1-MCP Improves Storability of 'Queen Cox' and 'Bramley' Apple Fruit

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Abstract. Better maintenance of firmness and suppression of ethylene production in 'Queen Cox' and 'Bramley' apple [*Malus × sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] fruit was achieved by prestorage applications of 1-MCP. 1-MCP concentration, exposure time and exposure temperature ranges of 0.1 to 10.0 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, 6 to 48 h, and 0 to 20 °C, respectively, were effective on fruit subsequently stored for 2 ('Cox') and 3 ('Bramley') months in air at 3 to 4 °C. However, 1-MCP had little effect on either firmness or ethylene production after 4 ('Cox') or 6 ('Bramley') months storage. Nonetheless, 1-MCP treated 'Bramley' fruit had reduced rot and superficial scald incidences compared with untreated control fruit. Chemical name used: 1-methylcyclopropene (1-MCP).

'Queen Cox' and 'Bramley' fruit [*Malus × sylvestris* (L.) Mill. var. *domestica* (Borkh.) Mansf.] are important apple cultivars, particularly in the U.K. 'Cox' is a dessert apple that softens quickly under retail conditions. 'Bramley' is a cooking apple that is susceptible to softening and development of the storage disorder superficial scald.

Maintenance of apple firmness is an aim of all apple storage management procedures, as soft fruit have reduced quality and commercial value. Softening is influenced by the internal ethylene concentration (IEC) of fruit. In general, firmness of fruit stored in ethylene concentrations below 44 $\mu\text{mol}\cdot\text{m}^{-3}$ (1 $\mu\text{L}\cdot\text{L}^{-1}$) is greater than for those stored in higher ethylene concentrations (Stow et al., 2000). However, for 'Cox' fruit, removal of atmospheric ethylene only delayed the accumulation to a critical IEC level of 0.1 $\mu\text{L}\cdot\text{L}^{-1}$ for 8 to 12 weeks. After this time, fruit softened; although less than for fruit stored in higher ethylene concentrations (Stow et al., 2000).

Superficial scald is a form of chilling injury of apple and pear fruit manifest as brown or discolored patches on the peel, with little or no physical damage to the pulp. Superficial scald is caused by the action of conjugated trienes (CTs) released as oxidative-breakdown products of α -farnesene (Rupasinghe et al., 2000). Production of α -farnesene in the skin tissue of apple fruit is influenced by ethylene levels in the fruit and storage atmosphere (Rupasinghe et al., 2000). Susceptibility to scald varies with cultivar, maturity, storage temperature and atmospheric ethylene levels (Huelin and Goggiola 1968, 1970).

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1-MCP binds to, and thus blocks, the ethylene receptor. As a result, ethylene is not able to bind to the receptor and exert an influence. 1-MCP treatment has been shown to reduce IEC (i.e., the suppression of autocatalytic ethylene production), maintain greater firmness, inhibit α -farnesene production and reduce superficial scald development in 'Delicious', 'Law Rome' and 'McIntosh' apples (Rupasinghe et al., 2000; Watkins et al., 2000). In addition, 1-MCP has also been shown to suppress other ripening-associated changes in apples. Reduced respiration rate and lower total soluble solids (TSS) have been found in 1-MCP treated apple cultivars, including 'Gala', 'Jonagold' and 'Delicious'.

The aim of this work was to determine the efficacy of 1-MCP applied at a range of concentrations, durations of exposure and exposure temperatures in reducing superficial scald development in 'Bramley' and softening of 'Bramley' and 'Cox' fruit during storage at 3 to 4 °C in air. Knowledge of the efficacy of 1-MCP applied under these conditions will be useful when designing commercial utilization strategies.

Materials and Methods

'Cox' and 'Bramley' apple fruit were harvested from a farm in Plaxtol, Kent, U.K. over the Sept. to Oct. 2000 season. Fruit were randomized, labelled and weighed on the day of harvest, and treated within 24 h (Table 1). 'Cox' apples were dipped in 10 $\text{g}\cdot\text{L}^{-1}$ Ridomil[™] mbc 60WP [carbendazim (5 $\text{g}\cdot\text{L}^{-1}$) and metalaxyl (1 $\text{g}\cdot\text{L}^{-1}$)] (Syngenta, Bracknell, U.K.) to control *Phytophthora* spp. 'Bramley' fruit were not dipped in fungicide, as per normal commercial practice. Fruit were equilibrated to treatment temperature and placed inside cardboard fruit trays (59 × 37 × 14 cm;

