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Suppression of ethylene perception after exposure to cooling conditions delays the progress of softening in 'Hayward' kiwifruit

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ABSTRACT

To investigate the physiological effect of ethylene and 1-methylcyclopropene (1-MCP) in the progress of 'Hayward' kiwifruit softening, fruit were treated with ethylene or 1-MCP right after harvest, or with 1-MCP at different ripening stages after 40, 80 or 120 d of cold (0 °C) storage. Treatment with ethylene right after harvest stimulated flesh softening, increased and advanced the ethylene production peak and the expression of the genes *KWACS1* and *KWACO1* involved in ethylene biosynthesis. In contrast, treatment with 1-MCP at the same stage markedly retarded softening, and inhibited ethylene production. Ripening-related increases in *KWACS1* and *KWACO1* transcript abundance were largely blocked by 1-MCP treatment thus indicating that these genes are positively regulated by ethylene. Nevertheless, ethylene and 1-MCP effect was highly dependent on the growing region that may influence kiwifruit constitution, quality, storage potential and subsequent responsiveness to postharvest technologies. Kiwifruit stored for 40, 80 or 120 d of cold storage and then treated with 1-MCP before rewarming to 20 °C for further ripening displayed a reduced flesh softening rate and an extended 'eating ripe' stage. These results clearly indicate that the application of 1-MCP can play a significant role in both the initiation and progress of the kiwifruit softening process. 1-MCP inhibited or severely decreased autocatalytic ethylene production at every ripening stage. *KWACS1* and *KWACO1* gene transcription was inhibited by 1-MCP treatment after 40 and 80 d of cold storage thus suggesting that there is a positive feedback regulation for ethylene production even after cold storage. In contrast, *KWACS2* transcript levels did not show a clear response to 1-MCP in every experiment. This work provides evidence that kiwifruit softening can be delayed by inhibiting ethylene perception, even when fruit have reached advanced stages of ripening. These findings point to the commercial usefulness of 1-MCP in 'Hayward' kiwifruit postharvest conservation and consumer acceptance.

1. Introduction

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var *deliciosa*] is a highly nutritious oblong berry, rich in vitamin C, Ca, K, Fe, Mg, amino acids, dietary fiber and antimutagenic compounds. In addition to its nutritive value, consumers buy kiwifruit based on appearance and textural quality. Their satisfaction and repeat purchases depend on flavor quality factors, mainly soluble solid concentrations (SSC) (Crisosto and Crisosto, 2001). In contrast, growers and handlers are concerned first with long storage and shelf-life along with appearance and textural quality. In fact, grading, packaging, transport and expenditure require kiwifruit resistant to physical damage. The 18-N threshold is the minimum kiwifruit firmness to avoid physical damage during standard postharvest handling when transferred to outlets (Crisosto et al., 1997). Kiwifruit between 13 and 18 N are considered as 'ready-to-buy' since they begin to yield to palm pressure. Finally, fruit between 4 and 13 N are usually regarded as 'ready-to-eat' according to different previously established firmness criteria (Stec et al., 1989; Crisosto and Crisosto, 2001), although those fruit ranging from 8 to 10 N may be considered optimum for consumer acceptance. Development of new postharvest storage protocols for kiwifruit should focus on the importance of delaying softening to extend kiwifruit postharvest life as well as ensuring good texture and flavor, major consumer acceptance issues.

Ethylene, a gaseous plant hormone responsible for climacteric fruit ripening, does not trigger any visible change in kiwifruit skin color or appearance but plays a major role in flesh softening. Kiwifruit is highly sensitive to ethylene and concentrations of this plant hormone of 0.1 $\mu L\,L^{-1}$ (McDonald and Harman, 1982) or even

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lower (Saltveit, 1999) are sufficient to induce premature ripening thus precluding long term storage and subsequent shelf-life. Techniques reducing ethylene responses in kiwifruit postharvest life are particularly valuable thus allowing some control over degradative processes associated with ripening and senescence. Cultural techniques that minimize the effects of ethylene on kiwifruit include temperature management (with a recommended range of 0–2 °C; Kader, 2002) and controlled atmospheres with 1 kPa O_2 and up to 7 kPa CO_2 (Mir and Beaudry, 2002). Kiwifruit has an excellent potential to respond to controlled atmospheres, which can reduce both the rate of flesh softening and *Botrytis* rot incidence. Nonetheless, there is little or no benefit if delays in establishing a controlled atmosphere exceed 7–10 d. Also, utilization of this technology may not be possible under every marketing condition.

In 2002, the strained alkene 1-methylcyclopropene (1-MCP, marketed as the compound SmartFresh™) was approved for use in the United States on kiwifruit, among other fruit crops (Warner et al., 2003). 1-MCP is a potent inhibitor of ethylene responses in many horticultural species (Blankenship and Dole, 2003; Watkins, 2006). When appropriate, 1-MCP may supplement proper postharvest management and result in kiwifruit decreased and delayed ethylene production, slowed down respiration rate and delayed pulp color change and softening (Kim et al., 2001; Fan and Zhang, 2001; Ding et al., 2003; Boquete et al., 2004; Koukounaras and Sfakiotakis, 2007). These benefits may translate into reduced quantitative and qualitative losses during postharvest handling and storage.

Climacteric tree-fruit crops respond to 1-MCP applications to various extents (Blankenship and Dole, 2003; Watkins, 2006; Sozzi and Beaudry, 2007). The influence of time from harvest to 1-MCP treatment varies with the crop species and advancing maturity and ripeness of fruit usually decreases the effect of 1-MCP (Harris et al., 2000; Trinchero et al., 2004; Kurahashi et al., 2005; Gutierrez et al., 2008). Nevertheless, softening of some climacteric fruit species such as tomato has been reported to be slowed down by 1-MCP at different ripening stages (Hoeberichts et al., 2002), and application of 1-MCP to ripe tomatoes results in an increase in postharvest life (Wills and Ku, 2002). The ethylene biosynthetic enzymes 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) and associated mRNA accumulation are inhibited by 1-MCP in various climacteric fruit species. Reduced ethylene production of 1-MCP-treated turning and pink tomatoes was accompanied to a large extent by inhibited *LE-ACS2*, *LE-ACS4*, *LE-ACO1* and *LE-ACO4* transcript accumulation while several other *ACS* genes were not affected (Nakatsuka et al., 1998). The degree to which the ripening stage influences the responsiveness to 1-MCP has not been totally elucidated in all species and cultivars.

