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Research note

1-Methylcyclopropene influx and efflux in 'Cox' apple and 'Hass' avocado fruit

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Abstract

Ethylene initiates and/or co-ordinates ripening associated processes in climacteric fruits. Among climacteric fruits, avocado is relatively high in lipid content and apple is comparatively low. The ethylene binding site blocker 1-methylcyclopropene (1-MCP) was sorbed faster and in greater amounts by avocado fruit (*Persea americana* Mill. 'Hass') and avocado oil than by apple fruit (*Malus sylvestris* var. domestica, 'Cox') and water, respectively. The ability of produce to sorb 1-MCP may have an influence on 1-MCP efficacy as a ripening inhibitor and should also be considered when prescribing commercial 1-MCP application strategies.

Keywords: *Malus sylvestris* var. *domestica*; 1-Methylcyclopropene; *Persea americana* Mill

1. Introduction

The use of the ethylene binding site blocker 1-methylcyclopropene (1-MCP) to extend the postharvest longevity of ethylene-sensitive produce has been reported widely. Most findings have been based on a single pre-storage application of 1-MCP. The efficacy of 1-MCP varies with the crop. Cut flowers have an immediate response to 1-MCP but typically show re-sensitivity to ethylene within a few days of 1-MCP treatment (Sisler et al., 1996; Macnish et al., 1999; Çelikel et al., 2002). Application of >0.1 μl l^{-1} 1-MCP has been shown to extend avocado fruit shelf life by 40% (Hofman et al., 2001; Pesis et al., 2002). Apples treated with >0.1 μl l^{-1} 1-MCP have been shown to maintain quality during air storage for as long as 9 months (Watkins et al., 2000; Dauny and Joyce, 2002).

Firmness of 'Cox' fruit treated with 10.0 μl l^{-1} 1-MCP has been shown to be slightly greater than that for 1-MCP concentrations of <1.0 μl l^{-1} (Dauny and Joyce, 2002). It was suggested that the higher concentration gradient at 10 μl l^{-1} 1-MCP might have enhanced diffusion of 1-MCP into the fruit. It was also thought possible that excess 1-MCP may be sorbed in some way by fruit tissue

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beyond saturation of ethylene-binding sites. If so, 1-MCP may be slowly desorbed during storage to become available to bind to a newly synthesized or regenerated ethylene-binding sites (Golding et al., 1998).

The ability of produce to retain 1-MCP may be directly related to plant tissue composition. 1-MCP should be preferentially sorbed into lipid versus aqueous compartments. Organic chemicals are typically more hydrophobic than hydrophilic. 1-MCP has no carboxylic or amino groups and thus would partition into oil/lipids and not water (K. Karim, Cranfield University, pers. comm.). Low polarity alkenes are much more soluble in non-polar than in polar solvents (Solomons 1978). Apples (0.1 g fat 100 g^{-1} edible portion; Wills, 1987) may have less 1-MCP sorbing capacity than oil-containing avocados (23.0 g 100^{-1} g). In order to test this hypothesis, apples and avocados were sealed in an atmosphere containing 1-MCP. The 1-MCP concentration of the atmosphere was measured repeatedly over a 48 h period to determine if 1-MCP was being taken up into the produce. To further test the proposition, oil was extracted from avocado and 1-MCP uptake was compared to a water control.

2. Material and methods

2.1. Whole fruit

'Cox' apple and 'Hass' avocado fruit were obtained from a local wholesaler (Wilkinsons Ltd, Bedfordshire, UK). Three of each type of fruit were labelled. Each fruit was placed into individual sealable 1.5-l jars. These jars also held a 200-ml Pyrex beaker containing a weighed amount of Ethylbloc™ (0.14% a.i.; AgroFresh Inc., Gessate, Italy). Water (20 ml) at 50 °C was added to the beaker to liberate 1-MCP gas and the jar was sealed immediately. This process achieved initial concentrations of approximately 120 μl l^{-1} 1-MCP within the jars. The jars were kept sealed for 48 h at 20 °C. Every hour after 1 h, 1-MCP concentration in a 1 ml sample of air extracted from each jar was quantified by GC. Air removed from the jar was replaced with 1 ml of nitrogen gas.

