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Control of ethylene responses in avocado fruit with 1-methylcyclopropene

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Abstract

Mature avocado fruit (*Persea americana* Mill. cvs 'Ettinger', 'Hass', 'Reed' and 'Fuerte'), harvested during the commercial harvesting season, were treated with various concentrations of 1-methylcyclopropene (1-MCP) for 24 h at 22°C and after ventilation, were exposed to 300 μl l^{-1} ethylene for 24 h at 22°C. The fruit were then stored at 22°C in ethylene-free air for ripening assessment. Ethylene production, firmness, cellulase (endo-1,4-β-glucanase) and polygalacturonase activity, as well as color change in 'Hass' fruit, were monitored during storage. 1-MCP was found to inhibit ethylene-induced ripening of avocado fruit at very low concentrations. Treatment for 24 h with 30–70 nl l^{-1} 1-MCP delayed ripening of avocado fruit by 10–12 days, after which the fruit resumed normal ripening. It is suggested that 1-MCP is a potent inhibitor of avocado fruit ripening which exerts its effect via inhibition of ethylene action. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: 1-Methylcyclopropene; Avocado fruit; Ripening; Ethylene; Cellulase; Polygalacturonase; Softening; Coloring

1. Introduction

Ethylene is a plant hormone that controls many plant responses, including growth, senescence, ripening, abscission and seed germination (Abeles et al., 1992). The hormone is a natural product of metabolism and plants respond to both endogenous and exogenous ethylene (Abeles et al., 1992). Ethylene responses can be controlled by regulation of its production or action. The use of inhibitors of ethylene production is limited because of the likelihood of exposure of agricultural produce to exogenous ethylene. Therefore, inhibitors of ethylene action are considered preferable for use in agriculture, since they provide protection against both exogenous and endogenous ethylene. However, some known inhibitors of ethylene action have certain drawbacks. For example, the use of silver (silver thiosulfate), a potent inhibitor of ethylene action (Veen and Overbeek, 1989), is

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limited because of its toxicity. The compound 2,5-norbornadiene effectively inhibits ethylene action (Sisler et al., 1985; Sisler and Serek, 1997), but its strong odor and corrosive nature render it unsuitable for agricultural purposes. The compound 1-methylcyclopropene (1-MCP) has been shown to compete with ethylene for the binding site on the ethylene receptor in plant tissue and to control ethylene responses (Sisler and Wood, 1988; Sisler and Serek, 1997). This compound is a highly strained olefin, which binds in an apparently irreversible manner to the ethylene receptor. After a certain period the tissue resumes sensitivity to ethylene (Sisler and Serek, 1997).

The aim of the present study was to learn more about the inhibitory nature of 1-MCP and to assess its ability to control the ripening of avocado fruit. Four cultivars of avocado fruit were used in the experiments. However, as the effect of the inhibitor on ripening was similar in all four cultivars, only the results of studies with the 'Hass' cultivar are presented.

2. Materials and methods

2.1. Fruit and treatments

Avocado fruit (*Persea americana* Mill. cvs Ettinger, Hass, Reed and Fuerte) were harvested in local orchards during the commercial harvesting season. Within 4 h of harvest the fruit were sealed in 3-l glass jars (one fruit in each jar) and exposed to different concentrations of 1-MCP for 24 h at 22°C. The concentrations of 1-MCP used were 0.5, 1, 5, 15, 30, 50 or 70 nl l^{-1} (0.02, 0.05, 0.22, 0.67, 1.34, 2.23 or 3.13 nmol l^{-1}). The jars were then vented for 30 min and thereafter the fruit in the jars were exposed to ethylene (300 μl l^{-1}) for 24 h at 22°C. The fruit were then stored in ethylene-free air at 22°C and their ripening was assessed by measuring the following parameters: ethylene production, firmness, cellulase and polygalacturonase (PG) activity and color change. Each treatment was carried out in six replicates and all experiments were repeated three times.

2.2. Measurements of ethylene production

Ethylene production was measured by a standard method. A 1-ml gas sample was withdrawn by syringe from each jar through a septum fitted in the jar lid. The sample was injected into a Varian 3300 gas chromatograph equipped with a flame ionization detector (FID) and an alumina column. Jars containing the fruit were sealed for 1 h prior to ethylene sampling and left open between measurements for ventilation.

2.3. Assessment of color change

Fruit skin color in the 'Hass' cultivar was assessed visually and recorded on a scale from 0 (no color change) to 10 (complete change).