In a previous study, we showed that 1-MCP application in kiwifruit is effective even after 30 d of cold storage, when the fruit are preclimacteric in terms of ethylene production (Boquete et al., 2004). However, the time that kiwifruit can be stored at low temperature and still elicit a response to 1-MCP has not been determined. The objective of this study was to evaluate the effects of 1-MCP on ethylene production and firmness of 'Hayward' kiwifruit previously exposed to 0, 40, 80 or 120 d of cold (0 °C) storage. ACS and ACO gene expression was also assessed.

2. Materials and methods

2.1. Plant materials

During the 2007 season, commercially mature 'Hayward' kiwifruit [mean weight = 102 g; mean SSC = 7%] were harvested from orchards located in two growing regions: Mar del Sur (38°19′S 57°59′W) and Baradero (33°49′S 59°24′W), province of Buenos Aires, Argentina. The distance between both orchards is approximately 517 km. Fruit from Mar del Sur had a mean firmness of 88.5 N while those from Baradero had 87 N. Kiwifruit were held at ambient temperature for 24 h after harvest so that the stem scar developed a physical barrier to *Botrytis cinerea* (Pennycook and Manning, 1992), and then kept at 20 ± 2 °C, or forced-air cooled to its storage temperature and held at 0 ± 0.5 °C and 90% RH for 40, 80 or 120 d. After treatment with 1-MCP (see below), kiwifruit were transferred to a room at 20 ± 2 °C and kept there for 28 d.

2.2. Experiment 1: application of 1-MCP or ethylene and subsequent storage at 20 °C

Fruit free from decay and defects were randomized to provide three 100-fruit experimental units per treatment and growing region. Each 100-fruit unit was placed in a 200 L stainless steel container.

The experimental units were kept as untreated controls or treated with 1 μL L^{-1} 1-MCP or 10 μL L^{-1} ethylene for 24 h. 1-MCP was released from 50-mL capped test vials containing weighed amounts of SmartFresh™ powder (0.14% active ingredient, Rohm and Haas, Argentina) by adding warm water (40 °C) through a septum. Each vial was vortexed and placed in the container. The vial was then opened followed by placement of the container lid within 10 s. The lid of each container was also taped to ensure a tight seal. The atmosphere in the container was circulated through a commercial CO_2 trapping system containing pellets of NaOH (CO_2 scrubber) as previously described (Boquete et al., 2004) to reduce CO_2 accumulation. The 1-MCP concentration was verified by means of gas chromatography using isobutylene as standard (Boquete et al., 2004). Ethylene-treated experimental units were placed in containers and exposed to a gaseous mixture of 10 μL L^{-1} ethylene in humidified air using a constant flow-through gas system, as previously described (Sozzi et al., 1999). Control fruit were kept in 200 L sealed containers having neither 1-MCP nor ethylene. After 24 h at 20 ± 2 °C, the containers were vented. The experimental units were then stored in air at 20 °C and 90% RH.

2.3. Experiment 2: application of 1-MCP after 40, 80 or 120 d storage at 0 °C

The experimental units, similar to those of experiment 1, were held at 0 ± 0.5 °C and 90% RH for 40, 80 or 120 d and then treated or not with 1 μL L^{-1} 1-MCP at 0 °C, following the previously described protocol. After that, kiwifruit were transferred to a room at 20 ± 2 °C for further ripening until used.

2.4. Experiment 3: application of 1-MCP after both 40 and 80 d storage at 0 °C

The experimental units, similar to those of experiment 1, were held at 0 ± 0.5 °C and 90% RH for 80 d. 1-MCP-treated fruit were exposed to 1 μL L^{-1} 1-MCP at 0 °C after 40 d storage. Following 1-MCP treatment, fruit were kept at 0 °C for another 40 d and treated once again with 1 μL L^{-1} 1-MCP at 0 °C. Then, fruit were transferred to a room at 20 ± 2 °C until used.

2.5. Ethylene and firmness assessment

Ethylene production was determined by introducing each fruit into a 1.5 L glass container which was in turn tightly sealed with a silicon septum. One milliliter of the head-space gas was extracted after 1 h and ethylene was quantified on a gas chromatograph (Hewlett Packard 5890 Series II) fitted with a FID and a stainless steel Porapak N column (3.2 mm × 2 m; 80/100 mesh) (Boquete et al., 2004). The injector, oven and detector temperatures were 110,

Table 1
Primers for amplification of cDNAs by RT-PCR.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Amplified fragment
KWACS1	CACCAATGACAAAAACATCCA	TTTACGAGCACACGAAGTCA	192 bp
KWACS2	ATTTACGCTGCCACGGTCT	GAAGCCCAAGTCCTTTGAGA	226 bp
KWACO	AGAGGCTGTTCAGTCCGAAA	TCTCTAGCCCCAGGTTCTCA	197 bp
AdAct	TGGCCGTGATCTAACAGATG	CTGAGGAGCTGCTCTTTGCT	164 bp

90, and 250 °C respectively. N_2 was used as the carrier gas at a flow rate of 0.37 mL s^{-1} (linear gas velocity = 4.5 cm s^{-1}). Ten independent replicates per unit, three units per treatment and date were evaluated.

Whole fruit firmness (flesh rupture force) was determined with an Instron Universal Testing Machine (Model 4442, Canton, MA) (Sozzi et al., 2003). Each fruit was placed on a stationary steel plate. On removal of the skin, two spots located on opposite sides of the fruit were punctured to a depth of 10 mm. Puncture tests involved the use of a 7.9-mm probe on a drill base with a crosshead setting of 0.83 mm s^{-1}. Ten independent replicates per unit, three units per treatment and date were evaluated.

