properties (Shezi et al., 2020), but its effects on the fruit quality and physiological parameters depend primarily upon factors such as cultivar, concentrations of ozone applied, storage temperatures and duration of ozone exposure (Tokala et al., 2018). The Cripps Pink and Granny Smith apple fruit stored in ozonised cold storage exhibited comparatively higher rates of ethylene production than the fruit cold-stored without ozone gas (Figure 1 and 2). Ozone is one of the strongest oxidising agent and exposure to very high concentrations can induce oxidative stress in the plant tissues (Pell et al., 1997). The elevated levels of ethylene in the apple fruit stored in ozonised storage could possibly be attributed to the stress-induced ethylene production (Liew and Prange, 1994).
The PLW was lowered in the Cripps Pink and Granny Smith apple fruit fumigated with BC, NC and 1-MCP when compared to control (Table 1). The PLW in the fruit is primarily caused due to the loss of water from the fruit, which in turn occurs as a result of physiological activities such as respiration and transpiration (Becker and Fricke, 1996). The reduced PLW in the fruit fumigated with the ethylene antagonist possibly be attributed to the retarded rates of respiration and ethylene (Martínez-Romero et al., 2007). The Cripps Pink and Granny Smith apple fruit stored in ozonised cold storage exhibited reduced PLW during storage (Table 1). A similar reduction in PLW in ozonised cold storage has been reported previously in various fruit such as strawberries (Zhang et al., 2011) and papaya (Ali et al., 2014).

The Cripps Pink and Granny Smith apple fruit fumigated BC, NC and 1-MCP retained higher fruit firmness when compared to the control fruit (Table 1). The reduction in fruit firmness during storage occurs mainly due to loss of cell turgor and cell wall hydrolysis. The activity of cell wall hydrolysing enzymes during the fruit ripening is activated mainly by the phytohormone ethylene (Giovannoni, 2008). The retention of the firmness in the fruit fumigated with ethylene antagonist may be associated with reduced rates of ethylene production and PLW during cold storage (Harker et al., 1997; Giovannoni, 2008). The application of ozone gas in the cold storage did not show a significant effect on the fruit firmness of Cripps Pink and Granny Smith apple fruit. Similarly, Skog and Chu (2001) also reported that ozone application in the cold storage did not show a significant effect on the firmness of apple fruit.

The Cripps Pink apple fruit fumigated with BC, NC and 1-MCP exhibited reduced levels of SSC, whilst Granny Smith apple fruit fumigated with ethylene antagonist had higher SSC when compared to control fruit (Supplementary material, Appendix 1, Table 1). Likewise, the levels of different sugars also exhibited random fluctuations with fumigation of different ethylene
antagonist. Earlier, Blankenship and Dole (2003) have also reported such contrasting effects of ethylene antagonist treatments on the levels of sugar content in different apple varieties. It was also mentioned that the effects of ethylene antagonist treatments could vary in different cultivars. Fan et al. (1999) also indicated that sugar accumulation in the apple fruit during the storage is not essentially associated with ethylene perception. The Cripps Pink and Granny Smith apple fruit stored in ozonised cold storage exhibited higher levels of sugars when compared to the fruit stored in cold storage without ozone gas. A similar increase in the sugar levels of the Fuji apple fruit stored in ozonised cold storage was previously reported by Rui-min (2009). No significant effect or a distinctive trend was recorded in the values of TA and SSC: TA ratio from the ethylene antagonist (BC, NC and 1-MCP) fumigation or by ozone application in cold-stored Cripps Pink and Granny Smith apples. Likewise, the non-significant effect of 1-MCP on TA values have been previously reported in different cultivars of apple viz., Redchief Delicious (Mir et al., 2001) as well as McIntosh, Empire, Delicious and Law Rome (Watkins et al., 2000).

The levels of total phenols and total antioxidant capacity in the cold stored apple fruit fumigated with ethylene antagonists were higher when compared to the control fruit (Table 4). The fruit ripening process is an oxidative process and involves the production of different reactive oxidative species (ROS) (Masia, 1998). The phenolics and flavanols form chief bioactive compounds in the apple fruit and actively degrade ROS produced during ripening processes (Łata and Tomala, 2007; Valero et al., 2016). The retention of higher levels of total phenols and total antioxidant capacity in the apple fruit fumigated with ethylene antagonist indicates decelerated ripening process due to retarded rates of ethylene and respiration (Masia, 1998).

5. Conclusions
The Cripps Pink apple fruit fumigated with BC and NC exhibited significantly lowest ethylene production and respiration than 1-MCP and control fruit. While, the Granny Smith fruit treated with 1-MCP exhibited lowest ethylene production followed by NC and BC treatments, compared to control. The BC and NC fumigation treatment possess the potential to be used as an ethylene antagonist in apple fruit, without any negative effects on quality. with new ethylene antagonists (BC and NC) as well as 1-MCP effectively retarded the ethylene production and also maintained the fruit quality in the cold-stored Cripps Pink and Granny Smith apple. Therefore, BC and NC possess the potential to be used as an alternative ethylene antagonist in the apple fruit industry. The ozone application in the cold storage aided in maintaining the postharvest fruit quality but increased ethylene production in both the cultivars. There was no significant interaction effect between the ethylene antagonist fumigation and ozone application in cold storage, on the ethylene production and respiration rates as well as on the other quality parameters studied. The magnitude of the effects caused by the ethylene antagonist fumigation and ozone application on postharvest physiology and quality parameters varied based upon the apple cultivar. The effects of different ozone concentrations on the fruit quality of apple cultivars and standardising the optimum gas concentration warrant future investigation to effectively exploit advantages of ozone.

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**Declaration of interest**

All the authors declare that there is no conflict of interests.

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Figure captions:

Figure 1. Changes in the ethylene production during ripening days (D) due to ozone and ethylene antagonist (BC, NC and 1-MCP) treatment (T) in Cripps Pink and Granny Smith apple fruits cold stored (0±2°C) for 90 and 120 d. Vertical bars represent standard error (SE) of mean values and are not visible when the values are smaller than the symbol. n=4 replicates (2 fruit per replication).

LSD (P ≤ 0.05) (A) T=0.05, D=0.08, TXD=0.21 (B) T=0.06, D=0.08, TXD=0.21 (C) T=0.11, D=0.14, TXD=0.39 (D) T=0.09, D=0.11, TXD=0.31

Figure 2. The ethylene climacteric peak onset (d) and climacteric peak rates (µmol kg⁻¹h⁻¹) influenced by the ethylene antagonists (BC, NC and 1-MCP) and ozone in the Cripps Pink and Granny Smith apple fruit cold stored (0±2°C) for 90 d and 120 d.

Figure 3. The respiratory climacteric peak onset (d) and climacteric peak rates (mmol kg⁻¹h⁻¹) influenced by the ethylene antagonists (BC, NC and 1-MCP) and ozone in the Cripps Pink and Granny Smith apple fruit cold stored (0±2°C) for 90 d and 120 d.

Figure 4. The levels of individual sugars (g kg⁻¹) influenced by the ethylene antagonists (BC, NC or 1-MCP) and ozone in the pulp of Cripps Pink and Granny Smith apple fruit cold stored (0±2°C) for 90 d and 120 d. Each bar represents the mean of four replicates with two fruit per replication. The vertical bars in the graph represent SE of the mean values.