In follow up experimentation, a further three apple and avocado fruit were treated with approximately 120 μl l^{-1} 1-MCP. 1-MCP was quantified after 1, 6, 12, 24 and 48 h. These fruit were then removed from the 1.5-l jars, and individually placed into 500-ml jars. These jars were connected

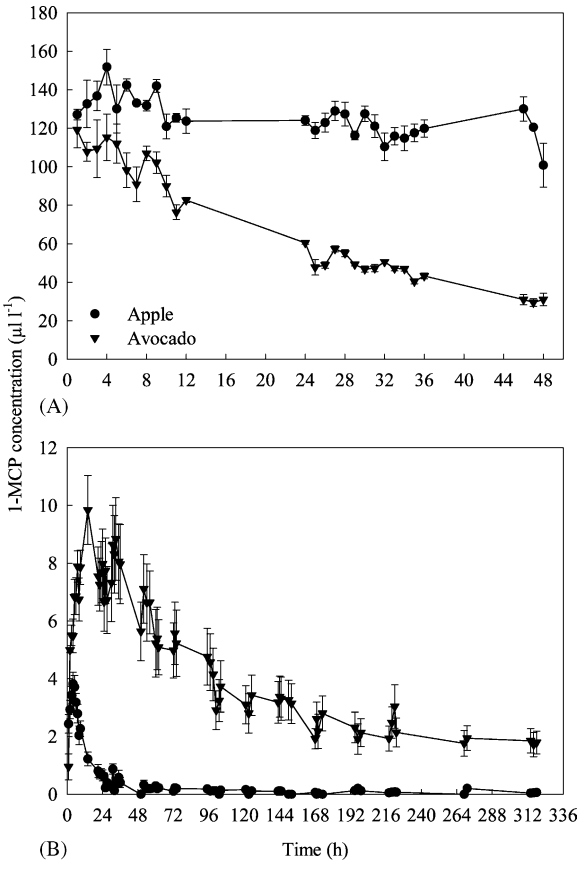

Fig. 1. (A) 1-MCP concentrations (μl l^{-1}) over 48 h in the headspace of sealed 1.5-l jars containing either an individual 'Cox' apple or a 'Hass' avocado fruit (influx). 120 μl l^{-1} 1-MCP was added as Ethylbloc at 0 h. (B) 1-MCP concentrations (μl l^{-1}) over 312 h in outflow air over either 'Cox' apple and 'Hass' avocado fruit stored in individual ventilated 0.5-l jars with an air flow-through rate of 4 ml min^{-1} (efflux). Fruit were previously exposed to 120 μl l^{-1} 1-MCP for 48 h, then transferred to ventilated jars within 1 h. Fruit were placed into ventilated jars at 0 h. Vertical bars show the standard errors of the means ($n = 3$). Where no vertical bars are visible the standard errors were smaller than the size of the symbols.

to a flow-through gas system, and the air supply regulated to give one air change every 8 h. A 1 ml sample of exhaust gas from each jar was removed after 1 h. 1-MCP concentrations in these samples were quantified by GC. 1-MCP was subsequently measured hourly for the first 48 h, then three times a day until a fruit showed signs of decay.