2.6. RT-PCR

After ethylene and firmness assessment, semi-quantitative RT-PCR reactions were conducted using the same kiwifruit from Mar del Sur. Three-millimeter thick outer cortex and core pieces obtained from the equatorial region of each fruit were sampled and the two tissue types separately bulked for the ten fruit, immediately frozen in liquid nitrogen and stored at −80 °C for RNA analysis. Transcript accumulation of two ACS genes [*KWACS1* (GenBank accession no. AB005722) and *KWACS2* (GenBank accession no. AB005723)] and one ACO gene [*KWACO1* (GenBank accession no. AB003514)] were estimated through RT-PCR reactions. Polyadenylated RNA was extracted from kiwifruit outer cortex and core using the mRNA Isolation Kit (Roche Molecular Biochemicals, GmbH, Mannheim, Germany) and cDNA was made up with at least 10 ng of mRNA sample by means of Revert Aid™ M-Mul V Reverse transcriptase system (Fermentas International Inc., Burlington, Ontario, Canada). mRNA concentration was quantified by measuring absorbance at 260 nm with an Agilent 8453 UV-Vis diode array spectrophotometer (Agilent Technologies, Inc., Santa Clara, CA, USA). The specific primers used are described in Table 1.

PCR conditions for each primer pair were optimized empirically to determine the linear range of amplification. PCR conditions for *KWACS1* were as follows: 4 min at 95 °C (first cycle); 45 s at 95 °C, 1 min at 56 °C and 1 min at 72 °C (35 cycles); and 7 min at 72 °C (last cycle). PCR conditions for *KWACS2* were as follows: 4 min at 95 °C (first cycle); 45 s at 95 °C, 1 min at 52 °C and 1 min at 72 °C (35 cycles); and 7 min at 72 °C (last cycle). PCR conditions for *KWACO1* were as follows: 4 min at 95 °C (first cycle); 45 s at 95 °C, 1 min at 52 °C and 1 min at 72 °C (25 cycles); and 7 min at 72 °C (last cycle). Actin was selected as a housekeeping gene, as it was found to be expressed at constant levels throughout fruit development and ripening (Atkinson et al., 2009). Actin (GenBank accession no. ABG74899) mRNA levels were utilized as internal controls to normalize the amount of starting template. PCR conditions were 4 min at 95 °C; 45 s at 95 °C, 45 s at 56 °C and 1 min at 72 °C (25 cycles); and 7 min at 72 °C (last cycle).

PCR products were separated on 1.5% agarose gels, stained with SYBR Green (Invitrogen, Carlsbad, CA, USA) and visualized by the UVP Doc-It LS Image Acquisition Software. A 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) was used as standard molecular marker. Each gel is representative of several replications. Amplified fragments were cloned into the pGEM®-T Easy vector system (Promega Corp., Madison, WI, USA).

2.7. Statistical analysis

For ethylene and firmness, results were expressed as the mean ± SD of three 10-fruit independent samples. Statistical significance was determined by one-way ANOVA using the PC-SAS software package (SAS Institute Inc., Cary, NC). In experiment 1, statistical significance was also determined between equally treated fruit harvested from different growing regions. The model assumptions of homogeneity of variance and normality were probed using the Levene's test and the Shapiro–Wilk's test, respectively. When these assumptions were not satisfied, data were transformed into ranks (Conover and Iman, 1981) for further analysis. When a significant *F*-value was found, treatment means were compared using the Tukey's test ($p < 0.05$) within each sampling date.

3. Results

3.1. Experiment 1: application of 1-MCP and subsequent storage at 20 °C

Kiwifruit from the growing regions of Baradero and Mar del Sur were harvested at the same maturation stage but their ripening behavior after harvest was different. Control kiwifruit from Mar del Sur reached the peak of ethylene production on day 24 (Fig. 1a) while kiwifruit from Baradero showed the ethylene peak on day 14 (Fig. 1c). Ethylene treatment advanced the onset of the climacteric, autocatalytic ethylene production by 10 d in kiwifruit from Mar del Sur (Fig. 1a) and by 6 d in kiwifruit from Baradero (Fig. 1c). In addition, ethylene treatment increased the magnitude of the climacteric peak 3.4-fold in comparison with that of the control in fruit from Mar del Sur but almost 6-fold in fruit from Baradero. These facts point to the dissimilar perecibility of kiwifruit harvested from different growing regions. As expected, ethylene treatment accelerated the normal progress of softening, a ripening-related event. Softening of kiwifruit harvested from both growing regions was greatly enhanced by ethylene but fruit from Baradero softened at a much higher rate (Fig. 1b and d). Fruit from Mar del Sur were found to be inappropriate for consumption after 21 d, but major deterioration was observed in fruit from Baradero 8 d after ethylene treatment.

Treatment with 1 μL L^{-1} 1-MCP inhibited kiwifruit ethylene production throughout the experiment regardless the growing region (Fig. 1a and c) and greatly delayed firmness loss (Fig. 1b and d). While fruit from Baradero was considered 'eating ripe' after 28 d, fruit from Mar del Sur did not reach an 'eating ripe' stage throughout the experimental period. No differences were detected between kiwifruit harvested from different orchards in Mar del Sur (data not shown).

RT-PCR analysis was performed to determine transcript accumulation of ethylene synthesis-related genes in two specific tissues (outer cortex and core) in kiwifruit from Mar del Sur (Fig. 2). The transcript abundance of *KWACS1* and *KWACO1* increased concomitantly with autocatalytic ethylene production in control fruit to reach a maximum on day 24, both in the outer cortex and in the core. *KWACS1* transcript levels were substantially greater in the outer cortex than in the core. *KWACS1* and *KWACO1* were highly responsive to the ethylene treatment. *KWACO1* transcript levels