30 fruit per tray). The trays were placed inside rigid polypropylene fumigation chambers (88 × 59 × 59 cm). 1-MCP was prepared according to the method of Macnish et al. (1999). Briefly, 5 mL of lithium diisopropylamide (LDA; Fluka, Buchs, Switzerland) was injected into a test tube flushed with N₂. A 0.3-mL aliquot of 3-chloro-2-methylpropene (CMP; Fluka) was injected into the test tube over a period of 15 to 30 min to produce the lithium salt of 1-MCP. The liquid and precipitate mixture was allowed to stand at room temperature for an additional 30 min with regular mixing using a vortex mixer. LiCl precipitated throughout this stage. Measured aliquots of the 1-MCP-evolving stock solution were placed into the fumigation chambers, which were immediately sealed.

1-MCP concentrations were quantified using a Carlo Erba GC8340 gas chromatograph fitted with an EL 980 FID and a DP800 integrator (Thermoquest, Hertfordshire, U.K.). Oven and detector temperatures were 100 °C. The 2-m-long stainless steel column was packed with Chromosorb PAW mesh range 80–100, liquid phase OV1701 30% loading (Jones Chromatography, Mid Glamorgan, U.K.). 1-MCP was calibrated against 10 $\mu\text{L}\cdot\text{L}^{-1}$ n-butane (British Oxygen Co. Gases, Guildford, U.K.).

Three experiments were conducted for each cultivar. In the 1-MCP concentration experiment, fruit were exposed to 0.0, 0.1, 0.5, 1.0, or 10 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at either 0 or 20 °C for 24 or 48 h. In the 1-MCP exposure time experiment, fruit were exposed to 1.0 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 6, 12, 24, or 48 h at either 0 or 20 °C. In the 1-MCP exposure temperature experiment, fruit were exposed to 1.0 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at 0, 5, 10, 15, or 20 °C for either 24 or 48 h.

After 1-MCP treatment, fruit were removed from the fumigation chambers, re-randomized and individually enclosed in perforated polypropylene bags (15- μm thickness, 400- μm -diameter holes, five holes per cm^2) [Cryovac 250 Y; Cryovac, U.K.). Fruit were then kept in cardboard fruit trays (59 × 37 × 14 cm; 30 fruit per tray) and stored in air at 3 to 4 °C and >95% RH. Temperature was logged throughout the storage periods. 'Cox' apples were stored for 2 and 4 months, with one additional set for 6 months in the 1-MCP concentration experiment. 'Bramley' apple fruit were stored for 3 and 6 months. Upon removal from cold-air storage, the fruit were taken out of their perforated bags and kept in a shelf-life room at 20 °C and 50% to 60% relative humidity (RH) for 0, 7, and 14 d.

In addition, 30 fruit were taken per harvest for maturity analysis (Table 1). Ten were analyzed 24 h after harvest. The remaining 20 fruit were placed in the shelf life room and 10 were taken for analysis after 7 d. The remaining 10 were analyzed 14 d after harvest.

After each storage and shelf life time, fruit were weighed and skin color ($L^* a^* b^*$) was measured using a CR200 colorimeter (Minolta, Osaka, Japan). Fruit IEC was determined as described by Saltveit (1982). A 1.1-mm-diameter × 40-mm-long stainless steel needle was inserted through the calyx and sealed in place

Table 1. Harvest dates and maturities of 'Cox' and 'Bramley' apple fruit for each experiment (n = 10).

1-MCP treatments (experiment)	Cultivar	Harvest date (year 2000)	Penetration force (N)	IEC ($\mu\text{L}\cdot\text{L}^{-1}$)
Concentration	Cox	13 Sept.	83.2 $\pm$ 2.7	0.5 $\pm$ 0.3
	Bramley	6 Sept.	74.4 $\pm$ 1.8	0.0
Exposure duration	Cox	20 Sept.	71.7 $\pm$ 1.9 ^a	0.4 $\pm$ 0.2 ^a
	Bramley	20 Sept.	73.6 $\pm$ 2.0	0.0
Exposure temperature	Cox	27 Sept.	66.5 $\pm$ 2.0	25.3 $\pm$ 12.4
	Bramley	4 Oct.	68.2 $\pm$ 1.8	0.2 $\pm$ 0.1

^aOne missing value (n = 9)

with Blu-Tack™ (Bostic, Leicester, U.K.). A 3-mL gas sample was withdrawn slowly from the apple with a syringe. The ethylene concentration in 1 mL of this sample was quantified by GC. Fruit firmness was measured on opposite sides of peeled fruit using a Digital Force Gauge (Mecmesin, West Sussex, U.K.) fitted with a 10.0-mm-diameter flat end probe. The descent of the probe was controlled at 50 mm/min using the cross head of an Instron 1122 Universal Tester (Instron, Buckinghamshire, U.K.). The TSS of fruit juice extracted by crushing the flesh around the penetration holes with a wooden spatula was measured using a PR-1 digital refractometer (Atago, Tokyo). The number of fruit affected with disease or superficial scald was recorded.

