Postharvest application of 1-MCP and ethylene influences fruit softening and quality of 'Arctic Pride' nectarine at ambient conditions

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Abstract

Fruit softening in nectarine is a limiting factor for their extended postharvest life with best quality. Effects of postharvest exogenous applications of 1-MCP (1 µL L^{-1}), ethylene (10 µL L^{-1}) or 1-MCP (1 µL L^{-1}) followed by ethylene (10 µL L^{-1}) for 12 h on 'Arctic Pride' nectarine were investigated for changes in fruit softening and quality during ripening at ambient temperature (20 ± 1° C; 60-65% RH). Untreated fruit were kept as control and stored at the same conditions i.e. 20 ± 1° C; 60-65% RH. 1-MCP application significantly reduced ethylene production and activities of fruit softening enzymes, including pectin esterase (PE), *endo*-1,4-*β*-glucanase (EGase), *endo*-polygalacturonase (*endo*-PG), *exo*-polygalacturonase (*exo*-PG) as compared to ethylene treatment or control. A significant reduction in fruit weight loss, fruit softening, total sugars and organic acids was also observed with the application of 1-MCP, as compared to ethylene-treated or control fruit. Exogenous application of 1-MCP maintained individual sugars (glucose, fructose and sucrose) and organic acids (malic, shikimic, succinic, and citric acid) at higher levels and delayed ripening of nectarine fruit, as compared to ethylene or untreated fruit during ripening. In conclusion, 1-MCP application delayed fruit ripening by inhibiting ethylene production and the activities of fruit softening enzymes and maintained the quality of nectarine fruit as compared to ethylene-treated or control fruit during ripening.

Keywords: *Endo*-1,4-*β*-glucanase; *Endo*-polygalacturonase; Ethylene; *Exo*-polygalacturonase; fruit ripening; 1-MCP; pectin esterase; *Prunus persica* (L.) Batsch. cv nectarine.
Abbreviations: EGase _*endo*-1,4-*β*-glucanase; *Endo*-PG_*endo*-polygalacturonase; *Exo*-PG_*exo*-polygalacturonase; 1-MCP_1-methylcyclopropene; PE_pectin esterase.

Introduction

Nectarine being climacteric fruit ripen very quickly at ambient conditions. Rapid fruit softening during ripening, consequently limit their postharvest storage and shelf life (Ortiz et al., 2011). Softening of these fruit involves series of changes in the polysaccharide of middle lamella and primary cell wall (Fischer and Bennett, 1991). Possible reasons for fruit softening are hydrolysis of polysaccharides and modification in the polymers bonds established with turgor alterations which results in increased cell separation and softening of the cell wall (Brummell, 2006). However, softening condition differs among fruits. In peach and nectarine, fruit softening has been found to be associated with a depolymerization of matrix glycans both loosely and tightly attached to cellulose and a loss of galacturonic acids from all cell wall fractions (Ortiz et al., 2011). Softening results from increased activities of cell wall degrading enzymes including pectin esterase (PE), *endo*-1,4-*β*-glucanase (EGase), *exo*-polygalacturonase (*exo*-PG) and *endo*-polygalacturonase (*endo*-PG) during ripening in peach (Brummell et al., 2004; Ullah et al., 2013).

Ethylene significantly regulates ripening of climacteric fruit which consequently influences their eating quality attributes such as appearance, texture, colour, flavour and fruit softening (Khan et al, 2007; Yang et al., 2013). Ethylene has been involved in partial control of fruit ripening as evidenced by its exogenous application in pears (Acuna et al., 2011) or

propylene in banana (Golding et al., 1998) thus enhancing the levels of endogenous ethylene production. Role of ethylene in promoting fruit softening has been demonstrated using inhibitors of ethylene action, such as polyamines (Khan et al., 2007; Khan and Singh, 2010), aminoethoxyvinylglycine (AVG) (Whale et al., 2008) and 1-methylcyclopropene (1-MCP) (Khan and Singh, 2007, 2008).

1-MCP is being used in the postharvest phase to delay ripening of various fruits including nectarine and peach. It has been classified as ethylene action inhibitor with a 10-fold more affinity to bind ethylene receptors than ethylene itself (Blankenship and Dole, 2003). Effects of 1-MCP application differ in relation to a number of factors including genotype, concentration of 1-MCP, method of application and ripening conditions (Dal-Cin et al., 2006; Watkins, 2006). Some fruit crops benefitted from 1-MCP regardless of the presence of exogenous ethylene, whereas, some others showed less response to 1-MCP application unless exogenous ethylene was present (Watkins, 2008).

Previously exogenous application of 1-MCP has been used to delay ripening in peaches and nectarine either alone (Bregoli et al., 2005) or in combination with CO_2, AVG, jasmonates andethephon, under controlled atmosphere and cold storage conditions (Mothooko et al., 2001; Hayama et al., 2008; Costa et al., 2008; Ortiz et al., 2011; Zhang et al., 2012;). However, in these studies the main focus was on management of fruit quality. However, the mode of action of 1-MCP in modulating nectarine fruit ripening particularly softening or the activities of fruit-softening enzymes at ambient conditions warrants to be investigated. It was hypothesized that 1-MCP treatment would retard ethylene production; consequently delay fruit softening while reducing activities of various fruit softening enzymes including exo-, endo-PG and EGase leading to extension of nectarine fruit shelf life. Therefore, the mode of action of 1-MCP in modulating nectarine fruit softening and ripening by employing 1-MCP fumigation or ethylene alone and 1-MCP followed by ethylene in regulating ethylene production and rate of respiration, fruit firmness and activities of fruit softening enzymes (exo-PG, endo-PG, PE and EGase) were investigated in pulp of nectarine during ripening at ambient temperature.

