

5-14-2021

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[10.1007/s00344-021-10387-2](https://doi.org/10.1007/s00344-021-10387-2)

This is a post-peer-review, pre-copyedit version of an article published in Journal of Plant Growth Regulation. The final authenticated version is available online at: <http://dx.doi.org/10.1007/s00344-021-10387-2>

Tokala, V. Y., Singh, Z., & Kyaw, P. N. (2021). 1H-cyclopropabenzene and 1H-cyclopropa[b]naphthalene fumigation suppresses climacteric ethylene and respiration rates and modulates fruit quality in long-term controlled atmosphere-stored 'gold rush' pear fruit. *Journal of Plant Growth Regulation*, 40(6), 2276-2285.

<https://doi.org/10.1007/s00344-021-10387-2>

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1H-cyclopropabenzene and 1H-cyclopropa[b]naphthalene fumigation suppresses climacteric ethylene and respiration rates and modulates fruit quality in longterm controlled atmosphere stored ‘Gold Rush’ pear fruit

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Abstract

‘Gold Rush’ pear (*Pyrus communis* L.) is a russet-coloured fruit with soft buttery textured flesh and is gaining wide popularity in Australia and other countries along with other pear cultivars. The fruit are sensitive to ethylene, and exposure even at very low concentrations significantly reduce the storage duration as well as fruit quality during storage. The efficacy of two new ethylene antagonist compounds, namely 1H-cyclopropabenzene (BC) and 1H-cyclopropa[b]naphthalene (NC), as well as 1-methylcyclopropene (1-MCP) in regulating ethylene production, respiration rates and maintaining the fruit quality of Gold Rush pear during 150 d and 200 d of controlled atmosphere (CA) storage (2.3 ± 0.5 % O₂ and 0.4 ± 0.15 % CO₂ and 0.50 ± 0.71 °C), was investigated. The pear fruit were fumigated with 1 µM BC ($0.09 \mu\text{L.L}^{-1}$) or 1 µM NC ($0.14 \mu\text{L.L}^{-1}$) or 18 µM ($1 \mu\text{L.L}^{-1}$) 1-MCP for 18 h at room temperature and the untreated fruit were considered as the control. Following 150 d and 200 d CA storage, the fruit fumigated with BC and NC exhibited significantly reduced ethylene and respiratory climacteric peak rates and were lowest in the fruit treated with 1-MCP. The pear fruit fumigated with ethylene antagonists (BC, NC

and 1-MCP) exhibited lower physiological loss of weight (PLW) (up to 2.06 times) and higher fruit firmness (up to 1.07 times) throughout the CA storage period, compared to the control fruit. The fruit fumigated with BC and NC had lower levels of SSC, glucose and sorbitol compared to other treatments. There was no significant effect of ethylene antagonist treatments on levels of individual organic acids, total phenols, ascorbic acid and total antioxidant capacity of the fruit. Therefore, new ethylene antagonist compounds, BC and NC, exhibit the potential to act as ethylene antagonists in longterm CA stored ‘Gold Rush’ pears to retard the fruit ripening process, extend storage life and maintain the fruit quality. The effectiveness of the different concentrations of BC and NC in suppressing ethylene production in different cultivars of pears warrants further investigation.

Keywords:

Ethylene, ethylene antagonists, 1-methylcyclopropene, Gold Rush pear, climacteric fruit

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

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

1. Introduction

‘Gold Rush’ cultivar of European pear (*Pyrus communis* L.) possess attractive russet-coloured pericarp with crisp and crunchy texture when not fully ripe. At the fully ripe stage, it turns to soft buttery textured flesh with high sugar content. The European pear fruit exhibit a steep rise in postharvest ethylene production and respiration rates, and therefore categorised as climacteric fruit (Biale and Young 1981). The climacteric fruits are sensitive to internal ethylene and exposure to external ethylene, even at a minute concentration promotes fruit ripening and also cause undesirable physical and physiological changes during storage (Iqbal et al. 2017). Ethylene accelerates the fruit softening, hydrolysis of cell wall materials, depletion of bioactive compounds and increases decay as well as the incidence of several postharvest physiological disorders (Tucker 2012; Iqbal et al. 2017). Several approaches have been investigated to downregulate the fruit ripening process such as manipulating the storage environment and regulating ethylene production and its action in the fruits (Gross et al. 2016). The controlled atmosphere (CA) storage comprised of higher levels of carbon dioxide and reduced levels of oxygen extend the storage of the fruit, due to reduced ethylene production as well as retarded respiration rate and the changes associated with the fruit ripening process (Keller et al. 2013; Saquet and Streif. 2017). However, Bai et al. (2009) reported a sudden increase