All six experiments were $5 \times 2 \times 3$ factorial arrangements. In the 1-MCP concentration experiment, n (number of fruit) = 20 for 2 months stored 'Cox' and 3 months stored 'Bramley' fruit after 24 h exposure to 1-MCP and n = 10 for 4 and 6 months stored 'Cox' and 6 months stored 'Bramley' after 48 h 1-MCP exposure. In all other experiments, n = 10 for each individual treatment. Batches of 10 fruit from each treatment were assessed 0, 7, and 14 d after removal from storage. All treatments were arranged in a completely randomized design (CRD). Data were subjected to analysis of variance (ANOVA) using Genstat 5.0 (IACR Rothamsted, U.K.).

Results and Discussion

1-MCP applied at 0.1 to $10.0 \mu\text{L}\cdot\text{L}^{-1}$ maintained fruit firmness for 2 and 3 months in 'Cox' and 'Bramley', respectively (Fig. 1 A and B). Firmness of 'Cox' treated with $10.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP was slightly greater than that at the other 1-MCP concentrations. The higher concentration gradient at $10 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP might have enhanced diffusion of 1-MCP into the fruit. Moreover, it is possible that excess 1-MCP may be sorbed in some way by fruit tissue beyond saturation of ethylene-binding sites. If so, 1-MCP may be slowly released during storage, becoming available to bind to newly synthesized or regenerated ethylene-binding sites (Golding et al., 1998).

IEC for fruit exposed to 1-MCP was reduced relative to that of untreated fruit (Fig. 1 A and B). An exception was the IEC for 'Cox' treated with $10.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP which increased compared with other 1-MCP concentrations.

Firmness and IEC of 'Cox' and 'Bramley' fruit were not affected by 1-MCP at 4 and 6 months and 6 months storage, respectively (data not shown). Loss of 1-MCP efficacy

over time during storage is probably due to increasing availability of ethylene-binding sites in the fruit tissue (Macnish et al., 2000; Sisler and Serek, 1999). There is debate as to the nature of this increase. It may be that binding sites slowly release bound 1-MCP after a period of time (regeneration), that 1-MCP is slowly metabolized, that new binding sites are formed (synthesis), or a combination of these possibilities. Involvement of plant metabolism is

suggested by an observation that *Pelargonium peltatum* L. plants treated with $1 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for 2 h at 22 °C and then grown at 12, 21, and 25 °C maintained resistance to ethylene-induced petal abscission for longer at the lower temperature (Cameron and Reid, 2001). Retreatment of re-sensitized pelargonium plants with 1-MCP restored their resistance to ethylene.

'Cox' and 'Bramley' fruit were as firm after 2 and 3 month air storage, respectively, after just 6 h exposure to $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP as after the longest exposure time of 48 h (Fig. 2 A and B). An absence of a differential exposure time effect suggests that 1-MCP rapidly permeates throughout apple fruit. As in the 1-MCP concentration experiment, differences in firmness and IEC for untreated and 1-MCP treated fruit were not significant ($P > 0.05$) (data not shown) for longer storage periods of 4 and 6 months for 'Cox' and 6 months for 'Bramley'.

Fig. 1. Fruit firmness (●) and IEC (○) main factor means (i.e., across 0, 7, and 14 d shelf life evaluations) for apple fruit exposed to 1-MCP for 24 h. Fruit were stored in air at 3 to 4 °C for 2 months ('Queen Cox', A) or 3 months ('Bramley', B). Data are the means for 60 individual replicate fruit. LSD ($P = 0.05$).

Fig. 2. Fruit firmness (●) and IEC (○) main factor means (i.e., across 0, 7, and 14 d shelf life evaluations) for apple fruit exposed to $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP for different exposure times. Fruit were stored in air at 3 to 4 °C for 2 months ('Cox', A) or 3 months ('Bramley', B). Data are the means for 30 individual replicate fruit. LSD ($P = 0.05$).

Table 2. Percentage of superficial scald and rot incidence in 'Bramley' apple fruit stored for 6 months in air at 3 to 4 °C with or without a prestorage 1-MCP application.