Results

Changes in ethylene production and respiration rate

1-MCP-treated nectarine fruit exhibited suppressed ethylene production, as compared to ethylene-treated fruit. Nectarine fruit of all treatments showed climacteric ethylene production peak on day-9 of ripening (Fig. 1A). However, the fruit treated with 1-MCP alone or in combination with ethylene exhibited about 1.8-fold and 2.5-fold less ethylene production as compared to control on day-9 of fruit ripening, respectively. On the other hand, about 7-fold and 11-fold more ethylene was produced in fruit treated with ethylene alone than 1-MCP alone and 1-MCP- treated fruit followed by exposure to ethylene, respectively.

A significant increasing trend was observed in respiration rate in 1-MCP-treated nectarine fruit during ripening at ambient conditions. However, reduced respiration rate was observed in 1-MCP-treated nectarine fruit, as compared to untreated control fruit. The fruit treated with 1-MCP followed by exposure to ethylene showed the least respiration rate which was about 1.1-fold and 1.5-fold less than the respiration rate of control fruit and ethylene-treated fruit, respectively (Fig. 1B).

Change in fruit weight loss

Fruit weight loss (FWL) was significantly ($P \leq 0.05$) increased during fruit ripening following treatment applications (Fig. 2A). Minimum FWL (1.6-fold less) was observed in fruit treated with 1-MCP followed by ethylene as compared to untreated nectarine fruit.

Changes in fruit softening and activities of softening enzymes

Postharvest 1-MCP application, significantly delayed nectarine fruit softening during ripening. Exogenous application of 1-MCP significantly ($P \leq 0.05$) reduced the fruit firmness as compared to control during ripening at ambient conditions (Fig. 2B). Fruit firmness was higher (around 1.2-fold and 1.1-fold) in the fruit treated with1-MCP alone and 1-MCP followed by ethylene as compared to ethylene- alone and control, respectively. Fruit firmness decreased rapidly with progression in fruit ripening time.

Activity of PE was significantly ($P \leq 0.05$) reduced in nectarine fruit by the application of 1-MCP as compared to control and ethylene-treated fruit (Fig. 3A). About 2.3-fold and 4-fold higher activities of PE were observed in untreated and ethylene-treated nectarine fruit on day-9 than in 1-MCP-treated fruit during ripening, respectively. Similarly, exogenous application of 1-MCP suppressed the EGase activity in pulp tissues of nectarine fruit compared to the control and ethylene-treated fruit (Fig. 3B). Activity of EGase was about 1.9-fold and 4.1-fold higher in control and ethylene-treated fruit on day-9 than 1-MCP-treated fruit during ripening, respectively.

Application of 1-MCP significantly ($P \leq 0.05$) reduced the activities of endo-PG and exo-PG enzymes in pulp tissue of nectarine fruit (Figs. 3C, D) compared to control and ethylene-treated fruit. In the pulp of control and ethylene-treated nectarine fruit, on day-9 of fruit ripening, endo-PG was about 2.1-fold and 2.8-fold higher than 1-MCP-treated fruit, respectively (Fig. 3C). Untreated fruit and ethylene-treated fruit on day-9, showed about 2.3-fold and 3.2-fold higher activity of exo-PG than 1-MCP-treated fruit, respectively (Fig. 3D).

Changes in fruit rheological properties

Number of days at ambient conditions significantly ($P \leq 0.05$) lessened the gumminess of nectarine fruit. However, 1-MCP application and its interaction with days after treatment showed non-significant effect. There was a steep decrease in the fruit gumminess on day-9 which was about 2-fold less than on day-0 of fruit ripening (Table 1). Cohesiveness of nectarine fruit was non-significantly affected by 1-MCP treatment, days at ambient conditions and their interactive affect (Table 1). Springiness of nectarine fruit was significantly ($P \leq 0.05$) reduced by 1-MCP treatment, time period at ambient and their interaction. Springiness of fruit decreased as the fruit ripening period progressed. Overall least springy fruit were observed at day-9 of fruit ripening, which was about 3-fold less springy than day-0. At day-9 of fruit ripening, 1-MCP-treated fruit were about 1.4-fold and 1.3-fold springier compared to ethylene and control fruit (Table 1).

Chewiness of nectarine fruit was significantly ($P \leq 0.05$) affected by 1-MCP- treatment during ripening. A rapid loss of chewiness was observed in nectarine fruit until day-9 of fruit ripening. However, 1-MCP treated fruit retained better chewiness than untreated and ethylene-treated fruit. On day-9

Table 1. Effect of exogenous application of 1-MCP and ethylene on rheological properties of 'Arctic Pride' nectarine fruit.