in ethylene production in the pear fruit after removing them from the CA storage. Previously, Watkins (2006) and Bai et al. (2009) reported that the pear fruit treated with ethylene antagonists such as 1-methylcyclopropene (1-MCP), reduced this sudden surge in rates of ethylene production. 1-MCP is a widely used ethylene antagonist and inhibits ethylene production and its action by irreversibly blocking the ethylene receptor sites in the fruit (Sisler 2006). The efficacy of 1-MCP varied among genotypes, concentrations applied, storage temperature and treatment duration (Zhang et al. 2020). Moreover, it is difficult to handle 1-MCP, as its boiling point is very low and readily vaporises at room temperatures (Sisler et al. 2006). Several commercial products involving different delivery methods of 1-MCP as fumigation have been developed by different companies such as AgroFresh (SmartFresh™, ProTabs, SmartFresh™ InBox tablets, SmartFresh™ SmartTabs™), Hazel®, Logfresh® etc (AgroFresh 2021; Hazel Tech 2021; Logfresh 2021). Similarly, the liquid form of 1-MCP (Harvista™) has also been developed by AgroFresh whilst, Logfresh® has developed 1-MCP in liquid as well as in dustable powder form (Logfresh 2021). A pre-harvest spray application of Harvista™ has exhibited positive responses in maintaining fruit quality in different pear cultivars (Defilippi et al. 2010; Sakaldas et al. 2016; Villalobos-Acuna et al. 2017; Escribano et al. 2017; Li et al. 2020). Singh et al. (2018) discovered the capacity of 1*H*-cyclopropabenzene (BC) and 1*H*-cyclopropa[*b*]naphthalene (NC) to antagonize the ethylene action in a similar mechanism to that of 1-MCP. Structurally these compounds are different from 1-MCP, making them more stable at room temperature than 1-MCP in natural form. Kyaw (2019) and Tokala et al. (2020) investigated different BC and NC formulations and reported that the fumigation treatments were relatively more effective in retarding the postharvest ethylene production and maintaining the postharvest quality of cold-stored fruit. Recently, Tokala et al. (2021 a, b) also reported the beneficial effects of BC and NC fumigation on postharvest fruit quality of ozonized cold stored ‘Cripps Pink’ and ‘Granny Smith’ apples as well as in controlled atmosphere storage. Our preliminary research findings claiming that BC and NC fumigation suppress climacteric ethylene production and maintain fruit quality of CA stored ‘Gold Rush’ pear have been presented at the 2019 ASHS Conference in Las Vegas, USA (Tokala et al. 2019). To the best of our knowledge, no detailed research work has been reported on the effects of BC, NC and 1-MCP on the rates of respiration and ethylene production as well as on the fruit quality of long term CA stored ‘Gold Rush’ pear. It was hypothesized that the fumigation treatment with ethylene antagonists (BC, NC and 1-MCP) may effectively reduce the rates of climacteric ethylene and respiration while maintaining optimum fruit quality of ‘Gold Rush’ pears following 150 d and 200 d of CA storage. The objective of this study is to investigate the effects of BC and NC as well as 1-MCP in retarding the climacteric ethylene production, respiration rate and maintaining fruit quality of long-term CA stored ‘Gold Rush’ pear fruit.

2. Materials and methods

2.1. Plant materials

Mature pear (*Pyrus communis* L. cv. Gold Rush) fruit (fruit firmness 82.03 ± 4.14 N; SSC 11.43 ± 0.05 %; TA 0.09 ± 0.01 %) were harvested from the commercial orchard in Beedelup, Western Australia ($34^{\circ}19'$ S, $116^{\circ}00'$ E) on 20th March 2018. A negligible amount of ethylene, undetectable by gas chromatograph (GC), was produced by the fruit at this stage. The pear trees were 19 years old, grafted on *Pyrus calleryana* D6 (Callery Pear) rootstock and trained to modified the central leader system. The trees were planted with a spacing of 5 m \times 1.5 m at North-South orientation and received uniform cultivation practices. The fruit were packed in corrugated cardboard boxes and immediately transported to Curtin Horticulture Research Laboratory, Perth using an air-conditioned vehicle. The pear fruit of relatively uniform size, free from mechanical injuries, bruises or any signs of pests or diseases, were used for the experiment.

2.2. Chemicals

The ethylene antagonist compounds (1-MCP, BC and NC) used in the experiment were synthesised at Chemistry Laboratory, Curtin University. The 1-MCP was synthesized following the procedure explained earlier by Fisher and Applequist (1965). The BC was synthesised from 1,3-cyclohexadiene and NC was synthesised from naphthene in anhydrous tetrahydrofuran, following the procedures explained previously by Davalian et al. (1980) and Billups and Chow (1973), respectively.

2.3. Fumigation treatments and CA storage conditions

The pear fruit were fumigated with 1 μ M BC ($0.09 \mu\text{L.L}^{-1}$) or 1 μ M NC ($0.14 \mu\text{L.L}^{-1}$) or 18 μ M ($1 \mu\text{L.L}^{-1}$) 1-MCP for 18 h using 60 L plastic drums at room temperature (20 ± 2 °C and 65 ± 5 % RH). The fruit were arranged in plastic drums and calculated volumes of respective ethylene antagonist solution dissolved in ethanol were poured on to the filter paper in a Petri-plate. Granular soda lime (30 g) to absorb any excess carbon dioxide (CO₂) and a battery-operated portable fan to uniformly distribute the ethylene antagonist vapours were placed inside the drum before hermetically sealing it. No fumigation treatment was given to control fruit but were placed in the same conditions as other treatments. The experiment was laid out by following a two-factor (ethylene antagonist treatments and CA storage times) factorial completely randomised design with four replications and fifteen fruit per replication. On completion of 18 h of fumigation treatment, the drums were unsealed in an open-air

environment and the fruit were immediately packed in corrugated cardboard boxes with softboard trays. All the boxes were labelled appropriately with respect to the treatment and transferred to CA storages at Carmel, Western Australia (32°00' S 116°06' E) and stored for 150 d and 200 d. The gas concentrations in the CA storages comprised of 2.3 ± 0.5 % O₂ and 0.4 ± 0.15 % CO₂ and 0.50 ± 0.71 °C temperature. After completion of designated CA storage duration, the pear fruit were transferred to the laboratory, to determine the rates of ethylene production, respiration and fruit quality parameters analysis.