2.2. Avocado oil

'Hass' avocado fruit were peeled and the flesh (200 g fresh weight (FW) per avocado) was cut into small pieces. Tissue was snap-frozen in liquid nitrogen and freeze-dried. Each freeze-dried sample was ground and approximately 3 ml g^{-1} FW of 99% (v/v) hexane at 20 °C was added. The mixture was homogenised at 20 500 rpm using an Ultra-Turrax T25 homogeniser (Janick and Kunkel, Stafen, Germany) for 5 min at 20 °C. The homogenate was filtered under vacuum through Whatman No. 3 filter paper using a 5.5 cm diameter Buchner funnel. The solvent was removed using a rotary evaporator (Buchi Rotovapor, Büchi Labortechnik AG, Flawil, Switzerland) under vacuum (0.6 kPa) at 35 °C.

Avocado oil (15 ml) was placed into each of three 250 ml volumetric flasks. Distilled water (15 ml) was placed into each of three other 250-ml volumetric flasks. Three more 250-ml volumetric flasks were left empty. All the flasks were sealed with Suba seals (Fisher Scientific, Leicestershire, UK). 1-MCP gas was prepared as a stock. Equal volumes of stock gas were injected into each of the flasks through the seal to give a concentration of approximately 10 μl l^{-1} 1-MCP. Every hour after 1 h, 1-MCP concentrations in a 2 ml sample of air extracted from each flask were quantified by GC. Air removed from the flasks was replaced with 2 ml of nitrogen gas. After 24 h, the seals were removed and the flasks flushed with nitrogen for 15 min and resealed. From that time and every 3 h after, 1-MCP concentrations in 2 ml samples of air extracted from each flask were quantified by GC. Air removed from the flasks was replaced with 2 ml of nitrogen gas.

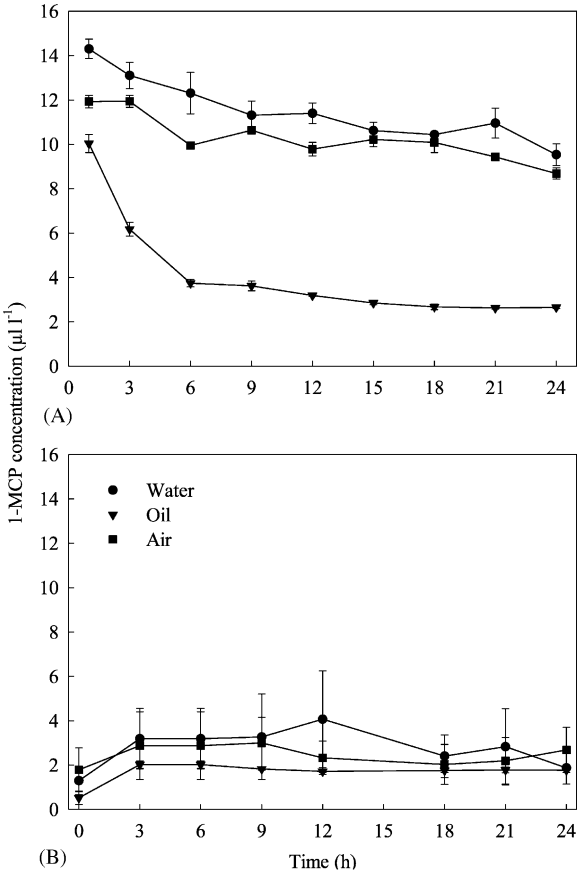

Fig. 2. (A) 1-MCP concentrations (μl l^{-1}) over 24 h in the headspace of sealed 250-ml volumetric flasks containing 15 ml of avocado oil or distilled water plus an air control (influx). 10 μl l^{-1} 1-MCP was injected into the flasks at 0 h. (B) 1-MCP concentrations (μl l^{-1}) over 24 h in the headspace of sealed individual 250-ml volumetric flasks containing 15 ml of either avocado oil or distilled water plus an air control (efflux). The flasks were previously injected with 10 μl l^{-1} 1-MCP and the contents left for 48 h followed by flushing with nitrogen gas for 15 min Vertical bars show the standard errors of the means ($n = 3$). Where no vertical bars are visible the standard errors were smaller than the size of the symbols.