Parameters	Treatment (T)	Ripening period (RP) (Days)			
		0	3	6	9
Gumminess (N)	Control	6.15±0.01	0.69±0.2	0.38±0.23	0.20±0.17
	1-MCP	6.15±0.01	0.75±0.26	0.62±0.18	0.26±0.02
	Ethylene	6.15±0.01	0.65±0.05	0.31±0.08	0.10±0.01
	1-MCP + Ethylene	6.15±0.01	1.08±0.38	0.45±0.11	0.15±0.01
	LSD ($P \leq 0.05$), T = NS, RP = 0.1298, T × RP = NS				
Cohesiveness	Control	0.09±0.02	0.08±0.01	0.08±0.0	0.02±0.01
	1-MCP	0.09±0.02	-1.42±0.33	-0.13±0.02	0.02±0.0
	Ethylene	0.09±0.02	0.20±0.05	0.04±0.01	0.02±0.01
	1-MCP + Ethylene	0.09±0.02	0.08±0.01	0.03±0.02	0.02±0.0
	LSD ($P \leq 0.05$), T = NS, RP = NS, T × RP = NS				
Springiness (mm)	Control	4.00±0.01b	2.13±0.03cd	1.60±0.026ef	0.93±0.05ij
	1-MCP	4.00±0.01b	2.35±0.05c	1.83±0.09de	1.27±0.07gh
	Ethylene	4.00±0.01b	4.73±0.33a	1.22±0.14ghi	0.81±0.04j
	1-MCP + Ethylene	4.00±0.01b	4.97±0.01a	1.50±0.15fg	1.03±0.06hij
	LSD ($P \leq 0.05$), T = 0.1617, RP = 0.1617, T × RP = 0.3234				
Chewiness (N mm)	Control	25.02±0.4 a	1.83±0.02de	0.83±0.08 efg	0.41±0.05 g
	1-MCP	25.02±0.4a	2.09±0.11 cd	1.53±0.02 def	0.46±0.01 fg
	Ethylene	25.02±0.4 a	3.16±0.15 c	0.52±0.06 fg	0.11±0.01 g
	1-MCP + Ethylene	25.02± 0.4a	5.39±0.19 b	0.83±0.05 efg	0.18±0.02 g
	LSD ($P \leq 0.05$), T = 0.5356, RP = 0.5356, T × RP = 1.0711				
Adhesiveness (N mm)	Control	2.23±0.01	0.61±0.01	0.41±0.07	0.36±0.06
	1-MCP	2.23±0.01	1.36±0.17	0.80±0.08	0.58±0.03
	Ethylene	2.23±0.01	1.02±0.06	0.23±0.01	0.38±0.01
	1-MCP + Ethylene	2.23±0.01	1.68±0.16	0.52±0.02	0.41±0.03
	LSD ($P \leq 0.05$), T = 0.2538, RP = 0.2538, T × RP = NS				
Stiffness (N mm^{-1})	Control	18.66±0.03	7.40±0.64	6.01±0.72	4.71±0.68
	1-MCP	18.66±0.03	8.45±1.03	5.34±0.09	4.89±0.28
	Ethylene	18.66±0.03	8.89±0.71	251.90±38.9	4.99±0.41
	1-MCP + Ethylene	18.66±0.03	10.67±0.42	91.87±12.2	4.52±0.16
	LSD ($P \leq 0.05$), T= NS, RP = NS, T × RP = NS				

Values within a row and column sharing different letter were significant ($P \leq 0.05$), NS = non-significant, values followed by ± denotes standard deviation of means (n=3).
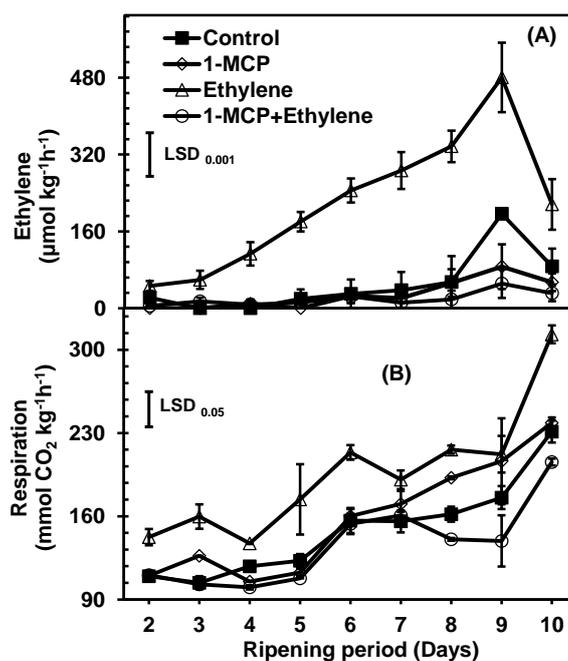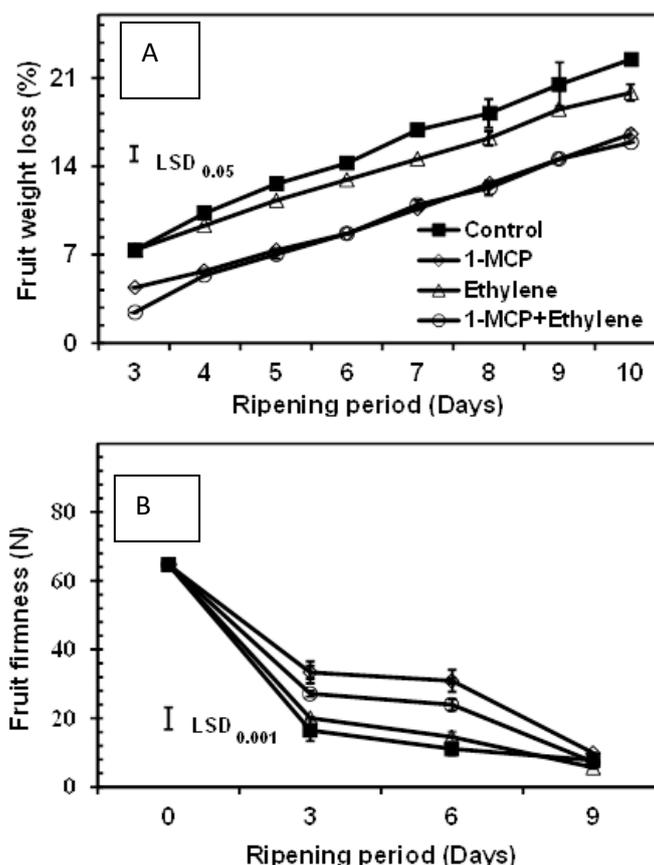