2.4. Determination of ethylene production and respiration rate

Two pear fruit were randomly selected from each replication to determine the ethylene production and respiration rate. The chosen fruit were sealed in 1 L glass jars for 1 h and then the gas samples were drawn from the headspace, through a rubber septum at the top. The 1 mL gas sample was injected into a gas chromatograph (Model 6890N, Agilent Technology, CA, USA) to determine the ethylene production and 2 mL gas sample was injected into the infrared gas analyser (Servomex Gas Analyser, 1450 Food Package Analyser, Servomex Limited, UK) to estimate respiration rate, as the production of carbon dioxide. The complete details of the instruments and the procedure have been earlier explained by Tokala (2019). The ethylene production and respiration rate were estimated daily until a post climacteric stage. The ethylene production and respiration rate were calculated as $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ ethylene and $\text{mmol.kg}^{-1}.\text{h}^{-1}$ CO₂, respectively.

2.5. Physiological loss of weight (PLW)

Fifteen fruit in each replication were weighed before transferring them into respective CA storage rooms and noted as initial weight. On completion of the respective storage periods, the final weight was then recorded. The PLW was calculated from initial and final weights using the following formula and expressed as %.

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

2.6. Fruit firmness

The fruit firmness was determined from ten fruit per replication, by puncturing the peeled portion of pear fruit on opposite sites at the equatorial region. The Texture Analyser (TA Plus, Ametek Lloyd Instruments Limited, UK) fitted with an 8 mm (5/16") Magnus-Taylor probe was used to puncture the fruit at 7 mm sample depth with 100

138 mm s⁻¹ test speed and 5 N trigger force. The fruit firmness was calculated using Nexygen® v.4.6 software interface
139 and expressed as newtons (N).

140 2.7. Soluble solids content (SSC), titratable acidity (TA) and SSC: TA ratio

141 The pooled juice sample extracted from the slices cut from thirteen fruit per replication was used to determine
142 SSC, TA and SSC: TA ratio. SSC was determined using an infrared digital refractometer (Atago – Palette PR 101,
143 Atago Co., Tokyo, Japan) and expressed as %. The diluted fruit juice sample was titrated against the 0.01 N sodium
144 hydroxide (NaOH) with 2-3 drops of phenolphthalein indicator till pale pink colour endpoint, to determine TA.
145 The calculated TA was expressed as a percentage of malic acid. The SCC: TA ratio value was calculated by
146 dividing SSC by TA values.

147 2.8. Individual sugars and organic acids

148 The levels of individual sugars and organic acids in the fruit pulp samples, from thirteen fruit per replication, were
149 determined using the reverse-phase high-performance liquid chromatography (RP-HPLC) system (Waters 1525,
150 Milford Corporation, USA) following the method detailed earlier by Tokala (2019). The Dual λ UV absorbance
151 detector (Water 2487, Milford Corporation, USA) at 214 nm was used to determine the individual organic acids
152 (citric acid, tartaric acid, malic acid, succinic acid and fumaric acid). The Refractive Index (RI) detector (Water
153 2414, Milford Corporation, USA) was used to estimate the levels of individual sugars (sucrose, glucose, fructose
154 and sorbitol). The values of individual sugars and organic acids were calculated for the area of the chromatographic
155 peaks using Breeze®2 software version 6.20 (Waters, Milford Corporation, USA) and are expressed as g.kg⁻¹ fresh
156 weight basis.

157 2.9. Total phenols

158 The levels of total phenols in the fruit pulp samples were determined using the Folin-Ciocalteu reagent method
159 and a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) using the procedure explained
160 earlier by Robles-Sánchez et al. (2009), with some modifications as detailed earlier by Tokala (2019). The levels
161 of total phenols were calculated from the standard curve drawn using pure gallic acid and were expressed as g
162 Gallic Acid Equivalent (GAE) kg⁻¹ fresh weight basis.

163 2.10. Ascorbic acid

The ascorbic acid levels in the fruit pulp samples were determined using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK) following the procedure earlier detailed by Tokala (2019). The standard L-ascorbic acid curve was used to calculate levels of ascorbic acid and expressed as g.kg⁻¹ fresh weight basis.

2.11. Total antioxidant capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay explained by Brand-Williams et al. (1995) was used to determine the total antioxidant capacity in the fruit pulp samples, following the procedure detailed by Tokala (2019). The absorbance of the samples prepared was recorded at 515 nm using a UV/VIS spectrophotometer (Jenway spectrophotometer Model 6405, UK). The levels of total antioxidant capacity were calculated using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) standard curve and expressed as µM kg⁻¹ Trolox fresh weight basis.

2.12. Statistical analysis

The data were analysed using a two-way analysis of variance (ANOVA) to evaluate the effects of ethylene antagonist treatments, CA storage duration and their interaction. The *GenStat* software version 14.0 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK) was used to analyse all the experimental data. The least significant difference (LSD) at 5 % error probability was calculated by F-test and treatment means were compared using Duncan multiple comparison tests. The results in the tables are presented as means ± standard errors (SE) of the means.

3. Results

3.1. Ethylene production and respiration rates

The ‘Gold Rush’ pear fruit fumigated with BC, NC and 1-MCP exhibited reduced rates of ethylene climacteric peak by 28 %, 33 % and 99 % in 150 d CA stored and by 28 %, 17 % and 99 % in 200 d CA stored, respectively, when compared to the control fruit (Figure 1 and 2C). Similarly, when compared to control fruit, the rates of the respiratory climacteric peak were reduced by 20 %, 17 % and 43 % following 150 d and by 23 %, 13 % and 55 % following 200 d of CA storage in the fruit fumigated with BC, NC and 1-MCP, respectively (Figure 2D). When compared to the control fruit, the onset of ethylene climacteric peak was delayed by 1.25, 1.5 and 6.25 d following 150 d and by 2.5, 0.5 and 5 d following 200 d of CA storage in the fruit fumigated with BC, NC and 1-MCP,

respectively (Figure 1 and 2A). However, the onset of the respiratory climacteric peak was not significantly affected by any of the ethylene antagonist treatments (Figure 2B).