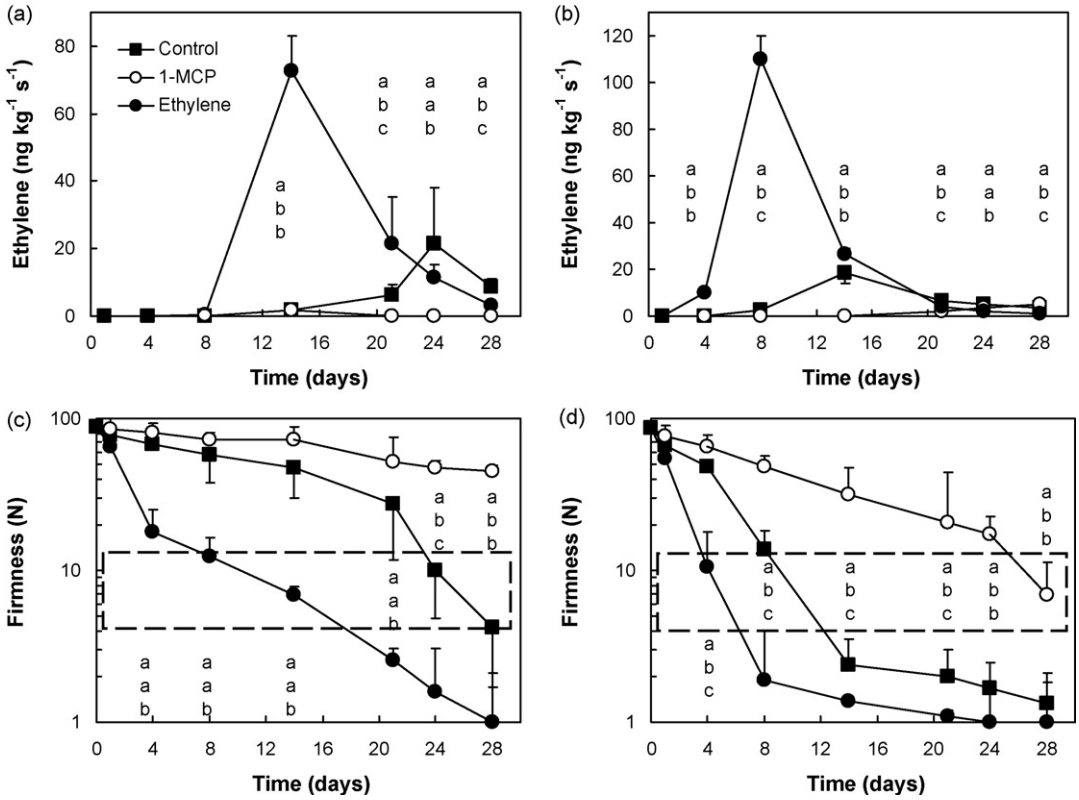

Fig. 1. Ethylene production (a and b) and fruit firmness (c and d) in kiwifruit harvested from two growing regions, Mar del Sur (a and c) and Baradero (b and d). Kiwifruit treated with 10 μL L^{-1} ethylene (Ethylene) or 1 μL L^{-1} 1-methylcyclopropene (1-MCP) and untreated (Control) kiwifruit were stored at 20 °C for 28 d. Values represent the means ± SD of three 10-fruit replicates. Where bars are not shown, the SD does not exceed the size of the symbol. Means with a different letter are significantly different within a single date according to the Tukey's test ($p < 0.05$). Dashed rectangles (c and d) include kiwifruit regarded as 'eating ripe' according to their firmness values (~4–13 N).

showed a transient increase in outer cortex 1 d after the ethylene treatment and rose significantly in the outer cortex and core on day 14. *KWACS1* transcripts accumulated on days 8 and 14 in the outer cortex, but only on day 14 in the core. *KWACS2* was the most consistently expressed gene throughout kiwifruit ripening (Fig. 2). No relationship could be identified between the *KWACS2* expression pattern and the autocatalytic ethylene synthesis, but *KWACS2* transcript levels were relatively abundant in the outer cortex from

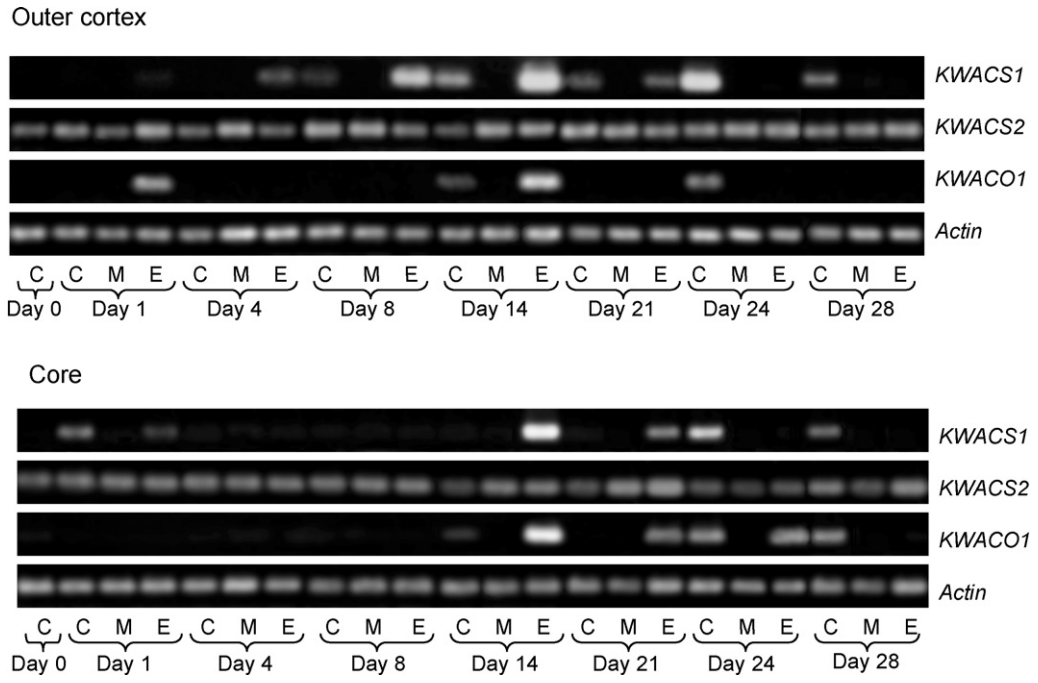

Fig. 2. *KWACS1*, *KWACS2* and *KWACO1* transcript accumulation in two different tissues (outer cortex and core) from Mar del Sur kiwifruit. Control fruit (C) and kiwifruit treated with 10 μL L^{-1} ethylene (E) or 1 μL L^{-1} 1-methylcyclopropene (M) were stored at 20 °C for 28 d. Actin was used to evaluate equal loading.

1-MCP-treated kiwifruit from day 4 through day 28. In contrast, the direct association of *KWACS1* and *KWACO1* gene expression with ethylene biosynthesis was confirmed by 1-MCP treatment. Neither the *KWACS1* nor *KWACO1* gene expression was detectable in 1-MCP-treated fruit throughout the experiment regardless the tissue considered. 1-MCP also inhibited the transient increase in *KWACS1* transcripts found in control fruit core on day 1 (Fig. 2).