Fig 1. Ethylene production (A) and respiration rate (B) of 'Arctic Pride' nectarine fruit as influenced by exogenous application of 1-MCP and ethylene (T) and ripening period (RP) at ambient temperature. Vertical bars represent S.E. of means and are invisible when the values are smaller than the symbol. n = 3, LSD (***, ** represents significantly different at $P \leq 0.001$, 0.01 and NS= Non significant). Ethylene production: T = 30.203***, RP = 45.305***, T × RP = 90.610***; respiration rate: T = 9.8044***, RP = 14.797***, T × RP = 29.593*.

Table 2. Relationship between ethylene production, fruit firmness and various softening enzymes as affected by exogenous application of 1-MCP and ethylene in 'Arctic Pride' nectarine fruit.

Variable compared	Pearson's correlation (*r*)
Ethylene vs. Firmness	-0.5833**[a]
Ethylene vs. pectin esterase	0.7929**
Ethylene vs. *endo*-1,4-*β*-d-glucanase	0.7795**
Ethylene vs. *endo*-polygalacturonase	0.6707**
Ethylene vs. *exo*-polygalacturonase	0.7404**
Firmness vs. pectin esterase	-0.6880**
Firmness vs. *endo*-1,4-*β*-d-glucanase	-0.6420**
Firmness vs. *endo*-polygalacturonase	-0.7281**
Firmness vs. *exo*-polygalacturonase	-0.7095**

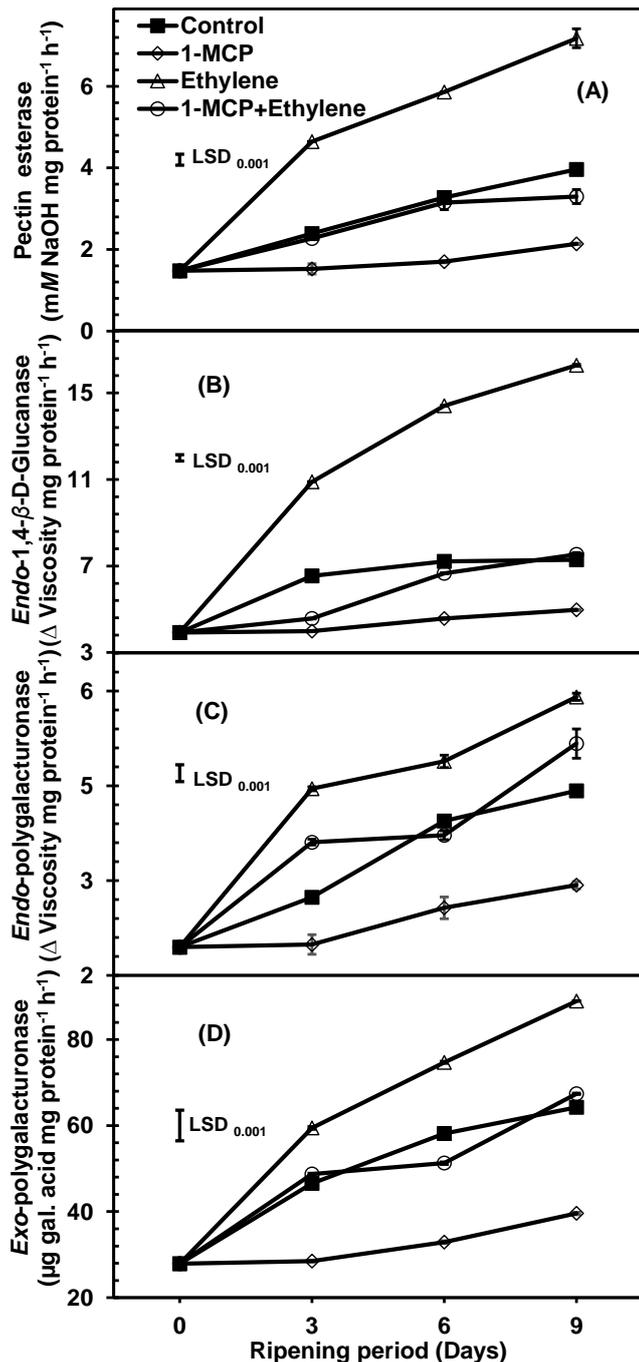[a]Significant at $P \le 0.01$.

