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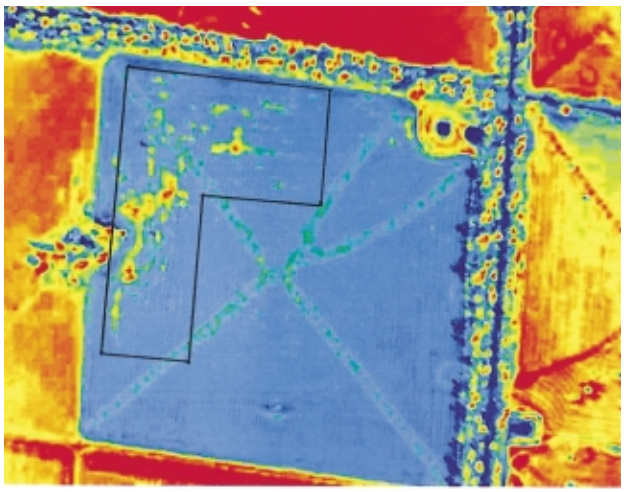

VOLUME 41, 2001

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Australian Journal of Experimental Agriculture, 2001, **41**, 567–572

Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1-methylcyclopropene

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Abstract. The potential for the ethylene binding inhibitor, 1-methylcyclopropene, to delay ripening of 'Hass' avocado, 'African Pride' custard apple, 'Kensington Pride' mango and 'Solo' papaya was examined. Fruit were gassed with 25 μL/L 1-methylcyclopropene for 14 h at 20°C, followed by treatment with 100 μL/L ethylene for 24 h, and then ripened at 20°C. Ethylene treatment alone generally halved the number of days for fruit to reach the ripe stage, compared with untreated fruit. 1-Methylcyclopropene treatment alone increased the number of days to ripening by 4.4 days (40% increase), 3.4 days (58%), 5.1 days (37%) and 15.6 days (325%) for avocado, custard apple, mango and papaya, respectively, compared with untreated fruit. Applying 1-methylcyclopropene to the fruit before ethylene prevented the accelerated ripening normally associated with ethylene treatment, so that the number of days to ripening for fruit treated with 1-methylcyclopropene plus ethylene was similar to the number of days to ripening for fruit treated with 1-methylcyclopropene alone. 1-Methylcyclopropene treatment was associated with slightly higher severity of external blemishes in papaya and custard apple, slightly higher rots severity in avocado, custard apple and papaya, and at least double the severity of stem rots in mango, relative to fruit not treated with 1-methylcyclopropene. Thus, 1-methylcyclopropene treatment has the potential to reduce the risk of premature ripening of avocado, custard apple, mango and papaya fruit due to accidental exposure to ethylene. However, additional precautions may be necessary to reduce disease severity associated with 1-methylcyclopropene treatment.

Introduction

Manipulating the ripening of fruit is important in ensuring appropriate quality of fruit to the customer. Unpredictable ripening during storage, transport and distribution can result in spoilage before consumption. Ethylene is known to trigger ripening in climacteric fruit, and senescence in non-climacteric fruit, vegetables and ornamentals (Burg and Burg 1962). This knowledge is used by commercial operators to promote rapid and predictable ripening of climacteric fruit, or to delay ripening by minimising exposure of these fruit to ethylene. Therefore, customer dissatisfaction due to under-ripe and over-ripe fruit can be reduced by careful manipulation of exposure to ethylene. However, handling conditions at wholesale, retail and consumer level, and ethylene concentrations in the atmosphere at these stages, can often negate the benefits of controlled ripening practices and reduce fruit life and quality (Wills *et al.* 1999, 2000).

The risks of accidental exposure to ethylene can be minimised by reducing ethylene concentrations in the storage environment with practices such as oxidation by potassium permanganate, or ultraviolet light. However, these systems, while being effective for certain commodities, have limited commercial application.

The development of the gas, 1-methylcyclopropene (1-MCP) provides a new approach to manipulation of ripening and senescence (Sisler and Serek 1997). This gas prevents the action of ethylene in plants by binding permanently to ethylene receptors in the tissue. Results have indicated that 1-MCP can significantly delay ripening and senescence in a range of fruit, vegetables and ornamentals (Serek *et al.* 1994; Golding *et al.* 1998; Fan and Mattheis 1999; Ku and Wills 1999; Macnish *et al.* 1999, 2000*b*), and extend the display life of potted plants and survival of cuttings (Serek *et al.* 1994, 1998).