2.4. Measurement of firmness

Fruit firmness was determined using a Chatillon digital force gauge (model DFG-50) fitted with a 6 mm diameter conical probe. The firmness of each fruit was measured at four points along the equatorial region of the fruit and expressed in newton (N).

2.5. Extraction and assay of cellulase and polygalacturonase activity

Cellulase (endo-1,4-β-glucanase; EC 3.2.1.4) and polygalacturonase (PG, EC 3.2.1.15) activity were extracted and assayed by the methods of Goren and Huberman (1979) and Pesis et al. (1978) with slight modifications. All extractions were performed at 4°C. Three samples of mesocarp tissue (2 g) were taken from the equatorial region of three fruits from each treatment. Each sample was homogenized for 2 min at 24 000 rpm (SEV homogenizer) in 20 ml of 0.2 M K-phosphate buffer, pH 5.5, containing 1 M NaCl and 0.05% L-cysteine. The homogenate was stirred for 30 min in an ice bath, filtered through four layers of cheesecloth and centrifuged for 10 min at 20 000 g (Sorvall RC 5C Plus centrifuge, rotor SS-34). The supernatant was dialyzed overnight against the above buffer, diluted 100 times, and recentrifuged for 10 min at 12 000 rpm.

Cellulase activity in the supernatant was assayed viscosimetrically at 37°C, using 1.3% Na-carboxymethylcellulose (CMC) dissolved in 0.02 M K-phosphate buffer pH 6 as substrate. Activity was expressed as the percentage change in viscosity of CMC.

The activity of PG in the supernatant was determined spectrophotometrically by measuring reducing groups liberated from Na-polypectate with the dinitrosalicylic acid reagent. D-galacturonic acid was used as a standard and 1 mmol Na-hydrosulfite was added as an inhibitor of uronic acid oxidase (Riov, 1974). The release of 1 mg of D-galacturonic acid in 6 h was defined as one unit of PG activity. The activity of both enzymes was calculated and expressed per fruit fresh weight.

2.6. Preparation and quantification of 1-MCP

1-MCP was synthesized according to the procedure described by Sisler and Serek (1997). In this publication the authors indicated that 'the preparation contains some impurities, but as it contains so much activity the impurities usually can be ignored'. In light of that, attention was paid to check possible presence of physiological active impurities in the 1-MCP preparation. It was found that employing iodine, which reacts with the double bond in 1-MCP to form the physiological inactive diiodine compound, renders the preparation physiologically inactive. Thus, 1-MCP was the only physiological active compound in the preparation capable of inhibiting ethylene action.

1-MCP was quantified by injecting a 1-ml gas sample of the inhibitor into a Varian 3300 gas chromatograph fitted with a 23% SP-1700 on 80/100 chromosorb P AW column and a FID. Ethylene and 1-butene were used as calibration standards.

Table 1
Days after harvest to reach the climacteric peak of ethylene production, fruit softening (15–20 N) and complete skin color change in 'Hass' avocado fruit[a]

Treatment (1-MCP nl l^{-1})	Ethylene	Softening days	Color
0	3.8a	4.8a	6.3a
0.5	4.0a	4.9a	6.5a
1.0	4.1a	6.0a	7.1ab
5.0	4.9a	6.1a	8.2b
15.0	9.2b	10.2b	10.2c
30.0	16.5c	16.3c	16.5d
50.0	17.2cd	17.7d	17.1d
70.0	18.7d	18.8d	18.0d

[a] Values with different letters indicate statistical differences at $P = 0.05$. Fruit were treated for 24 h with the indicated concentrations of 1-MCP, then treated for 24 h with 300 μl l^{-1} ethylene and stored at 22°C for ripening assessment. Fruit that received no treatment ripened 5–9 days after harvest depending on the harvest date.

3. Results and discussion

1-MCP is considered a putative inhibitor of ethylene action, exerting its effect by binding to the ethylene receptor sites (Sisler and Serek, 1997). In the present study, several measures were undertaken and extreme conditions were employed to determine the potency of 1-MCP in delaying avocado fruit ripening. (i) The effect of the inhibitor on ethylene-induced ripening rather than on spontaneous ripening was studied. (ii) A high concentration of ethylene (300 μl l^{-1}) was used to induce ripening. This concentration was 40 000-fold that of the highest concentration of 1-MCP used. This measure was taken in case the inhibition of ethylene binding by 1-MCP is competitive rather than non-competitive in nature. (iii) During follow-up of ripening the fruit were held at 22°C, which is considered the optimal temperature for acceleration of ripening.