Fig 2. Fruit weight loss (A) and fruit firmness (B) of 'Arctic Pride' nectarine fruit as influenced by exogenous application of 1-MCP and ethylene (T) and ripening period (RP) at ambient temperature. Vertical bars represent S.E. of means and are invisible when the values are smaller than the symbol. n = 3, LSD (**,* represents significantly different at $P \le 0.01$, 0.05 and NS= Non significant). Fruit weight loss: T = 0.4228**, RP = 0.5979**, T × RP = 1.1958*; Fruit firmness: T = 1.5964**, RP = 1.5964**, T × RP = 3.1928***.

of fruit ripening, about 1.7-fold and 1.5-fold higher chewiness was retained by 1-MCP-treated fruit as compared to ethylene-treated and untreated fruit, respectively (Table 1). A significant reduction in adhesiveness of nectarine fruit was noticed until day 9 of fruit ripening which was about 80% on day- 0. Adhesiveness of nectarine fruit was significantly reduced by 1-MCP treatment during ripening. About 1.3-fold and 1.2-fold more adhesiveness was found in 1-MCP-treated fruit as compared to ethylene treated and untreated fruit on day-9 of fruit ripening, respectively (Table 1). Stiffness of nectarine fruit during ripening was non-significantly affected by all the treatments (Table 1).

Changes in SSC, TA and SSC: TA ratio

Soluble solid content (SSC) of nectarine fruit juice was significantly ($P \le 0.05$) reduced by treatment with 1-MCP; however control and ethylene-treated fruit showed increased levels of SSC during fruit ripening at ambient conditions (Fig. 4A). The highest SSC was observed in ethylene-treated fruit, while the lowest SSC was recorded in 1-MCP + ethylene-treated fruit. TA and SSC: TA ratio of nectarine fruit was not significantly affected by exogenous application of 1-MCP or in combination of ethylene during ripening.

Fig 3. Activities of pectin esterase (PE) (A), endo-(1-4)-β-d-glucanase (EGase) (B), endo-polygalacturonase (endo-PG) (C) and exo-polygalacturonase (exo-PG) (D) enzymes of 'Arctic Pride' nectarine fruit as influenced by exogenous application of 1-MCP and ethylene (T) and ripening period (RP) at ambient temperature. Vertical bars represent S.E. of means and are invisible when the values are smaller than the symbol. n = 3, LSD (***, ** represents significantly different at $P \le 0.001$, 0.01 and NS= Non significant). PE activity: T = 0.1332**, RP = 0.1332**, T × RP = 0.2665***; EGase activity: T = 0.0713**, RP = 0.0713***, T × RP = 0.1427**; endo-PG activity: T =0.1331**, RP = 0.1331***, T × RP = 0.2661***; exo-PG activity: T =0.3555**, RP = 0.3555**, T × RP = 0.711***.

Relation of ethylene production, fruit firmness and fruit softening enzymes

A significant ($P \le 0.001$) negative correlation ($r = -0.5833$) was observed between ethylene production and fruit firmness as influenced by ethylene and 1-MCP treatment. Activity of PE enzyme was significantly ($P \le 0.001$) and positively correlated ($r = 0.7929$) with ethylene production; however PE was significantly ($P \le 0.001$) and negatively correlated ($r = -0.6880$) with fruit firmness of nectarine fruit. Similarly, a significant ($P \le 0.001$) positive ($r = 0.7795$) and a significant ($P \le 0.001$) negative correlation ($r = -0.6420$) was exhibited by EGase activity with ethylene and fruit firmness of nectarine fruit, respectively. The *endo*-PG and *exo*-PG activities, exhibited significant ($P \le 0.001$) positive ($r = 0.6707$ and 0.7404) correlations with ethylene, while significant ($P \le 0.001$) negative ($r = -0.7281$ and -0.7095) correlations with fruit firmness, respectively (Table 2).

Discussion

Exogenous application of 1-MCP- significantly ($P \le 0.05$) suppressed the ethylene production in nectarine fruit as compared to the untreated fruit. Reduced level of ethylene production in treated fruit is due to irreversible blockage of sites of autocatalytic ethylene production with 1-MCP-(Sisler et al., 1996). Similarly, findings of Khan and Singh (2007) in plum and Mathokoo et al. (2001) in peach support our results.

However, the climacteric rise of ethylene production was not delayed in 1-MCP-treated nectarine fruit during ripening. It is possible that fruit have the capacity to overcome the ethylene inhibition caused by 1-MCP- by synthesising new receptors (Sisler and Serek, 1997). Therefore, 1-MCP-treated fruit in study may have generated new ethylene receptors within the short time after the application of 1-MCP. On the other hand, nectarine fruit exogenously treated with ethylene showed rapid rise in ethylene peak as compared to other treatments. This may be due to the fact that different climacteric fruit show variation in response to exogenous application of ethylene. Melting and non-melting cultivars of peaches and nectarines exhibit differences in ethylene production in response to its exogenous application (Biggs et al., 1982). 1-MCP- treated fruit showed reduced respiration rate as compared to ethylene- treated and untreated fruit. As ethylene is strongly associated with respiration rate of nectarine fruit during ripening, enhanced respiration rate in ethylene-treated fruit is attributed to the role of ethylene in triggering their respiratory climacteric. Khan and Singh (2007) have also reported reduced respiration rate along with reduced level of ethylene in 1-MCP treated plum.