3.2. PLW and fruit firmness

The PLW was significantly reduced by 34 %, 35 % and 52 % in the fruit fumigated with BC, NC and 1-MCP when compared to control fruit, respectively, irrespective of the CA storage period (Table 1). The firmness in the pear fruit fumigated with BC, NC and 1-MCP was maintained each 1.07 times higher than that of the firmness of control fruit, irrespective of the CA storage period (Table 1). There was no significant interaction effect between the ethylene antagonist treatment and the storage duration on the PLW and fruit firmness.

3.3. SSC, TA and SSC: TA ratio

The pear fruit fumigated with BC and NC exhibited significantly lower SSC values (11.61 % and 11.70 %, respectively) when compared to the fruit treated with 1-MCP and control fruit, irrespective of the CA storage period (Table 1). The SSC values increased by 1.04 folds with the extension of CA storage duration from 150 d to 200 d (Table 1). The pear fruit fumigated with BC and stored for 150 d exhibited significantly lowest SSC values (11.35 %) when compared to all other treatments and control (Table 1). The values of TA as well as SSC: TA ratio were not significantly affected by any of the treatments (Supplementary, Appendix 1, Table 1).

3.4. Individual sugars and organic acids

Glucose, fructose, sucrose and sorbitol were determined from the treated and control fruit following 150 d and 200 d CA storage, but fructose was the predominant sugar (Table 2). The pear fruit fumigated with 1-MCP exhibited significantly highest levels of glucose (5.18 g.kg⁻¹) and sorbitol (11.86 g.kg⁻¹) but lowest levels of sucrose (6.68 g.kg⁻¹), when compared to all other treatments and control (Table 2). The levels of sucrose were significantly higher (9.91 g.kg⁻¹) in 150 d CA stored fruit than those stored for 200 d (7.86 g.kg⁻¹). Whilst the levels of sorbitol were higher (11.37 g.kg⁻¹) in the 200 d CA stored fruit than 150 d stored (10.27 g.kg⁻¹) (Table 2). BC, NC and 1-MCP fumigation did not significantly affect the levels of fructose as compared to the control in 150 d and 200 d CA stored fruit. Malic acid, succinic acid and fumaric acid were quantified from the treated and control fruit following 150 d and 200 d CA storage, but succinic acid was the predominant organic acid (Table 2). The levels of malic acid, succinic acid and fumaric acid were also not significantly affected by BC, NC and 1-MCP fumigation treatments or CA storage duration (Supplementary, Appendix 1, Table 2).

3.5. Total phenols, ascorbic acid and total antioxidant capacity

BC, NC and 1-MCP fumigation treatments did not significantly influence the levels of total phenols, ascorbic acid and total antioxidant capacity as compared to the control in 150 and 200 d CA stored fruit. The levels of ascorbic acid and total antioxidant capacity were reduced by 15 % and 30 %, respectively, with an extension of CA storage duration from 150 d to 200 d. The interactions between BC, NC and 1-MCP fumigation treatments and CA storage periods were non-significant for total phenols, ascorbic acid and total antioxidant capacity (Supplementary, Appendix 1, Table 3).

4. Discussion

The efficacy of the two new ethylene antagonist compounds (BC and NC) and 1-MCP fumigation in downregulating the climacteric ethylene production, respiration rate and maintaining postharvest fruit quality of long-term CA stored ‘Gold Rush’ pear fruit has been investigated for the first time. BC, NC and 1-MCP fumigation treatments have effectively reduced the rates of the ethylene and respiratory climacteric peak in the ‘Gold Rush’ pear fruits during CA storage (Figure 1 and 2). The 1-MCP inhibits the ethylene action in the fruit at the cellular level, by irreversibly blocking ethylene receptor sites and interfering with the expression of ethylene-responsive genes (Sisler et al. 2003; Apelbaum et al. 2008). Pirrung et al. (2008) proposed a cyclopropene ring-opening reaction mechanism forming a copper carbenoid intermediate to explain ethylene antagonistic action of 1-MCP. The intermediate formed blocks the ethylene action by irreversibly reacting with amino acids of the ethylene receptor protein domain. The BC and NC compounds also react with copper (I) cofactor situated with the ETR1 ethylene receptor to antagonize the ethylene action in fruit and thereby retard ethylene production and respiration rates (Musa 2016; Singh et al. 2018; Tokala et al. 2020, 2021 a, b). BC and NC are structurally different from 1-MCP, but the proposed mode of antagonising ethylene action in the fruit is similar to 1-MCP (Musa 2016; Singh et al. 2018). The fruit fumigated with the 1-MCP exhibited very low levels of ethylene production ranging between 0 to 0.02 $\mu\text{mol.kg}^{-1}.\text{h}^{-1}$ in both the storage durations studied. Xie et al. (2016) reported that European pear fruit fumigated with 1-MCP exhibited an inability to produce ethylene and ripen normally after extended low-temperature storage. There is a scope to investigate the necessity of post-storage ethylene application in “Gold Rush” pear fruit. Similar to ethylene production, when compared to control, the rates of the respiratory climacteric peak in 200 d of CA stored fruit was suppressed in the fruit fumigated with BC, NC and 1-MCP (Figure 2D). The ethylene antagonist action also inhibits or retard the respiration rates along with other ripening associated

physiological changes in climacteric fruits (Zhang et al. 2020). This reduction implies that the ethylene antagonist treatments effectively blocked the ethylene receptor sites and inhibited ethylene action in the fruit (Sisler 2006).

