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Cultivar differences in gaseous 1-methylcyclopropene accumulation in whole and fresh-cut apple fruit

Xiaoqing Dong[a,b], Donald J. Huber[a,*], Maricruz Ramírez-Sánchez[a], Jingping Rao[b], Jinsu Lee[a], Christopher B. Watkins[c]

[a] Horticultural Sciences Department, IFAS, University of Florida, Gainesville, FL 32611-0690, USA
[b] College of Horticulture, Northwest A&F University, Yangling, Shaanxi Province 712100, PR China
[c] Department of Horticulture, Cornell University, Ithaca, NY 14853-0001, USA

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ABSTRACT

A number of studies have shown that responses of apple fruit to 1-methylcyclopropene (1-MCP) vary considerably among cultivars. This study was designed to determine if cultivars show differences in accumulation of gaseous 1-MCP. Apple fruit were placed in 1.76 L jars that were sealed and injected with 20 $\mu L L^{-1}$ 1-MCP. After 12 h, samples of intercellular atmosphere were removed and analyzed for 1-MCP concentration. Accumulation of internal gaseous 1-MCP varied markedly among cultivars, ranging from 0.14 ± 0.06, 0.22 ± 0.03, and 0.77 ± 0.30 in 'Redcort', 'McIntosh', and 'Empire', respectively, to 2.10 ± 0.28, 3.33 ± 0.13, and 6.93 ± 0.35 $\mu L L^{-1}$ in 'Gala', 'Cameo', and 'Honeycrisp', respectively. Accumulation of gaseous 1-MCP was reduced an average of 51% in fruit treated with Sta-Fresh 8711 fruit wax. The role of the epidermis in modulating 1-MCP ingress was determined by measuring gaseous 1-MCP accumulation in fresh-cut tissue. Fresh-cut cortical tissue rapidly depleted headspace 1-MCP (>95%) over a 1-h exposure yet accumulated negligible quantities of internal gaseous 1-MCP. By contrast, cortical tissue treated with ascorbic acid or hypotaurine, or aged for several hours prior to exposure to 1-MCP, showed reduced consumption of headspace 1-MCP and high accumulation of internal gaseous 1-MCP. Levels of internal 1-MCP in cortical tissue from the cultivars generally paralleled those for intact fruit, ranging from 0.23 ± 0.07, 0.37 ± 0.18 and 1.09 ± 0.14 $\mu L L^{-1}$ in 'Empire', 'McIntosh' and 'Redcort', respectively, to 2.40 ± 0.71, 4.55 ± 0.15, and 6.24 ± 0.85 in Gala', 'Cameo', and 'Honeycrisp', respectively. Although commercial fruit wax influences gaseous 1-MCP accumulation, the comparable accumulation patterns in unwaxed whole and fresh-cut apple fruit suggest that epidermal tissue/native waxes alone do not account for cultivar differences.

1. Introduction

The efficacy of 1-methylcyclopropene, a potent ethylene antagonist (Sisler, 2006), at influencing ripening is known to vary among horticultural commodities, particularly climacteric fruit (Blankenship and Dole, 2003; Huber, 2008; Watkins, 2008). Responses to 1-MCP can also vary among cultivars. Studies addressing multiple cultivars of tomato have shown relatively similar responses to 1-MCP applied to fruit of similar maturity or ripening classes (Krammes et al., 2003; Guillén et al., 2006; Sabir et al., 2012). By contrast, in terms of dosage responses, internal ethylene levels (IEC) and firmness retention (Watkins et al., 2000; Calderon-Lopez et al., 2005; Watkins and Nock, 2005), and impact bruise susceptibility (Jung and Watkins, 2009), responses of apple cultivars to 1-MCP vary quite markedly.

The objective of the present study was to determine if apple cultivars exhibit differences in accumulation of internal gaseous 1-MCP. The effect of commercial wax on accumulation was also examined. The role of the epidermis in modulating 1-MCP ingress was determined by performing parallel experiments with fresh-cut tissue.