FWL was decreased in the fruit with 1-MCP alone and 1-MCP treated fruit exposed to ethylene treated as compared to control fruit. Reduced FWL might be due to the fact that 1-MCP suppresses respiration rate resulting in lower water loss from produce. However, results of some studies showed that application of 1-MCP did not delay the FWL as inhibition of ripening in 1-MCP treated fruit was not persistent (Liu et al., 2005) and it depends on the concentration and exposure time as reported in peaches (Hayama et al., 2005). Our results were supported by those reported by Valero et al. (2003) who also mentioned delayed FWL in 1-MCP treated plum fruit. However, contradictory findings of Fan et al. (2000) stated that there was no or limited effect of 1-MCP application on apricot FWL.

The results suggest that loss of fruit firmness in nectarine is closely related to the activities of fruit softening enzymes.
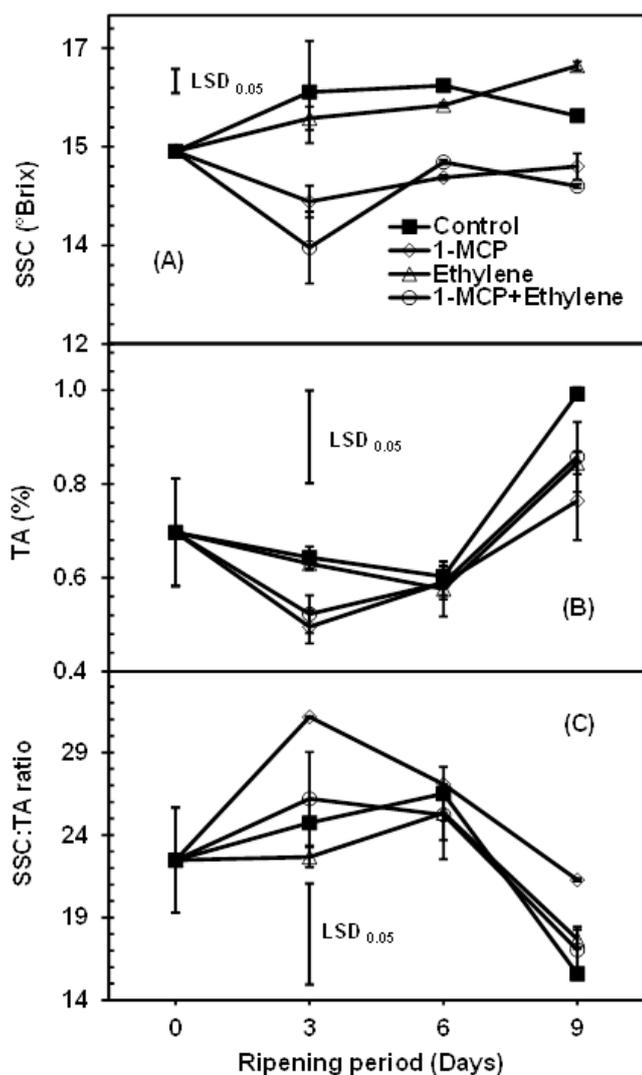
Fig 4. Change in soluble solids concentration (SSC) (A), titratable acidity (TA) (B) and SSC: TA ratio (C) of 'Arctic Pride' nectarine fruit as influenced by exogenous application of 1-MCP and ethylene treatment (T) and ripening period (RP) at ambient temperature. Vertical bars represent S.E. of means and are invisible when the values are smaller than the symbol. n = 3, LSD (**,* represents significantly different at $P \leq 0.01$, 0.05 and NS= Non significant). SSC: T = 0.0175**, RP = 0.0175**, T × RP = 0.7389*; TA: T = 0. 1482*, RP = 0. 1482*, T × RP =NS; SSC:TA: T = NS, RP = 3.051*, T × RP = NS.

Loss of fruit firmness was inversely related to activities of fruit softening enzymes. Higher the activity of fruit softening enzymes, lower was the firmness of fruit (Table 2). The results further suggested that ethylene was involved in loss of fruit firmness loss as it was concomitant with increase in ethylene production. There was a significant negative correlation between fruit firmness and fruit softening enzymes (Table 2). Fruit firmness showed a significant ($P \leq 0.001$) negative ($r = -0.5833$) correlation with ethylene during the ripening of nectarine fruit. Higher firmness recorded in 1-MCP-treated fruit may be due to inhibition of ethylene by the action of 1-MCP in nectarine fruit. Moreover, ethylene-treated fruit were softer compared to untreated fruit. Reduction in fruit firmness with the application of 1-MCP have also been reported during ripening in peaches and

nectarine (Ziosi et al., 2007), plum (Khan and Singh, 2007; 2008) and papaya (Fabi et al., 2007). Moreover, respiration rate also play an important role in maintenance of fruit firmness during storage. Similarly, Chen et al. (2011) also reported that plum fruit with reduced level of respiration rates exhibited higher firmness during cold storage.