At 22°C, untreated fruit of all of the harvested cultivars ripened after 5–9 days, depending on the harvest date. In fruit treated with ethylene, the time to complete ripening was significantly shortened by 3–5 days (Table 1).

In fruit that were exposed to 1-MCP, at concentrations above 30 nl l^{-1} for 24 h prior to ethylene treatment, ethylene-induced ripening was considerably delayed. The delaying effect of 1-MCP on fruit ripening was concentration dependent (Table 1). Treatments with 1-MCP at 0.5, 1, or 5 nl l^{-1} did not affect the ethylene-induced

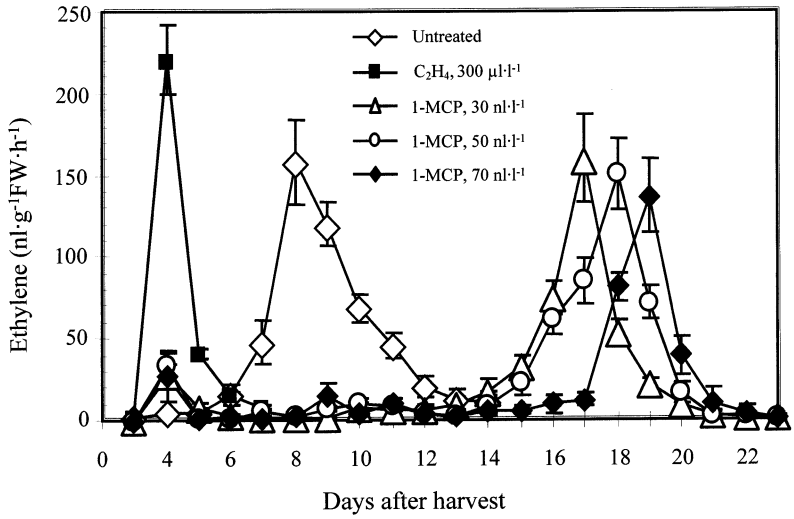

Fig. 1. Effect of pretreatment with 1-MCP on ethylene production in 'Hass' avocado fruit. The fruit were treated for 24 h with the above indicated concentrations of 1-MCP, then exposed to ethylene (300 μl l^{-1}) for additional 24 h and stored at 22°C for ripening assessment. For ethylene treatment (■), fruit were closed in the jars for 24 h with air instead of with 1-MCP before exposure to ethylene for an additional 24 h. Each value is a mean of six replications ± SE.

ripening (Table 1). Treatment with 1-MCP at 15 nl l^{-1} had a moderate effect, delaying the climacteric peak, softening and skin color change by 6, 5 and 3 days, respectively. Increasing the concentration of 1-MCP to 30, 50, or 70 nl l^{-1} caused a marked delay in ripening (Table 1). The peak of ethylene production was delayed by 12–13 days, softening was delayed by 12–13 days and color change by 10–11 days, respectively. Increasing the concentration of 1-MCP to 70 nl l^{-1} (Table 1) or higher did not result in a further delay in ripening. The results indicate that treatment with 50 nl l^{-1} 1-MCP is sufficient to exert maximal delay of avocado fruit ripening.

The concentration dependence of the inhibitory effect of 1-MCP on ethylene-induced ripening of avocado fruit is shown in Figs. 1–5. In untreated fruit, the ethylene climacteric peak appeared on day 7 after harvest (Fig. 1). Treatment with ethylene for 24 h resulted in advanced fruit ripening, and the ethylene climacteric peak appeared on day 4. However, treatment of the fruit with 1-MCP before exposure to ethylene delayed the ethylene-induced fruit ripening. The postharvest climacteric ethylene peak occurred 17 days after harvest in fruit pretreated with 30 nl l^{-1} 1-MCP and 18 or 19 days after harvest in fruit pretreated with 50 or 70 nl l^{-1} 1-MCP, respectively. It should be noted that all fruit treated with 1-MCP exhibited a minor peak of ethylene production 24 h after 1-MCP application. The magnitude of this peak was not found to be related to the concentration of 1-MCP applied. Nevertheless, ripening was always substantially delayed by 1-MCP treatments. A similar finding was obtained with other inhibitors of ethylene action, such as Ag^{2+} (Beyer, 1979) and 2,5-norbornadiene (Sisler et al., 1985; Abeles et al., 1992). This phenomenon appeared consistently in all the avocado cultivars tested. In a separate study with citrus leaf explants, we found a similar response to 1-MCP where treatment with the inhibitor resulted in an increase in ethylene production by the explants (data not published). Therefore, it is suggested that the minor peak of ethylene production displayed by fruit treated with 1-MCP is a response of the fruit to the chemical.