3.2. Experiment 2: application of 1-MCP after 40, 80 or 120 d storage at 0 °C and subsequent storage at 20 °C

After 40 d of cold storage, control kiwifruit from Mar del Sur required 12 d at 20 °C before apparent autocatalytic ethylene production began, and reached a peak on day 18 (Fig. 3a). Nonetheless, control kiwifruit showed 0.48 ± 0.3 ng kg^{-1} s^{-1} after 1 d at 20 °C, and 1.25 ± 0.8 ng kg^{-1} s^{-1} after 5 d. In contrast, 1-MCP-treated fruit only displayed basal levels of ethylene production: 0.32 ± 0.5 ng kg^{-1} s^{-1} on day 1, 0.2 ± 0.4 ng kg^{-1} s^{-1} on day 5, and a maximum of 0.52 ± 0.4 ng kg^{-1} s^{-1} on day 18 (Fig. 3a).

Control fruit softened at a higher rate than 1-MCP-treated fruit and showed lower firmness values throughout the experiment (Fig. 3b). Control fruit reached an 'eating ripe' stage after 4 d while 1-MCP-treated fruit was considered 'eating ripe' after ~18–22 d (Fig. 3b).

KWACS2 transcripts were found at a more abundant level in the outer cortex than in the core (Fig. 3c). Contrasting with results of experiment 1, a higher *KWACS2* transcript accumulation was found in control fruit on days 15 and 18 along with the ethylene production peak. *KWACS1* was strongly expressed in control fruit on day 18, both in the outer cortex and the core, and more slightly in the core on days 1 and 22 and in the outer cortex on day 15 (Fig. 3c). *KWACO1* had a peak in expression in the outer cortex and core on day 18 (Fig. 3c). As in experiment 1, the association of *KWACS1* and *KWACO1* with ethylene perception was confirmed by the 1-MCP treatment which inhibited their expression on every date tested, both in the outer cortex and in the core (Fig. 3c).

After 80 or 120 d of cold storage, control kiwifruit from Mar del Sur showed an increased ethylene production by days 9 and 5 respectively, and reached the highest values on days 18 and 9 (Fig. 4a and b). 1-MCP applied at the end of cold storage restrained autocatalytic ethylene production throughout the experiment.

Control fruit firmness declined rapidly after 80 d of cold storage, dropping from 11 to 4 N in 18 d while 1-MCP-treated fruit softened at a significantly lower rate, from 11 to 6 N in 28 d (Fig. 4c). The decline in control fruit firmness was very rapid after 120 d of cold storage, the kiwifruit showed to be severely deteriorated after 5 d (Fig. 4d). On the other hand, 1-MCP-treated fruit softened gradually and were 'eating ripe' for 15 d.

3.3. Experiment 3: application of 1-MCP after both 40 and 80 d storage at 0 °C and subsequent storage at 20 °C

When 1-MCP was applied twice, after 40 and 80 d of cold storage, kiwifruit displayed far lower ethylene production than control fruit, although mean levels of up to 2.8 ng kg^{-1} s^{-1} were detected on day 18 (Fig. 5a). On day 0, firmness mean values for 1-MCP-treated fruit were higher in this experiment (Fig. 5b) than in the event kiwifruit was treated with a single dose (Fig. 4c). Nevertheless, softening rate was similar in both experiments. In any case, 1-MCP-treated fruit displayed higher firmness values than control fruit, particularly on days 18 through 28 (Fig. 5b).

In the outer cortex of control fruit, *KWACS1* transcripts were found on days 1 and 15 (Fig. 5c), but not on day 18 when maximum ethylene production was measured. In control core, *KWACS1* revealed a transcript accumulation on days 18 and 22. The outer cortex and the core showed relatively low levels in *KWACS2* on day

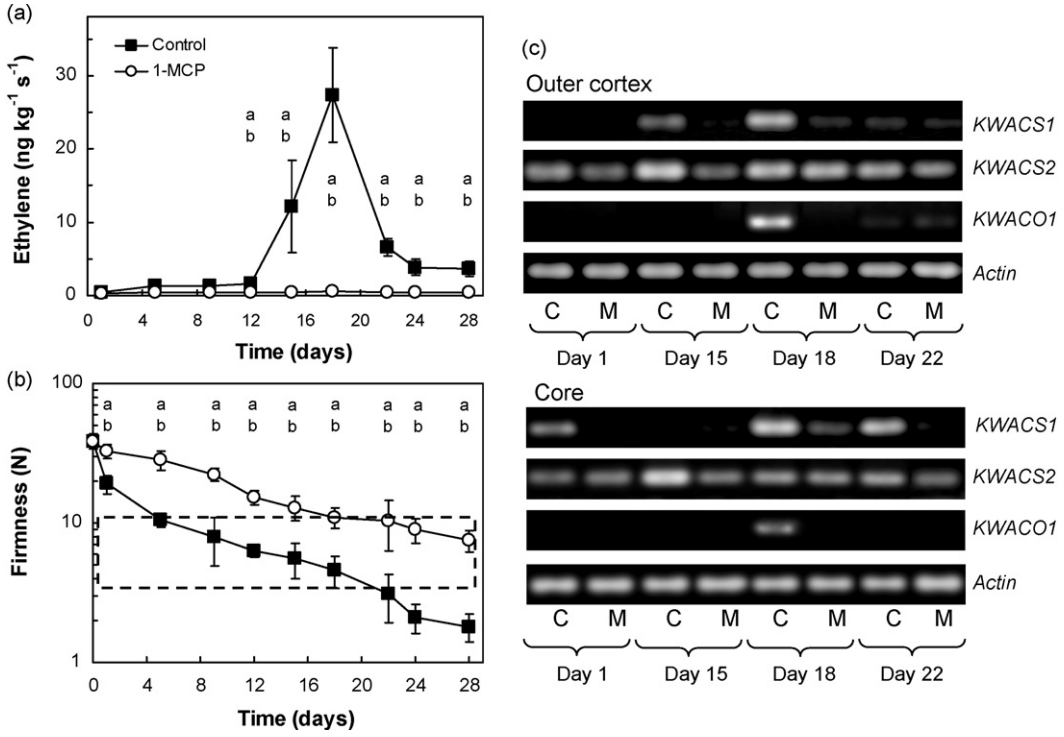

Fig. 3. Ethylene production (a) and fruit firmness (b) in kiwifruit stored at 0 °C for 40 d and then treated (1-MCP) or not (Control) with 1 μL L^{-1} 1-methylcyclopropene. After treatment, kiwifruit were transferred to a room at 20 ± 2 °C until used. Values represent the means ± SD of three 10-fruit replicates. Where bars are not shown the SD does not exceed the size of the symbol. Means with a different letter are significantly different within a single date according to the Tukey's test ($p < 0.05$). Dashed rectangles in (b) include kiwifruit regarded as 'eating ripe' according to their firmness values (~4–13 N). (c) *KWACS1*, *KWACS2* and *KWACO1* transcript accumulation in the outer cortex and core of control kiwifruit (C) and kiwifruit treated with 1 μL L^{-1} 1-methylcyclopropene (M) as previously described. Actin was used to evaluate equal loading.