2.3. 1-Methylcyclopropene quantification

1-MCP concentrations were quantified using a Carlo Erba GC8340 gas chromatograph fitted with an EL 980 FID and a DP800 integrator (Thermoquest, Hertfordshire, UK). Oven and detector temperatures were 100 °C. The 2 m long, 6 mm external diameter, 4 mm internal

diameter stainless steel column was packed with Chromosorb PAW mesh range 80–100, liquid phase OV1701 30% loading (Jones Chromatography, Mid Glamorgan, UK). 1-MCP was calibrated against 10 μl l^{-1} *n*-butane (British Oxygen Company Gases, Guildford, UK).

3. Results and discussion

1-MCP concentrations in a sealed atmosphere decreased both faster and to a greater extent for avocado fruit than for apple fruit (Fig. 1A). Thus the avocado fruit sorbed more 1-MCP than the apple fruit. When 1-MCP treated fruit were placed into a flow-through system, 1-MCP concentrations in the outflow air stream showed that avocado fruit exposed to 1-MCP released more 1-MCP than apple fruit (Fig. 1B). Avocado fruit contain oil (Wills, 1987), which may act as a sink for the cycloalkene 1-MCP.

1-MCP concentrations in sealed containers decreased both faster and to a greater extent over oil extracted from avocado fruit than over distilled water (Fig. 2A). When the headspace over oil or water exposed to 1-MCP was then flushed with nitrogen gas, 1-MCP concentrations in the headspace were similar. Although apparently inconsistent with the whole fruit experiment, this result suggests that 1-MCP preferentially partitioned into the oil versus the air in the headspace over the oil (Fig. 2B). The apparent inconsistency may be due to the different set-up for the two experiments. The whole fruit experiment was continually ventilated. Thus there was no opportunity for the 1-MCP in the oil and the headspace to equilibrate according to the partition coefficient.

To facilitate measurements of 1-MCP influx and efflux (in a flow-through system) into and from fruit, respectively, an initial treatment of >100 μl 1-MCP l^{-1} was used. The relative differences between avocado and apple were clear in terms of greater 1-MCP sorption by the high-oil avocado fruit. Similar differences were evident when extracted avocado oil and water were compared using a 1-MCP treatment concentration of 10 μl l^{-1}, which is closer to the recommended commercial treatment concentration of <1 μl l^{-1} (G. Regiroli, AgroFresh Inc., pers. comm.). Preferential partitioning into oil is to be expected at all 1-MCP concentrations.

These results pose questions about the commercial application of 1-MCP for different fruit types. For example, the high oil content of avocados may allow avocados to be treated with a lower 1-MCP concentration than apples due to their greater ability to store 1-MCP. In this case the oil may release 1-MCP to bind to ethylene binding sites when 1-MCP is no longer present in the surrounding atmosphere.

Differences among products in 1-MCP treatment responses in terms of degree of effect (e.g. on ripening retardation or abscission prevention) and the loss of 1-MCP efficacy over time may be due primarily to the synthesis of new ethylene binding sites in plant tissues (Sisler and Serek, 1999; Macnish et al., 2000; Dauny and Joyce, 2002). Catabolism of 1-MCP remains a possibility. In addition, product physiological (e.g. magnitude and duration of the ethylene climacteric) and physicochemical characteristics (e.g. cuticular and tissue resistance to gas diffusion) could have effects. However, non-specific sorption in lipids could also modulate efficacy, for example, if there were subsequent desorption of 1-MCP to bind to newly formed ethylene binding sites. In an applied context, non-specific sorption helps to explain the disappearance of 1-MCP over time in commercial fumigation rooms (G. Regiroli, AgroFresh Inc., pers. comm.) and also in laboratory treatment systems (unpublished observations) at greater rates than expected due to either leakage or specific binding. Unless trickle delivery systems are developed, commercial treatment recommendations may need to be made on the basis of the concentration of the initial dose.

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