Ethylene-treated fruit produced ethylene during ripening at a relatively high rate, with a peak production 40% higher than that of fruit not exposed to ethylene. However, the peak of ethylene production after treatment with the various concentrations of 1-MCP, even though they were

subsequently treated with ethylene, was similar to that of fruit neither treated with ethylene nor with 1-MCP (Fig. 1). The reason for the higher rate of ethylene production by ethylene-treated fruit is not yet known. However, it should be noted that fruit exposed to ethylene were well ventilated with ethylene-free air, in order to ensure no ethylene residue in the fruit. The increased ethylene pro-

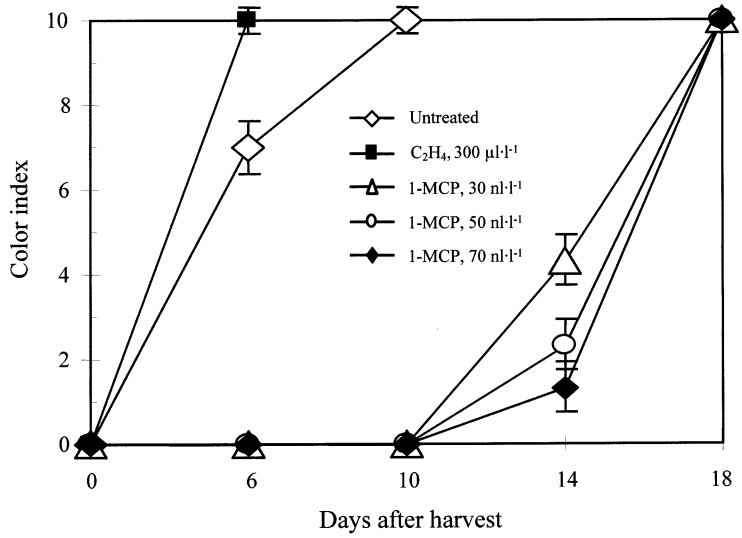

Fig. 2. Effect of pretreatment with 1-MCP on skin color in 'Hass' avocado fruit. The fruit were treated for 24 h with the above indicated concentrations of 1-MCP, then exposed to ethylene (300 µl l^{-1}) for additional 24 h, and stored at 22°C for ripening assessment. For ethylene treatment (■), fruit were closed in the jars for 24 h with air instead of with 1-MCP before exposure to ethylene for an additional 24 h. Each value is a mean of six replications ± SE.

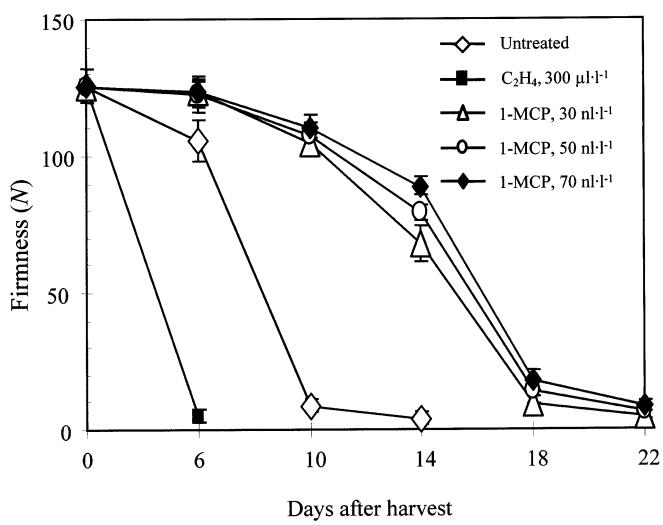

Fig. 3. Effect of pretreatment with 1-MCP on softening in 'Hass' avocado fruit. The fruit were treated for 24 h with the above indicated concentrations of 1-MCP, then exposed to ethylene (300 µl l^{-1}) for additional 24 h, and stored at 22°C for ripening assessment. For ethylene treatment (■), fruit were closed in the jars for 24 h with air instead of with 1-MCP before exposure to ethylene for an additional 24 h. Each value is a mean of six replications ± SE.