BC, NC and 1-MCP fumigation treatments have significantly reduced the PLW during CA storage (Table 1). The loss of weight in the fruit during storage is primarily due to water loss through continuous physiological processes such as respiration and transpiration (Becker and Fricke 1996). The rate of transpiration from the fruit surface during storage increases with an increase in the rate of respiration (Dhillon and Mahajan 2011). The reduction in PLW could be associated with decreasing trends of ethylene production and respiration in the fruit (Martínez-Romero et al. 2007). The maintenance of higher fruit firmness in BC, NC and 1-MCP fumigated pear fruit may be attributed to the downregulation of ethylene production and its action, which consequently reduced fruit softening and PLW (Giovannoni 2008). The phytohormone ethylene plays a key role in activating the cell wall hydrolysing enzymes during the fruit ripening process (Giovannoni 2008). The fruit firmness in pear fruit is closely related to the degree of ripeness, internal quality and possible shelf-life (Zhang et al. 2018).

The SSC values were maintained significantly lower in fruit fumigated with BC and NC, while the SSC was higher in the 1-MCP treated fruit. Inconsistencies of the SSC values in the fruit treated with ethylene antagonists have also been previously reported by Blankenship and Dole (2003). Fan et al. (1999) also indicated that the accumulation of sugars in the fruit during storage is not essentially associated with ethylene perception. The levels of individual sugars (glucose, fructose and sorbitol) in the fruit treated with 1-MCP were highest as compared to those fumigated with BC or NC and control fruit (Table 2). Similarly, Mahajan et al. (2010) also reported that the levels of sugars in ‘Patharnakh’ pear fruit treated with 1-MCP were higher than the control fruit. BC, NC and 1-MCP fumigation treatments did not significantly affect the levels of TA and individual organic acids in CA stored fruit. Similarly, 1-MCP fumigation did not significantly regulate the levels of TA in different cultivars of pear such as ‘Blanquilla’ (Larrigaudière et al. 2004), ‘Red Clapp’s (Calvo and Sozzi 2004), and ‘Bartlett’ (Trinchero et al. 2004).

5. Conclusions

The fumigation treatment with novel ethylene antagonists (BC and NC) as well as 1-MCP were effective in downregulating ethylene production and respiration rate in the long-term CA stored ‘Gold Rush’ pear fruit but 1-MCP was more efficient. The BC and NC fumigation were at par with 1-MCP treatment in reducing PLW and loss of fruit firmness. Therefore, BC and NC possess the potential to be used as an ethylene antagonist in ‘Gold

273 Rush' pear without causing any undesirable effects on the fruit quality during long-term CA storage. The effects
274 of the different concentrations of these new ethylene antagonists in comparison with 1-MCP on suppressing
275 ethylene production in different cultivars of Asian and European pears warrants further investigation.

276 **Acknowledgements**

277 V.Y. Tokala would like to thank the Australian Government and Curtin University for the Australian Government
278 Research Training Program Scholarship (formerly known as International Postgraduate Research Scholarship) to
279 pursue his PhD research program. Casuarina Valley Orchards, Beedelup, WA is gratefully acknowledged for
280 providing the experimental fruit and access to CA storage facilities at Carmel, WA. The authors are obliged to Dr
281 Alan D. Payne, for synthesizing the BC, NC and 1-MCP and Ms Susan Petersen for the technical support provided
282 during the experiment. The authors are thankful to Dr Satvinder Dhaliwal, Professor of Biostatistics, Curtin
283 University for providing insights into the statistical analysis.

284 **Conflict of interest**

285 The authors declare that they have no known competing financial interests or personal relationships that could
286 have appeared to influence the work reported in this paper.

287 **Credit authorship contribution statement:**

288 **Vijay Yadav Tokala:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing -
289 original draft.

290 **Zora Singh:** Conceptualization, Methodology, Supervision, Resources, Writing - review and editing.

291 **Poe Nandar Kyaw:** Investigation, Methodology, Writing - review and editing.