2. Materials and methods

2.1. Plant material

Fruit of 'Redcort', 'McIntosh', 'Empire', 'Gala', 'Cameo', and 'Honeycrisp' cultivars were harvested based on maturity guidelines

* Corresponding author at: Horticultural Sciences Department, PO Box 110690, University of Florida, IFAS, Gainesville, FL 32611-0690, USA. Tel.: +1 352 273 4779; fax: +1 352 392 6479.
E-mail address: djhuber@ufl.edu (D.J. Huber).

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for New York (Blanpied and Silsby, 1992) at Cornell research orchards between 4 and 26 September, 2012. Freshly harvested fruit were packaged in boxes with cut-out foam inserts and shipped via overnight courier to postharvest facilities at the University of Florida, Gainesville, FL. Internal ethylene concentrations (Watkins and Nock, 2005) in all cultivars at time of arrival were $\leq 1.8\ \mu L\ L^{-1}$, indicating that fruit were at a preclimacteric or early climacteric stage. Fruit of each cultivar were received from two harvests at 7- to 10-day intervals. 'Red Delicious' fruit from local retail sources were used in some of the experiments with fresh-cut tissue.

2.2. 1-MCP accumulation in intact apple fruit

Prior to treatment with 1-MCP, fruit were dipped for 2 s in diH_2O or a suspension prepared from Sta-Fresh 8711 vegetable oil-based fruit wax (JBT Corporation, Lakeland, FL) [50:50 Sta-Fresh/water (v/v)]. Fruit were placed over PTFE Laboratory Matting (Saint Gobain Chemware) on trays and held overnight at 20 °C for drying. Afterward, coated and uncoated fruit were placed individually in 1.76 L jars (one fruit per jar, three fruit per treatment), the jars sealed, and injected through septa with 1-MCP at $20\ \mu L\ L^{-1}$. After 12 h at 20 °C, one jar of each treatment was opened and the fruit immersed in diH_2O and sampled for internal 1-MCP. From each fruit, four 1 mL samples were obtained from four equidistant points on the equator of the submerged fruit using a 1 mL syringe equipped with a 2.5 cm, 23-gauge needle. The other jars were opened in treatment pairs in succession and fruit sampled as described. The aliquots of internal atmosphere were injected into 5 mL React-vials fitted with Teflon-lined septa (Thermo Scientific, Catalog No. TS-12718). For all cultivars, data are reported as average [three fruit per cultivar per treatment (coated/uncoated) × four samplings per fruit] internal 1-MCP ($\mu L\ L^{-1}$) ± standard error of the mean (SEM). In experiments examining duration of exposure to 1-MCP, separate sets of fruit/jars were used at each measurement interval. Experiments with each cultivar were performed a minimum of two times.

2.3. Sorption of gaseous 1-MCP to Sta-Fresh 8711 fruit wax

Twenty milliliter (around 19.5 g) of Sta-Fresh 8711 fruit wax were placed in each of three 244 mL jars and held in a drying oven at 45 °C until no further weight reduction was observed (4–5 d). The residual material (average 4.2 g) should consist largely of vegetable oil and vegetable-derived fatty acid salts, the two primary ingredients listed on product label. Empty jars served as controls. The jars were sealed and injected with $20\ \mu L\ L^{-1}$ 1-MCP. After 1, 3, 6 and 24 h, 1 mL headspace samples were removed and analyzed for 1-MCP concentration. Data are expressed as average 1-MCP content (% of initial) of three values (jars) ± SEM.

2.4. Oil content of Sta-Fresh 8711 fruit wax

Ten milliliter of chloroform–MeOH (2/1, v/v) were added to samples of dried Sta-Fresh 8711 (around 4.2 g derived from 20 mL formulation) in 100 mL beakers. After manual stirring for 10 min, the liquid was removed with a pipette and filtered through Miracloth into tared Petri plates. Another 5 mL of chloroform–MeOH were used to rinse the insoluble residue and were transferred to the Petri plates. The Petri plates were held in an oven at 45 °C for 12 h and again weighed. The non-volatile, chloroform–MeOH–soluble material (around 3.5 g of clear, viscous fluid) was taken as an estimate of the vegetable oil and fatty acid content (83%, g/g wax dry weight).