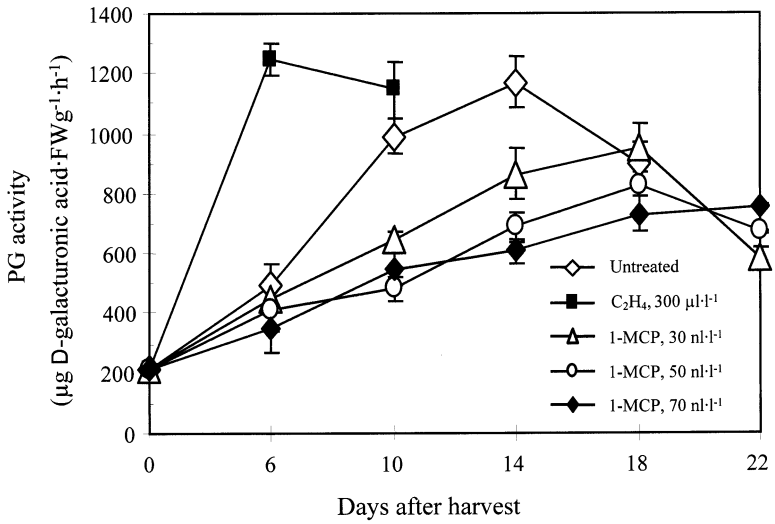

Fig. 4. Effect of pretreatment with 1-MCP on PG activity in 'Hass' avocado fruit. The fruit were treated for 24 h with the above indicated concentrations of 1-MCP, then exposed to ethylene (300 μl l^{-1}) for additional 24 h and stored at 22°C for ripening assessment. For ethylene treatment (■), fruit were closed in the jars for 24 h with air instead of with 1-MCP before exposure to ethylene for an additional 24 h. Each value is a mean of six replications ± SE.

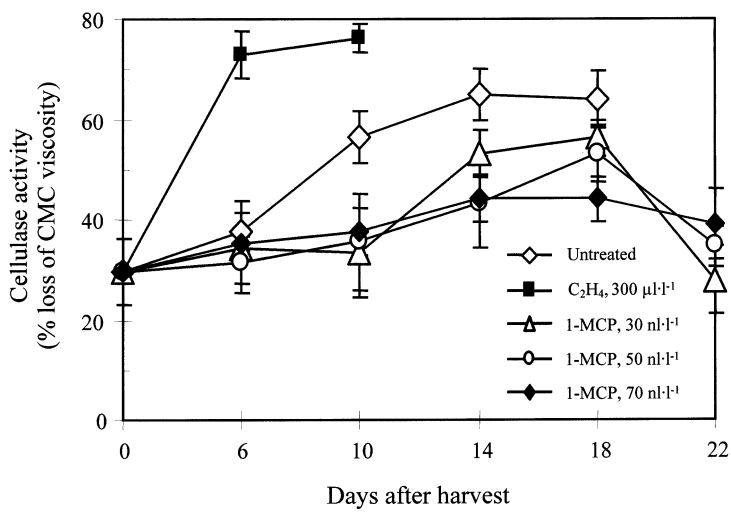

Fig. 5. Effect of pretreatment with 1-MCP on cellulase activity in 'Hass' avocado fruit. The fruit were treated for 24 h with the above indicated concentrations of 1-MCP, then exposed to ethylene (300 μl l^{-1}) for additional 24 h, and stored at 22°C for ripening assessment. For ethylene treatment (■), fruit were closed in the jars for 24 h with air instead of with 1-MCP before exposure to ethylene for an additional 24 h. Each value is a mean of six replications ± SE.

duction due to ethylene absorbed by the fruit treated with high concentration of ethylene can be ruled out, since fruit treated with 1-MCP received the same ethylene treatment as well, but did not show increased ethylene production over the control. Therefore, this response could be physiological rather than merely absorbed ethylene released by the fruit.

Pretreatment of fruit with 1-MCP prior to their exposure to ethylene delayed skin color change (Fig. 2). Untreated fruit showed a complete color change between 6 and 10 days after harvest, but treatment with ethylene shortened that period by 4 to 5 days. Treatment of fruit with 1-MCP at 30, 50, or 70 nl l^{-1} prior to exposure to ethylene delayed the ethylene-induced color change, which occurred between 14 and 18 days after harvest.