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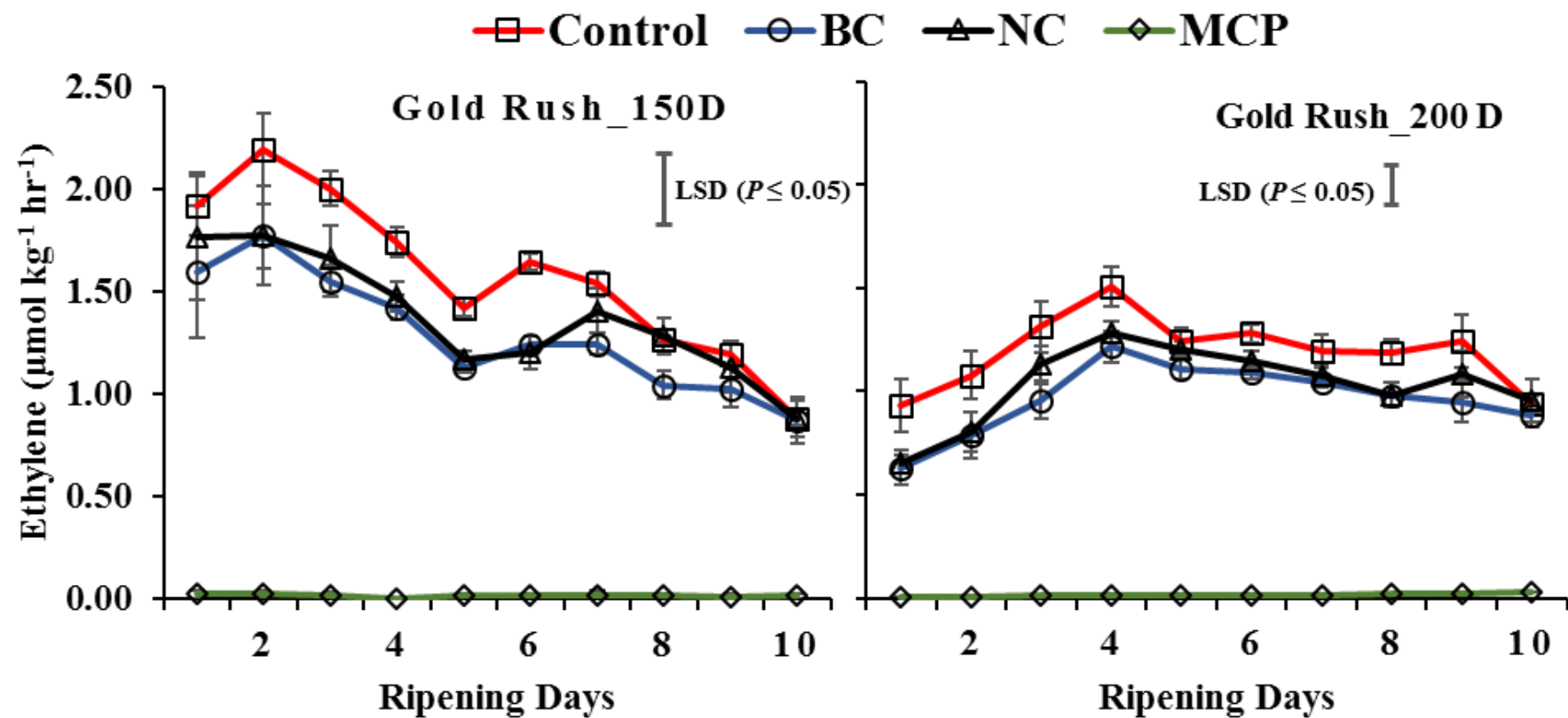


Figure 1. Effects of BC, NC and 1-MCP fumigation treatments (T) on ethylene production during ripening days (D) following 150 d and 200 d CA storage of ‘Gold Rush’ pear fruit. Vertical bars represent SE of mean values and are not visible when values are smaller than the symbol. $n = 4$ replicates (2 fruit per replication). LSD ($P \leq 0.05$) $T=0.11$, $D=0.17$, $TXD=0.35$ for 150 d and $T=0.06$, $D=0.10$, $TXD=0.19$ for 200 d.