The reduced SSC in 1-MCP- treated fruit might be due to delay in ripening of nectarine fruit as outlined by the report in plum where 1-MCP significantly delayed the rise in total soluble solids and decline in the TA during ripening and storage with lower SSC:TA ratio (Khan and Singh, 2008). 1-MCP treatment reduced the loss of springiness in nectarine fruit as compared to untreated and ethylene-treated fruit (Table 2). In addition, it might be ascribed that reduced activities of fruit softening enzymes in 1-MCP-treated fruit maintained intercellular tissue integrity and adhesiveness at higher level. It had been known that fruit softening enzymes are involved in the reduction of intercellular adhesiveness and tissue rigidity during fruit ripening (Alonso et al., 1997). Therefore less rigidity and intercellular adhesiveness in flesh tissues of untreated nectarine fruit lead to less springy fruit. Increased chewiness in 1-MCP-treated nectarine fruit might be attributed to reduced activities of fruit softening enzymes as compared to untreated fruit. Increase in fruit softening is correlated with increased activities of fruit softening enzymes such as *endo*-PG, *exo*-PG and PE during ripening (Table 2), which consequently increased the activities of these enzymes to produce higher levels of soluble pectins through pectin degradation (Kays, 1997), thus making fruit less chewable. 1-MCP treatment had been reported to reduce activities of fruit softening enzymes in plum (Khan and Singh, 2007) and peach (Ortiz et al., 2011).

The reason that more adhesion was found in 1-MCP-treated fruit than untreated and ethylene-treated fruit might be due to the reduced activities of fruit softening enzymes in response to 1-MCP application. Adhesion is the most critical factor influencing the perception of fruit texture and considered to be related with different structure of temperate fruit -melting and non-melting type of fruit (Harker et al., 1997). Most cultivars of the peaches and nectarine are classified as melting type of fruit. Activities of fruit softening enzymes are responsible for fruit softening in melting flesh peaches and nectarine. The above results suggested a strong role of ethylene in fruit tissue softening in nectarine during ripening. Similar relationship was reported in 1-MCP-treated plum fruit between ethylene and fruit softening enzymes during ripening (Khan and Singh, 2007). Multiple role of ethylene is known to regulate different ripening related process including fruit softening (Khan and Singh, 2007, 2008) and ethylene biosynthesis (Acuna et al., 2011). In our results, reduced fruit softening by 1-MCP-treated nectarine fruit might be due to reduced ethylene production and action, leading to direct reduction in the activities of fruit softening enzymes. However, 1-MCP treatment followed by ethylene exposure was not able to reduce the activities of fruit softening enzymes in nectarine as compared to the sole application of 1-MCP especially in *endo*-PG and *exo*-PG enzymes activities. The reason might be the induced competition of ethylene with 1-MCP for binding to ethylene receptors in tissue of nectarine fruit resulting in reduced ability of 1-MCP to regulate ethylene responses during ripening. Similar reports are available on tomato indicating the direct role of 1-MCP to delay fruit ripening (Zhang et al, 2009). However, findings of Fan et al. (2002) contradicts our results, as there was limited response of 1-MCP in delaying peach fruit ripening. It might be due to single or multiple factors as effectiveness of 1-MCP depends upon fruit pulp temperature

at the time of application and concentration and exposure time (Liguori et al., 2004). It has also been reported that 1-MCP is steadier in controlling fruit softening with repeated application (Liu et al., 2005).

Materials and Methods

Fruit source

Six-year-old nectarine (*Prunus persica* (L.) Batsch var. Nectarina 'Arctic Pride') trees grafted on 'Nemaguard' rootstock, planted at 2.4 m × 4.5 m in the East-West row direction, trained as a central leader, cultivated at Perth Hills (34°15'S; 116°09'E), in the South West region of Western Australia were used in the experiment. All the experimental trees received uniform commercial cultural practices and plant protection measures. During 2013, uniform sized nectarine fruit, free from visual symptoms of any disease or blemishes at commercial maturity (14.93 ± 0.4% SSC and 64.6±1.2 N firmness) were harvested from the experimental trees and transported to the laboratory immediately after harvest.

Treatments and storage

On arrival in the lab, fruit were equally divided into four lots of each 360 fruit. Each lot was further subdivided into three sub lots of 90 fruit which served as one replication. Following four treatments were used in the experiment (i) untreated control fruit, (ii) fruit treated with either 1 μL L^{-1} 1-MCP, or (iii) 10 μL L^{-1} ethylene, or (iv) 1 μL L^{-1} 1-MCP followed by 10 μL L^{-1} ethylene at 20 ± 1°C for 12 h.