Treatment with 1-MCP delayed ethylene-induced softening of the fruit (Fig. 3). Compared to a firmness of 127 N at harvest time, the firmness of untreated fruit held at 22°C was about 20 N when measured 8 days after harvest. In fruit exposed to ethylene, the time taken to reach a firmness of 20 N was shortened by 5 days. Pretreatment with 1-MCP considerably delayed fruit softening: in fruit treated with 30, 50, or 70 nl l^{-1} 1-MCP, fruit firmness of about 20 N was recorded between 14 and 18 days after harvest (Fig. 3).

The activity of polygalacturonase (PG) in avocado fruit at harvest time was 200 μg D-galacturonic acid g^{-1} FW h^{-1} (Fig. 4). In untreated fruit, PG activity increased 5-fold within 10 days after harvest. In fruit exposed to ethylene, the activity of PG increased 6-fold within 6 days after harvest. Treatment with 1-MCP prior to ethylene treatment reduced the rate of increase in PG activity: by 18 days after harvest, the activity of PG increased 4.5-fold in fruit treated with 30 nl l^{-1}1-MCP and only 3.5-fold in fruit treated with 50 or 70 nl l^{-1} 1-MCP. It should be noted that although in 1-MCP-treated fruit the PG activity during ripening was lower than in fruit that were exposed to ethylene or left untreated, softening of 1-MCP-treated fruit progressed normally.

Cellulase activity at harvest time showed a 30% loss of CMC viscosity (Fig. 5). In untreated fruit held at 22°C, cellulase activity increased 1.5-fold within 10 days after harvest. In fruit exposed to ethylene, the activity increased 2.5-fold within 6 days. Treatment with 1-MCP prior to exposure to ethylene delayed the increase in cellulase activity; an increase of 1.8-fold after treatment with 1-MCP at 30 or 50 nl l^{-1} and of 1.4-fold after treatment with 70 nl l^{-1} 1-MCP were recorded only 18 days after harvest. It appears that in fruit exposed to ethylene, both the level of cellulase activity and its rate of increase were higher than in untreated fruit. In contrast, the level of cellulase activity in fruit treated with 1-MCP (70 nl l^{-1}) was low throughout the storage period. Despite the lower enzyme activity, however, fruit treated with 1-MCP ripened and softened normally.

The results of the present study showed that 1-MCP was capable of protecting the tissue against ethylene, probably by blocking the binding site on the ethylene receptor, as suggested by Sisler and Wood (1988) and Sisler and Serek (1997). Pretreatments with 1-MCP delayed ethylene-induced fruit ripening of all the avocado cultivars tested. The inhibitory effect of 1-MCP lasted about 2 weeks and thereafter the fruit resumed normal ripening. The resumption of sensitivity to ethylene suggests that at about 12–13 days after the treatment, free binding sites on the ethylene receptor are present in the tissue. Whether these are newly formed binding sites or sites that have become dissociated from 1-MCP is not yet clear. In addition, possible metabolism of 1-MCP in the tissue could be considered. Maximal activity of 1-MCP was achieved within a concentration range of 30–70 nl l^{-1}. On the assumption that 1-MCP competes with ethylene for the binding site, we used a relatively high physiological concentration of ethylene (300 μl l^{-1}). This was done to verify that 1-MCP can be active even under extreme conditions. Our findings imply that 1-MCP acts as a non-competitive inhibitor of ethylene action only if applied before ethylene application. In order to test the inhibitor under extreme ripening conditions we conducted our experiments at 22°C, a temperature at which ripening is hastened.

Studies over the last few years have shown that 1-MCP can delay fruit ripening (Serek et al., 1995a,b; Sisler and Serek, 1997; Song et al., 1997; Golding et al., 1998; Sisler and Wood, 1988) and flower senescence (Porat et al., 1995, 1999). In the present study we showed that 1-MCP delays ethylene-induced ripening in different cultivars of avocado fruit. The advantage of using inhibitors of ethylene action over inhibitors of ethylene production lies in the ability of the inhibitors of ethylene action to protect the tissue against both

endogenous and exogenous ethylene, thus providing better overall protection.

Our findings indicate that 1-MCP is a potent inhibitor, capable of providing good protection of avocado fruit against ethylene for a period of up to 13 days. The fact that 1-MCP is a gas and is non-phytotoxic, odorless, and effective at low concentrations, renders it a promising candidate for commercial use.

Acknowledgements

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