10.1071/EA00152 0816-1089/01/040567

The objectives of this study were to determine the potential for 1-MCP to delay ripening of several subtropical and tropical fruit, and to reduce the undesirable effects of accidental exposure to ethylene when delayed ripening is required. This was investigated by treating avocado, custard apple, mango and papaya fruit with 1-MCP, either without or with subsequent ethylene treatment.

Materials and methods

Fruit

Fruit were harvested at commercial harvest maturity from commercial orchards. 'Hass' avocados (*Persea americana* Mill) and 'African Pride' custard apples (*Annona squamosa* × *Annona cherimola* L.) were harvested from orchards near Nambour (south-eastern Queensland; 26°37′S, 152°56′E) on 15 June and 10 June 1997, respectively, 'Kensington Pride' mangoes (*Mangifera indica* L.) from Childers (south-eastern Queensland; 25°15′S, 152°30′E) on 10 January 1998, and 'Solo' papaya (*Carica papaya* L.) from Innisfail (northern Queensland; 17°52′S, 146°02′E) on 1 June 1997. Avocado, mango and custard apple fruit were transported to the laboratory within 3 h of harvest and treated on the same day, while papaya fruit were air freighted to the laboratory and treated the day after harvest. The fruit were treated with a fungicide dip of 1 mL/L Sportak (Prochloraz; 0.05% v/v) for 30 s immediately on arrival at the laboratory. About 100 of each fruit were harvested, and 20 unblemished fruit randomly selected for each treatment.

1-Methylcyclopropene and ethylene treatment

Twenty fruit were either ripened at 20°C without any additional treatment (untreated), or treated with ethylene only (ethylene), or treated with 1-MCP only (1-MCP), or treated with 1-MCP, then ethylene (1-MCP + ethylene). The fruit were then ripened at 20°C.

A 1-MCP stock was synthesised by the method of Macnish *et al.* (1999) and quantified by gas chromatography (Jiang *et al.* 1999*a*). The fruit were treated in a 60-L glass container held at 20°C. A 100-mL beaker of KOH was placed in the container to reduce accumulation of CO_2, and 2 filter paper wicks were stood in the beaker to increase the surface area for absorption. A saturated solution of ammonium sulfate was used as a water trap vent for pressure equalisation.

Fruit were placed into the container, the lid sealed with petroleum jelly, and the required volume of 1-MCP stock to produce 25 μL 1-MCP/L was injected through a rubber septum in the container lid. A small fan was used for the first 10 min after injection to circulate the 1-MCP. Untreated fruit were placed into a similar container without 1-MCP. The fruit were exposed to 1-MCP for 14 h and then aerated for about 1 h. Half of the 1-MCP and untreated fruit were then treated with 100 μL ethylene/L for 24 h at 20°C in a controlled temperature room. The remaining fruit were placed in air without ethylene at 20°C. Fruit in all treatments were then ripened at 20°C and about 80% RH.

Fruit quality

Individual fruit mass was determined at arrival at the laboratory and when ripe, and the average percentage weight loss per day calculated.

Fruit were examined daily by gentle hand pressure to determine the approximate stage of ripeness. When close to ripe, fruit firmness for avocado and mango was determined daily as the force (N) required to push an 8 mm diameter hemispherical probe 2 mm into the unpeeled fruit, using an Instron Universal Testing Machine model 1122 (Instron LTD, High Wycombe, UK). Days to ripe (DTR) was judged as the time (days) after harvest for the fruit to attain a firmness of 5–7 N. For custard apple and papaya, the eating soft stage was determined by gentle hand pressure only, corresponding to a force of 2–4 N and 5–7 N, respectively, using the same procedure as above.

When ripe, the percentage of the fruit surface area with green skin colour was recorded for mango and avocado. Skin colour of ripe mangoes was quantified on 2 opposite points on the largest diameter of the fruit using a Hunter Labscan 6000 Spectrocolourmeter (Hunter Associates Laboratories, Reston, USA) fitted with a 25-mm orifice, D65 illuminant, and 10-degree observer. L^*, a^* and b^* values were recorded, and Chroma (C) and hue angle (h°) calculated (Voss 1992).

The severity of diseases was recorded as the percentage of the cut surface area of the flesh affected by lesions. The diseases were described (based on visual symptoms) as body rots (caused mainly by *Colletotrichum* spp.) and stem rots (caused mainly by *Dothiorella* spp.) on mango and avocado, and anthracnose (caused mainly by *Colletotrichum* spp.), black rots (caused mainly by *Phoma caricae-papayae* and *Asperisporium caricae*) and stem rots (caused mainly by *Lasiodiplodia theobromae*) on papaya (Coates *et al.* 1995). The percentage of the skin area affected by discolouration of the protruding tips of custard apple fruit (called 'black tips'), and external blemishes (mainly seen as skin damage) on papaya, were also recorded.