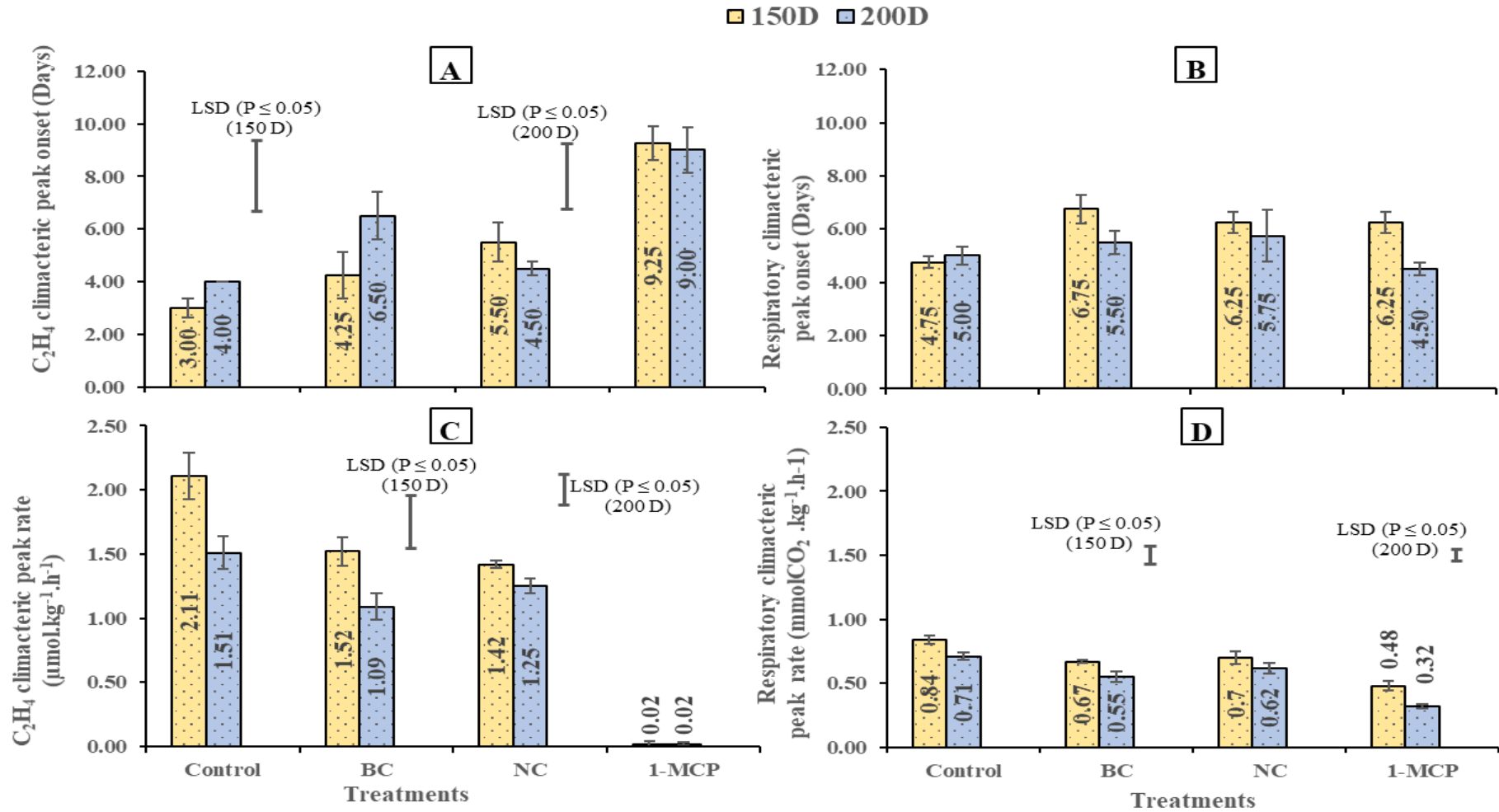


Figure 2. Effects of BC, NC and 1-MCP fumigation treatments on the climacteric peak onset (days) (A); peak rates of ethylene ($\mu\text{mol.kg}^{-1}.\text{h}^{-1}$); (B) a peak rates ethylene ($\mu\text{mol.kg}^{-1}.\text{h}^{-1}$) (C); climacteric respiration peak onset (days) (D) a peak rate ($\text{mmolCO}_2.\text{kg}^{-1}.\text{h}^{-1}$) in 150 and 200 d CA stored Gold Rush pear fruit. Vertical bars represent SE of mean values. n=4 replicates (2 fruit per replication). LSD ($P \leq 0.05$): (A) 2.69 for 150 d and 2.47 for 200 d (B) non-significant for 150 d and 200 d (C) 0.41 for 150 d and 0.23 for 200 d (D) 0.14 for 150 d and 0.09 for 200 d

Table 1. Effects of BC, NC and 1-MCP fumigation on the physiological loss of weight (PLW) (%), fruit firmness (N) and soluble solids concentration (SSC) (%) of the ‘Gold Rush’ pear fruit following 150 and 200 d CA storage.

	CA storage period (days)		
Treatment	150	200	Mean (T)
	PLW (%)		
Control	2.15±0.31	2.43±0.21	2.29 ^B
BC	1.34±0.19	1.71±0.08	1.52 ^A
NC	1.32±0.40	1.67±0.20	1.49 ^A
1-MCP	1.11±0.38	1.11±0.30	1.11 ^A
Mean (D)	1.48	1.73	
LSD (<i>P</i> ≤ 0.05)	T = 0.63	D = ns	TXD = ns
	Fruit firmness (N)		
Control	70.34±0.54	69.76±1.65	70.05 ^A
BC	77.70±2.14	72.17±1.24	74.94 ^B
NC	76.69±1.81	72.81±0.80	74.75 ^B
1-MCP	77.61±0.83	72.90±1.19	75.25 ^B
Mean (D)	75.58 ^B	71.91 ^A	
LSD (<i>P</i> ≤ 0.05)	T = 3.32	D = 2.35	TXD = ns
	SSC (%)		
Control	11.63±0.02 ^b	12.05±0.02 ^d	11.84 ^B
BC	11.35±0.02 ^a	11.88±0.02 ^c	11.61 ^A
NC	11.58±0.02 ^b	11.83±0.02 ^c	11.70 ^A
1-MCP	11.60±0.09 ^b	12.05±0.02 ^d	11.83 ^B
Mean (D)	11.54 ^A	11.95 ^B	
LSD (<i>P</i> ≤ 0.05)	T = 0.10	D = 0.07	TXD = 0.14

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (15 fruit (PLW), 10 fruit (fruit firmness), 13 fruit (SSC) 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 a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Table 2. Effects of BC, NC and 1-MCP fumigation on the levels of individual sugars (g kg⁻¹) in the pulp of 150 and 200 d CA stored ‘Gold Rush’ pear fruit.

CA storage period (days)			
Treatment	150	200	Mean (T)
Glucose (g.kg ⁻¹)			
Control	4.55±0.19 ^b	4.99±0.18 ^{bc}	4.77 ^B
BC	4.50±0.06 ^b	4.53±0.11 ^b	4.54 ^{AB}
NC	4.80±0.07 ^b	3.96±0.15 ^a	4.38 ^A
1-MCP	5.05±0.19 ^{bc}	5.32±0.01 ^c	5.18 ^C
Mean (D)	4.73	4.70	
LSD (<i>P</i> ≤ 0.05)	T = 0.34	D = ns	TXD = 0.48
Fructose (g.kg ⁻¹)			
Control	30.53±0.40	30.87±0.16	30.70
BC	31.39±0.21	30.20±0.49	30.80
NC	30.15±0.49	30.87±0.43	30.51
1-MCP	31.24±1.23	31.63±0.73	31.43
Mean (D)	30.83	30.89	
LSD (<i>P</i> ≤ 0.05)	T = ns	D = ns	TXD = ns
Sucrose (g.kg ⁻¹)			
Control	10.42±0.6.67	8.71±0.69	9.56 ^B
BC	11.22±0.5.75	8.76±0.24	9.99 ^B
NC	9.95±0.1.76	8.66±0.24	9.31 ^B
1-MCP	8.04±0.7.24	5.31±0.20	6.68 ^A
Mean (D)	9.91 ^B	7.86 ^A	
LSD (<i>P</i> ≤ 0.05)	T = 1.2.04	D = 0.8.51	TXD = ns
Sorbitol (g.kg ⁻¹)			
Control	9.90±0.26	11.13±0.21	10.51 ^A
BC	9.84±0.12	10.94±0.21	10.39 ^A
NC	9.57±0.26	11.44±0.12	10.50 ^A
1-MCP	11.76±0.53	11.96±0.50	11.86 ^B
Mean (D)	10.27 ^A	11.37 ^B	
LSD (<i>P</i> < 0.05)	T = 0.78	D = 0.55	TXD = ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The 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 a similar letter are not significantly different within the columns or rows. The lower case was used for mean interactions and the upper case was used for treatment and storage period means. Mean values without letters within columns or rows are non-significant.