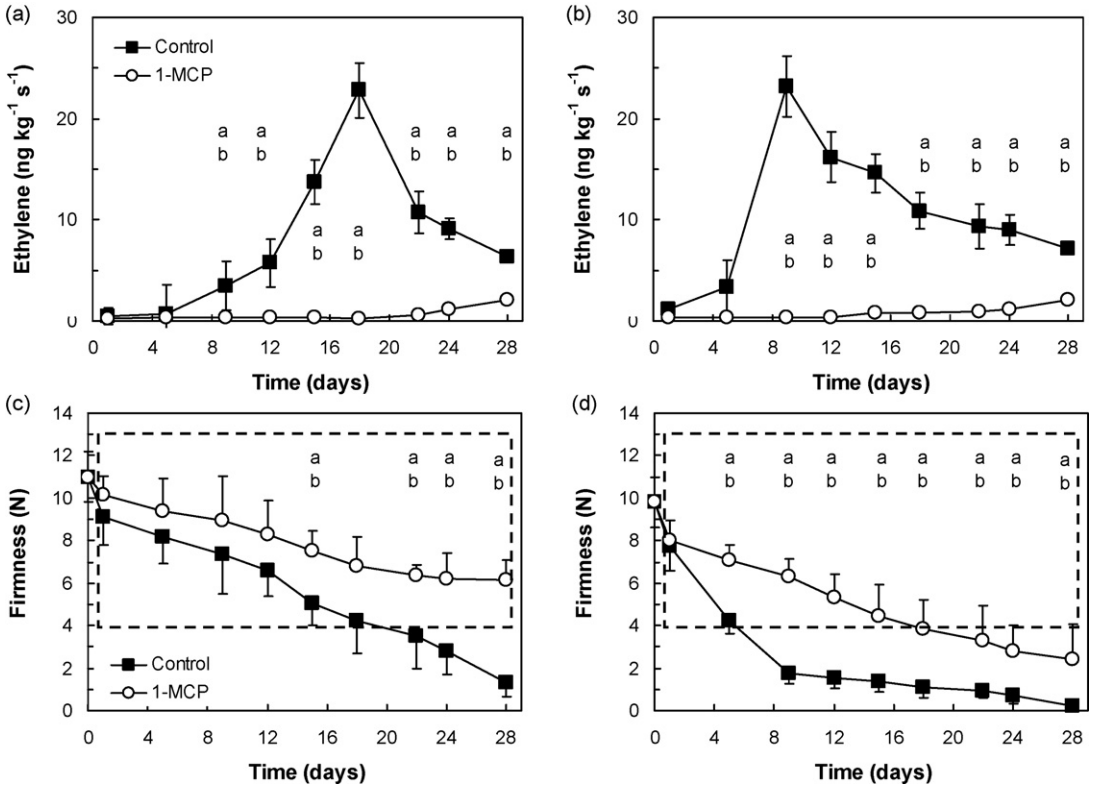

Fig. 4. Ethylene production (a and b) and fruit firmness (c and d) in kiwifruit stored at 0 °C for 80 d (a and c) or 120 d (b and d) and then treated (1-MCP) or not (Control) with 1 μL L^{-1} 1-methylcyclopropene. After treatment, kiwifruit were transferred to a room at 20 ± 2 °C until used. Values represent the means ± SD of three 10-fruit replicates. Where bars are not shown, the SD does not exceed the size of the symbol. Means with a different letter are significantly different within a single date according to the Tukey's test ($p < 0.05$). Dashed rectangles in (c) and (d) include kiwifruit regarded as 'eating ripe' according to their firmness values (~4–13 N).