Parameter and treatments (experiment)	Nontreated	1-MCP treated
<i>Superficial scald incidence</i>		
1-MCP concentration	17 ^z	1 ^y
1-MCP exposure time	32 ^z	0 ^y
1-MCP exposure temperature	1 ^x	1 ^w
<i>Disease incidence</i>		
1-MCP concentration	4 ^z	2 ^y
1-MCP exposure time	10 ^z	1 ^y
1-MCP exposure temperature	33 ^x	4 ^w

^zn = 180.

^yn = 720.

^xn = 150.

^wn = 300.

Application temperature did not affect efficacy of 1-MCP on 'Cox' and 'Bramley' fruit after 2 and 3 months storage in air, respectively (Fig. 3 A and B). Thus, 1-MCP may be administered to 'Cox' and 'Bramley' apple fruit while in cold storage. This result is in agreement with work on banana fruit (*Musa* sp. 'Williams'; Cavendish subgroup AAA), grevillea 'Sylvia' (*Grevillea banksii* R. Brown x *G. whiteana* D.J. McGillivray) and Geraldton waxflower (*Chamelaucium uncinatum* Schauer) flowers (Macnish et al., 2000). 1-MCP efficacy at $0.1 \mu\text{L}\cdot\text{L}^{-1}$ was not affected at 2 °C. However, at $0.01 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP, efficacy was reduced in the fruit and flowers at the lower temperature. Differences in firmness and IEC between control and 1-MCP treatments were nonsignificant ($P > 0.05$) (data not shown) for longer storage periods of 4 and 6 months for 'Cox' and 6 months for 'Bramley'.

1-MCP treatment prevented superficial scald development in 'Bramley' fruit after 6 months storage in both the 1-MCP concentration experiment and the 1-MCP exposure time experiment (Table 2). Inhibition of scald development in 'Bramley' by 1-MCP confirms similar findings for 'Delicious', 'Granny Smith', 'Law Rome', and 'McIntosh' (Fan et al., 1999a, 1999b; Watkins et al., 2000). Scald incidence was <1% in the 1-MCP exposure temperature experiment. This overall reduction in scald compared with the preceding 1-MCP concentration and exposure time experiments may have been due to the later harvest of these fruit (Table 1). Similarly, a fruit maturity effect on scald development has been reported for 'Cortland', 'Delicious', 'Granny Smith', and 'Crofton' (Huelin and Coggiola, 1968; Watkins et al., 1993).

Disease incidence was low in both 'Cox' and 'Bramley' fruit following storage for 2 and 3 months, respectively (data not shown). Nonetheless, storage disease incidence in 'Bramley' was reduced by 1-MCP application regardless of the 1-MCP concentration, exposure time, or exposure temperature (Table 2). Proportionally greater disease incidence in both 1-MCP treated and control fruit was observed in the final 1-MCP exposure temperature experiment compared to the two earlier experiments. This tendency for more disease in the last 'Bramley' experiment may have been a feature of lower inherent disease resistance in late harvested fruit. Immature apple fruit are relatively resistant to diseases, and subsequently lose this resistance during maturation and ripening (Ndubizu, 1976). Reduced decay in association with 1-MCP treatment of 'Bramley' apple fruit contrasts with some other 1-MCP studies. For avocado, custard apple, mango, orange, papaya, and strawberry fruit, certain 1-MCP treatments enhanced disease compared to untreated controls (Hofman et al., 2001; Jiang et al., 2001; Ku et al., 1999; Porat et al., 1999).

For both 'Cox' and 'Bramley', 1-MCP treatment resulted in slightly ($P < 0.05$) higher TSS ($\approx 1^\circ\text{Brix}$) and reduced weight loss ($\approx 2\%$ in real terms) compared with untreated control fruit (data not shown). There were no significant ($P > 0.05$) effects of 1-MCP on peel color (data not shown).

Fig. 3. Fruit firmness (closed symbols) and IEC (open symbols) main factor means (i.e., across 0, 7, and 14 d shelf life evaluations) for apple fruit exposed to $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP at different temperatures for 0 (●, ○), 24 (▼, ▽) or 48 h (■, □). Fruit were stored in air at 3 to 4 °C for 2 months ('Cox', A) or 3 months ('Bramley', B). Data are the means for 30 individual fruit. LSD ($P = 0.05$).

In summary, 1-MCP enhanced storability for 'Cox' and 'Bramley' apple fruit. Fruit firmness was better retained, disease incidence was reduced, and, for 'Bramley' fruit, superficial scald was inhibited. 1-MCP treatments ranging over 0.1 to $10 \mu\text{L}\cdot\text{L}^{-1}$ applied for 6 to 48 h at 0 to 20 °C were effective.

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