Supplementary Table 1. Effects of BC, NC and 1-MCP fumigation on the changes in the titratable acidity (TA) (%) and SSC: TA ratio in the juice of 150 and 200 d CA stored ‘Gold Rush’ pear fruit.

	CA storage period (days)		
Treatment	150	200	Mean (T)
	Titratable acidity (TA) (%)		
Control	0.06±0.01	0.07±0.01	0.07
BC	0.07±0.01	0.09±0.01	0.08
NC	0.08±0.00	0.09±0.01	0.09
1-MCP	0.08±0.01	0.09±0.01	0.08
Mean (D)	0.07	0.08	
LSD (<i>P</i> ≤ 0.05)	T=ns	D=ns	TXD=ns
	SSC: TA		
Control	217.04±36.38	174.85±12.50	195.95
BC	176.31±30.25	132.96±7.53	154.63
NC	143.97±0.27	132.40±7.50	138.18
1-MCP	161.55±17.48	142.41±6.65	151.98
Mean (D)	174.71	145.66	
LSD (<i>P</i> < 0.05)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The 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 without letters within columns or rows are non-significant.

Supplementary Table 2 Effects of BC, NC and 1-MCP fumigation on the levels of individual organic acids (g.kg⁻¹) in the pulp of 150 and 200 d CA stored ‘Gold Rush’ pear fruit.

	CA storage period (days)		
Treatment	150	200	Mean (T)
Malic acid (g.kg ⁻¹)			
Control	1.25±0.13	0.98±0.06	1.11
BC	1.22±0.08	1.25±0.14	1.23
NC	1.31±0.30	1.35±0.06	1.33
1-MCP	1.36±0.22	1.22±0.38	1.29
Mean (D)	1.28	1.20	
LSD (<i>P</i> ≤ 0.05)	T=ns	D=ns	TXD=ns
Succinic acid (g.kg ⁻¹)			
Control	4.30±0.20	2.66±0.60	3.48
BC	4.52±0.26	3.55±0.32	4.03
NC	4.13±0.52	2.83±0.33	3.48
1-MCP	3.33±0.55	3.93±0.26	3.63
Mean (D)	4.07B	3.24A	
LSD (<i>P</i> ≤ 0.05)	T=ns	D=0.73	TXD=ns
Fumaric acid (g.kg ⁻¹)			
Control	0.29±0.04	0.28±0.05	0.29
BC	0.19±0.00	0.21±0.02	0.20
NC	0.24±0.01	0.26±0.03	0.25
1-MCP	0.20±0.01	0.23±0.04	0.22
Mean (D)	0.23	0.25	
LSD (<i>P</i> < 0.05)	T=ns	D=ns	TXD=ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The 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 without letters within columns or rows are non-significant.

Supplementary Table 3. Effects of BC, NC and 1-MCP on the levels of total phenols, ascorbic acid and total antioxidant capacity in the pulp of 150 and 200 d CA stored ‘Gold Rush’ pear fruit.

	CA storage period (days)		
Treatment	150	200	Mean (T)
Total phenols (g.GAEkg ⁻¹)			
Control	11.41±0.73	12.16±1.23	11.79
BC	12.16±0.82	12.82±2.58	12.49
NC	11.04±0.49	10.20±0.87	10.62
1-MCP	10.29±0.82	10.29±0.50	10.29
Mean (D)	11.23	11.37	
LSD (<i>P</i> ≤ 0.05)	T=ns	D=ns	TXD=ns
Ascorbic acid (g.kg ⁻¹)			
Control	5.26±0.18	4.56±0.44	4.91
BC	5.12±0.29	4.88±0.34	5.00
NC	4.81±0.24	4.14±0.05	4.48
1-MCP	5.47±0.19	3.97±0.25	4.72
Mean (D)	5.17B	4.39A	
LSD (<i>P</i> ≤ 0.05)	T=ns	D=0.44	TXD=ns
Total antioxidant capacity (µM.kg ⁻¹ Trolox)			
Control	5.31±0.46	3.95±0.19	4.63
BC	5.72±0.33	3.76±0.22	4.74
NC	4.68±0.22	3.60±0.13	4.14
1-MCP	5.50±0.28	3.65±0.10	4.57
Mean (D)	5.31B	3.74A	
LSD (<i>P</i> ≤ 0.05)	T=ns	D=0.45	TXD=ns

ns = non-significant, T = treatments, D = CA storage period, n = 4 replicates (13 fruit per replication), mean ± SE. The 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 without letters within columns or rows are non-significant.