2.5. 1-MCP accumulation in fresh-cut apple

Prior to experiments with tissue from the six cultivars, 'Delicious' from retail sources were employed to establish protocols for measurement of internal 1-MCP in excised cortical tissue. Apple fruit with epidermis removed using a vegetable peeler were processed into cubes (dimensions 27 mm × 27 mm × 25 mm) of cortical tissue of approximately 15 g. Two pieces from each of three fruit per treatment were placed in separate 244 mL jars that were sealed and injected with 1-MCP at $20\ \mu L\ L^{-1}$. After 1 h, one of the jars was opened and the two tissue cubes immersed in diH_2O. A 1 mL sample of internal atmosphere was removed from each cube using a 1 mL syringe equipped with a 2.5 cm 23-gauge needle injected into the center of the tissue. The aliquots of internal atmosphere were injected into 5 mL React-vials fitted with Teflon-lined septa. After processing the first jar, the remaining two jars were opened in succession and the tissue treated as described. The effects of antioxidants on 1-MCP ingress were tested by dipping freshly prepared 'Delicious' tissue cubes in 360 mM ascorbic acid or hypotaurine (2-aminoethanesulfinic acid) for 3 min prior to sealing in the treatment jars. In experiments examining duration of exposure to 1-MCP, separate sets of tissue cubes were used at each measurement interval. Data are reported as average (three fruit per treatment × two cubes per fruit/jar × one sampling per cube) internal 1-MCP ($\mu L\ L^{-1}$) ± SEM.

The effects of tissue aging on accumulation of 1-MCP were examined by maintaining freshly prepared cubes (no ascorbic acid or hypotaurine treatment) derived from three fruit in unsealed jars (two cubes per fruit, 3 jars containing two cubes per aging time) with loose-fitting lids at 20 °C for 0, 0.5, 2, 6, 9 and 12 h. Afterward, the jars were sealed and 1-MCP injected with $20\ \mu L\ L^{-1}$. After 1 h, jars were opened and samples of internal atmosphere obtained as described above. Data are reported as average (three fruit per aging time × two cubes per fruit × one sampling per cube) internal 1-MCP ($\mu L\ L^{-1}$) ± SEM.

2.6. Preparation and measurement of gaseous 1-MCP

Stock 1-MCP gas was prepared using solutions of AFXRD-038 powder (3.8% active ingredient) as described in detail in Choi and Huber (2009). The solutions were prepared in side-arm flasks with septa, sealed, and gently stirred for 2 h prior to use. After measuring headspace 1-MCP concentrations, volumes of stock gaseous 1-MCP were injected into the treatment flasks or jars at a concentration of around $20\ \mu L\ L^{-1}$.

1-MCP concentrations were measured using gas chromatography as described in Lee et al. (2012).

2.7. Statistical analysis

Data were analyzed using SAS statistical software (Version 9.2, SAS Institute, Cary, NC, USA) and subjected to analysis of variance (ANOVA). Results among treatments were compared using Fisher's least significant differences (LSD, $P = 0.05$). Data are presented as the mean ± SEM.

3. Results

Internal 1-MCP levels in 'Cameo', 'Empire', 'Gala', 'Honeycrisp', 'McIntosh', and 'Redcort' apples and the influence of commercial fruit wax are shown in Fig. 1. Internal levels in uncoated fruit exposed to $20\ \mu L\ L^{-1}$ 1-MCP for 12 h ranged from a low of $0.14 \pm 0.06\ \mu L\ L^{-1}$ in 'RedCort' to a high of $6.93 \pm 0.35\ \mu L\ L^{-1}$ in 'Honeycrisp'. Exposure periods ranging from 6 to 24 h for both low and high 1-MCP accumulators revealed relatively constant levels of internal gaseous 1-MCP (not shown), indicating that