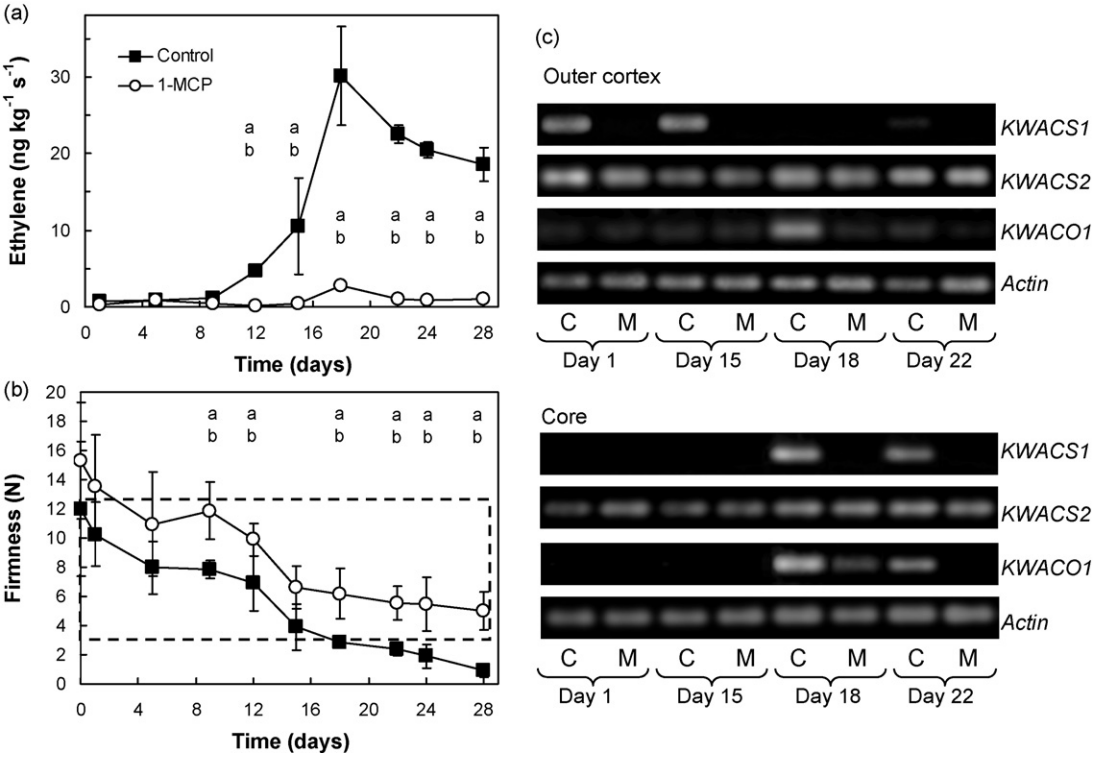

Fig. 5. Ethylene production (a) and fruit firmness (b) in kiwifruit stored at 0 °C for 80 d. Fruit were treated (1-MCP) or not (Control) with 1 μL L^{-1} 1-methylcyclopropene after 40 and 80 d of cold storage. After that, kiwifruit were transferred to a room at 20 ± 2 °C for further ripening until used. Values represent the means ± SD of three 10-fruit replicates. Where bars are not shown, the SD does not exceed the size of the symbol. Means with a different letter are significantly different within a single date according to the Tukey's test ($p < 0.05$). Dashed rectangles in (b) include kiwifruit regarded as 'eating ripe' according to their firmness values (~4–13 N). (c) *KWACS1*, *KWACS2* and *KWACO1* transcript accumulation in the outer cortex and core of control kiwifruit (C) and kiwifruit treated with 1 μL L^{-1} 1-methylcyclopropene (M), as previously described. Actin was used to evaluate equal loading.

15, both in control and in 1-MCP-treated fruit. Accumulation of *KWACO1* gene transcripts was detected in control outer cortex on day 18 and in control core on days 18 and 22. *KWACS1* and *KWACO1* transcripts did not accumulate in 1-MCP-treated fruit (Fig. 5c).

4. Discussion

At harvest, fruit from Baradero and Mar del Sur had similar firmness values and SSC but control fruit from Baradero ripened and softened more rapidly than that from Mar del Sur (Fig. 1). Applied ethylene shortened the postharvest life of Baradero kiwifruit more severely than that of Mar del Sur (Fig. 1c and d). When statistical analysis was performed to compare autocatalytic ethylene production of ethylene-treated fruit from those locations (data not shown), major differences were found, both in timing and magnitude of the ethylene peak, with Baradero kiwifruit showing an earlier peak and a higher ethylene production rate (Fig. 1a and b). 1-MCP inhibited ethylene production in fruit from both locations but was less effective in preventing kiwifruit softening in Baradero fruit, which softened to an 'eating ripe' stage by day 28 (Fig. 1d). 1-MCP brought about an excessive postponement of softening in Mar del Sur kiwifruit which reached 45 N at the end of the experiment. In previous experiments, application of 0.5 $\mu L\,L^{-1}$ 1-MCP slowed down kiwifruit softening with only a small decrease from 85 to 61.3 N after 25 d at 20 °C (Yin et al., 2008). Major differences between kiwifruit from two orchards have been reported in total starch concentration, in the pattern of change in starch in the core, and in the sugar/starch and sugar/acid ratios (MacRae et al., 1989). Preharvest conditions may lead to different composition and ripening rates which in turn can be possible causes for fruit response variability to 1-MCP (Sozzi and Beaudry, 2007). The fact that responsiveness to 1-MCP or other postharvest technologies may vary from one location to another can be related to soil characteristics, preharvest meteorological conditions and/or practices that determine fruit constitution and quality, ethylene production and subsequent storage potential. This research confirms that orchard location can modify 1-MCP effectiveness in controlling ethylene action in 'Hayward' kiwifruit and that dissimilar responses to 1-MCP may be expected during the postharvest period.

Koukounaras and Sfakiotakis (2007) demonstrated that 1-MCP application before cold storage delays kiwifruit softening for 14 d during shelf-life at 20 °C after short (4 weeks) and medium term (10 weeks) cold storage. Also, 1-MCP-treated fruit showed higher firmness values during long term (20 weeks) cold storage though no differences were detected during shelf-life at 20 °C. Boquete et al. (2004) provided evidence that kiwifruit treated with 1-MCP after 30 d of cold storage and then held at 20 °C softened at a lower rate than control fruit. However, the length of time kiwifruit can be stored at low temperature and still elicit a response to 1-MCP was not clear (Kim et al., 2001). In our work, 1-MCP applied just before removal from short (40 d), medium (80 d) or long term (120 d) cold (0 °C) storage proved to be still effective to delay softening though the performance of 1-MCP in retarding firmness loss decreased as cold storage duration increased. Nonetheless, 1-MCP may very well complement cold storage, since it has the potential to extend kiwifruit postharvest life within the limits of consumer acceptability. For example, kiwifruit exposed to 80 d of cold storage and subsequently treated with 1-MCP prior to rewarming to 20 °C softened gradually during ripening and were 'eating ripe' throughout the 28 d shelf-life (Fig. 4c). Additional evidence supports the fact that suppression of ethylene perception delays the progress of softening in 'Hayward' kiwifruit at different ripening stages. Exposure of partially ripe (15–20 N) fresh-cut kiwifruit to 1 $\mu L\,L^{-1}$ 1-MCP for 6 h at 10 °C effectively slowed down its softening rate during storage for 7 d at 5 °C, whether 1-MCP was applied before or after processing (Vilas-Boas and Kader, 2007). Kiwifruit shows a good response to 1-MCP under cooling conditions (Boquete et al., 2004; Koukounaras and Sfakiotakis, 2007; Vilas-Boas and Kader, 2007) but 1-MCP has also been applied at 20 °C in other laboratory-based experiments (Kim et al., 2001). In our work, kiwifruit stored for short, medium or long term cold storage were exposed to 1-MCP prior to rewarming to 20 °C for further ripening and this may partially explain the different results obtained. Alternatively, responsiveness to 1-MCP may also vary according to fruit characteristics.