The fruit were kept in a hermetically sealed plastic drum of 60 L capacity. 1-MCP concentrations (1.0 μL L^{-1}) were obtained by mixing the calculated amount of freshly prepared 1-MCP solution with ethanol in a petri dish with a filter paper according to volume of closed container. The required ethylene concentration (10 μL L^{-1} ethylene) was injected into the drums through rubber septum by using a syringe. Fruit were treated with 1-MCP for 12 h at 20 ± 1°C and were kept at ambient conditions (20 ± 1°C) with 60-65% RH. The experiment was designed as two factors (treatments and ripening period) with tree replications. In order to check, if there was any delayed climacteric ethylene production in 1-MCP-treated fruit, ethylene production and respiration rate were determined daily up to ten days of fruit ripening; whereas, the activities of fruit softening enzymes including pectin esterase (PE; EC 3.1.1.11), *endo*-1, 4-β-d-glucanase (EGase; EC3.1.1.4), *exo*-polygalacturonase (*exo*-PG; EC 3.2.1.67) and *endo*-polygalacturonase (*endo*-PG; EC 3.2.1.15) were determined in fruit pulp only on days 0, 3, 6 and 9 days after treatment (DAT) at ambient conditions.

Determination of fruit weight loss

Fruit weight loss was determined by a gravimetric method and calculated as percentage of the initial fresh weight as described by Ullah et al. (2013).

Determination of fruit ethylene production and respiration rate

Ethylene production was determined by enclosing 6 fruit from each treatment in airtight jars of 1000 mL for 1 h at 20°C. Gas samples (1.0 mL) taken from jars were injected into gas chromatograph (Agilent Technologies, 6890N netwrok GC system, Palo Alto, CA, USA) at 110°C.

Temperature of 2 m-long stainless steel supelco column (Porapack-Q 1/8″, mesh size 80/100) and a flame ionization detector were kept at 150 and 250°C, respectively. Ethylene production was expressed in μ moL kg^{-1} h^{-1} as described by Khan and Singh (2007). Respiration rate of nectarine fruit was determined on the basis of amount of CO_2 evolved following incubation, using an infrared gas analyser (Servomex, Gas Analyser, Analyser Series 1450; Servomex Ltd., East Sussex, UK). Respiration was expressed in mmole CO_2 kg^{-1} h^{-1} as reported by Khan and Singh (2007).

Determination of rheological properties

Texture analyser (TPA Plus, AMETEK Lloyd Instruments Ltd, Hampshire, UK) was used to determine rheological properties of nectarine fruit such as chewiness, springiness, hardness, stiffness, cohesiveness and adhesiveness were determined as described earlier by Razzaq et al. (2015). These properties were further defined and expressed as outlined by Bourne (1978).

Determination of SSC, TA and SSC:TA

Pulp of fruit was used to extract juice using a Mini Wizz® fruit juicer (WT 400, Breville, Sydney, Australia). SSC was determined using a digital refractometer (Atago-Palette PR 101, Atago Co. Ltd., Tokyo, Japan) and expressed as °Brix. Ten mL of freshly extracted juice was diluted with 20 mL distilled water. Titratable acidity (TA) was measured by titrating an aliquot (5.0 mL) of juice against 0.1 N NaOH solution using phenolphthalein as an indicator to a pink colour end point and expressed as % malic acid (Khan and Singh, 2010). The calculation of SSC:TA ratio was done by dividing SSC (%) with the corresponding TA (%).

Determination of fruit softening enzymes

To determine activity of softening enzymes fruit pulp samples were frozen in liquid nitrogen (-196 °C) and stored in ultra-low freezer (-86 °C ULT, Thermo Fischer Scientific, Australia) at -80°C until further analysis. For enzyme extraction nectarine frozen fruit flesh (13 g) was blended in a precooled pestle and mortar with 13 mL cold 12% polyethylene-glycol (PEG) and 0.2% sodium bisulfite with 400 mg white quartz sand until a homogenous mixture was obtained. The homogenate was centrifuged in a refrigerated centrifuge (Eppendorf 5810R, Hamburg, Germany) for 20 min at 13000 × g and the pellet washed with 4°C aqueous 0.2% sodium bisulfite. Pellets (13 g) extracted for each softening enzymes including PE, *endo*-PG, *exo*- PG, and EGase were stored in ultra-low freezer (-86 °C ULT, Thermo Fischer Scientific, Australia) at -80 °C until further analysis. The method outlined by Khan and Singh (2007) was used for the determination of *exo*-PG and *endo*-PG, PE and EGase activities and were expressed as μg of galacturonic acid mg protein^{-1} h^{-1}, viscosity changes in mg protein^{-1} h^{-1} mM NaOH mg protein^{-1} h^{-1} and viscosity changes mg protein^{-1} h^{-1}, respectively. Protein content from fruit pulp tissue were estimated using the method of Bradford (1976) and were expressed as mg protein mL^{-1} of enzyme extract.

Statistical analysis

The data were analysed by analysis of variance (ANOVA) using GenStat Release 13 (VSN International Ltd., Hemel Hempstead, UK). The treatment effects on various parameters were assessed within ANOVA and the least

significant differences (LSD) were calculated following significant F-test at $P \leq 0.05$. Relationship between fruit firmness, ethylene production and fruit softening enzymes were also determined through Pearson correction using same software at $P \leq 0.05$.

Conclusion

1-MCP application alone or 1-MCP treated fruit exposed to ethylene resulted in significantly reduced ethylene production, reduced activities of softening enzymes, fruit softening, and maintained quality of nectarine fruit during ripening at ambient temperature.

Acknowledgments

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