Control 'Hayward' kiwifruit shows a climacteric behavior by starting autocatalysis of ethylene synthesis approximately 19 d after harvest when placed at 20 °C in ethylene- or propylene-free air (Arpaia et al., 1994; Antunes et al., 2000) though starting time may depend on orchard location (Fig. 1a and b). Control Mar del Sur kiwifruit placed at 20 °C reached its maximum ethylene production on day 24 after harvest (Fig. 1b), with concurrent expression of *KWACS1* and *KWACO1* in the outer cortex and core (Fig. 2). Previous literature shows increased transcript accumulation of *KWACS* and *KWACO* during kiwifruit ripening (Whittaker et al., 1997; Xu et al., 2000). In our work, timing of *KWACS1* and *KWACO1* transcription correlated with the corresponding ethylene production peak, both in control and ethylene-treated fruit (Figs. 1b and 2). The association of gene expression with ethylene production was confirmed by 1-MCP treatment. While *KWACS1* and *KWACO1* transcript accumulation was totally arrested by 1-MCP both in the outer cortex and in the core, ethylene production at 20 °C was hardly noticeable over the whole experimental period (Fig. 1b), thus agreeing with Yin et al. (2008) who did not detect ethylene production in 1-MCP-treated fruit after 25 d. While exogenous ethylene enhanced and 1-MCP clearly inhibited *KWACS1* and *KWACO1* transcription in kiwifruit, regardless the tissue considered, the *KWACS2* gene expression pattern did not show a clear response to ethylene or 1-MCP (Figs. 2, 3c and 5c).

Kiwifruit exposure to chilling conditions (0 °C) for 40, 80 or 120 d advanced ethylene biosynthesis and ripening when compared with fruit held continuously at 20 °C (Figs. 3a and 4a and b). Antunes and Sfakiotakis (2002) found that this advanced ethylene biosynthesis in kiwifruit is due to the increase in both ACO and ACS activities upon fruit rewarming. Our results show that exposure to chilling conditions for 40 d advanced the peak of ethylene production (day 18), with a concomitant increase in *KWACS1* transcript accumulation in the outer cortex (days 15 and 18) and in the core (days 18 and 22), and in *KWACO1* transcript accumulation in both tissues on day 18. Our results also demonstrate that 1-MCP effectively inhibits or greatly lessens ethylene production by kiwifruit during shelf-life at 20 °C, even when applied under cooling conditions after cold storage (Figs. 3a and 4a and b). Exposure to chilling conditions for 40 d did not hinder 1-MCP effectiveness to inhibit *KWACS1* and *KWACO1* transcript accumulation at every date tested (Fig. 3c).

In kiwifruit, the inhibition of accumulation of *KWACS1* and *KWACO1* mRNAs by 1-MCP suggests that there is a positive feedback regulation for ethylene production, in line with a previous proposal that ethylene production and the expression of its related genes are under a system-2 regulation (Whittaker et al., 1997; Xu et al., 1998). On the other hand, *KWACS2* gene expression does not show a clear response to 1-MCP. In many fruit species such as tomato, ACS and ACO activity has been shown to be related with transcript levels of multigene families that comprise members regulated differentially (Nakatsuka et al., 1998), and this may also be the case for kiwifruit.

Recently published data on genes associated with the ethylene signaling pathway in kiwifruit proved a range of expression changes with ripening and in response to 1-MCP (Yin et al., 2008) and to chilling conditions (Yin et al., 2009). Interestingly, 1-MCP has other effects than largely blocking *KWACS1* and *KWACO1* transcript abundance since it is capable of inhibiting the ethylene response of the components of the ethylene signal transduction pathway. According to Yin et al. (2008), only *AdETR2* and *AdCTR1* transcript

levels are transiently down-regulated by 1-MCP, and this ethylene-action inhibitor has no effect on any receptor gene expression in the core. The receptor gene *AdERS1b*, identified by Yin et al. (2008) as having a possible relationship with kiwifruit softening, shows no ethylene response and maintains relatively high expression levels in 1-MCP-treated fruit concomitantly with delayed softening.

Kiwifruit shows a particular behavior: (a) it displays a climacteric ethylene production pattern when ripened at 20 °C but not at 10 °C (Antunes et al., 2000); (b) it softens extensively in the absence of significant increases in autocatalytic ethylene production and ethylene synthesis peaks at 20 °C when fruit has already softened to the 'eating ripe' stage (Arpaia et al., 1994; Kim et al., 1999; Ritenour et al., 1999; Boquete et al., 2004; Yin et al., 2008; Figs. 1 and 3–5); (c) it is very sensitive to ethylene and very low concentrations may induce extensive softening (McDonald and Harman, 1982); and (d) it is capable of sensing ethylene long before its autocatalytic biosynthesis (Whittaker et al., 1997; Antunes et al., 2000; Yin et al., 2008), and a degree of ethylene sensitivity is retained even at low temperature (Yin et al., 2009). Our results lead to the following conclusions:

(1) 1-MCP treatment right after kiwifruit harvest markedly retards fruit softening and inhibits ethylene production but the magnitude of these effects is highly dependent on the growing region; thus, differential responses upon application of this postharvest technology can be expected depending on preharvest factors;
(2) Kiwifruit held at 0 °C for 40, 80 or 120 d and then treated with 1-MCP before fruit are allowed to rewarm to 20 °C, shows inhibition of ethylene production and a concomitant postponement of softening when transferred to 20 °C for further ripening, thus increasing the extension of kiwifruit 'eating ripe' stage;
(3) 1-MCP effectiveness is lower as kiwifruit softens, but kiwifruit still elicits a response to 1-MCP at relatively advanced stages of firmness loss (~10–15 N);
(4) 1-MCP largely blocks the ripening-related increases in *KWACS1* and *KWACO1* transcript abundance in kiwifruit previously exposed or not to cooling conditions, thus suggesting that there is a positive feedback regulation for ethylene production;
(5) 1-MCP delays firmness loss at different partially ripe preclimacteric stages in terms of ethylene production but does not inhibit softening. Thus, 1-MCP application sized to develop the appropriate concentration can be a useful tool as it does not compromise kiwifruit eating quality in terms of excessive firmness retention.

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