The fruit were cut longitudinally, and the severity of mesocarp discolouration and vascular browning in avocado (Cutting *et al.* 1992) was rated as the percentage of the cut surface area affected. Internal blackening, grey flesh and core rots in custard apple were rated using the same scale.

Total soluble solids in custard apple, mango and papaya fruit was determined on an equatorial slice of flesh from each fruit using an Atago bench refractometer corrected to 20°C, and the results expressed as °Brix. Titratable acidity (TA; expressed as the percentage citric acid) was measured on 1 g fruit pulp from the equatorial section of mango by titrating with 0.1 mol sodium hydroxide/L to pH 8.2.

Statistical analysis

Data were analysed using analysis of variance of a completely randomised design with 4 treatments and 20 replications (individual fruit). The treatments consisted of 2 factors, ethylene and 1-MCP, each at 2 levels, absent and present. When there was a significant interaction between ethylene and 1-MCP (P<0.05) the 4 individual treatment means are presented. Otherwise main effects of any significant factor are included. The protected least significant difference (l.s.d.) procedure at P = 0.05 was used to test for differences in treatment means. Only significant differences are discussed, unless otherwise stated. Skewed data were angular transformed before analysis. Means of the angular transformed data are presented, with back-transformed means presented in brackets.

Results

Avocado

Ethylene-treated fruit, without 1-MCP, ripened about twice as fast as untreated fruit, while 1-MCP treatment alone increased the DTR by about 40% compared with untreated fruit (Table 1). 1-Methylcyclopropene treatment prevented the ethylene stimulation of ripening, so that the 1-MCP + ethylene-treated fruit ripened more slowly than untreated fruit. Ethylene treatment alone increased the percentage weight loss/day compared with all other treatments.

There were no significant effects of ethylene or 1-MCP on ripe fruit skin colour, and no flesh disorders were observed in any of the treatments (data not shown). There was no significant treatment interaction between MCP and ethylene on disease severity nor a significant ethylene effect, thus only the main effects for 1-MCP are presented. 1-Methylcyclopropene treatment was associated with

Table 1. Days for fruit to reach the eating soft stage (days to ripe at 20°C), percentage weight loss per day and the percentage of cut surface area of flesh affected with body + stem rots, in 'Hass' avocado fruit treated with 25 µL 1-MCP/L for 14 h at 20°C followed by exposure to 100 µL/L ethylene for 24 h at 20°C and finally ripening at 20°C

Means within each column followed by the same letter are not significantly different at $P = 0.05$ ($n = 20$)
Rots data are angular transformed, with back-transformed means in parentheses

Treatment	Days to ripe	Weight loss/day (%)	Rots
Untreated	11.2b	0.63a	—
Ethylene	6.0a	0.85b	—
1-MCP	15.6d	0.59a	—
1-MCP + ethylene	14.9c	0.59a	—
l.s.d. ($P = 0.05$)	0.6	0.05	
Nil 1-MCP	—	—	5.7a (1.0)
1-MCP	—	—	6.5b (1.3)
l.s.d. ($P = 0.05$)			0.4

slightly increased rots severity regardless of whether fruit were also treated with ethylene (Table 1).

Custard apple

Treatment with ethylene alone significantly reduced the DTR by 42% compared with untreated fruit (Table 2). 1-Methylcyclopropene increased the DTR by 58 and 167% compared with untreated and ethylene-treated fruit, respectively. 1-Methylcyclopropene prevented the typical ethylene effect of reducing the DTR, resulting in no difference between 1-MCP alone and ethylene after 1-MCP. Ethylene alone increased, and ethylene + 1-MCP reduced the percentage weight loss/day, compared with untreated fruit.

1-Methylcyclopropene-treated fruit had a higher percentage of black tips compared with untreated fruit or to those treated with ethylene alone. All ethylene and 1-MCP treatments increased the severity of grey flesh (Table 2). There was no treatment interaction between ethylene and 1-MCP for internal black discolouration or core rots, nor any significant effect of ethylene, thus only the main effects for 1-MCP are presented. 1-Methylcyclopropene-treated fruit had more severe black discolouration and core rots than those not treated with 1-MCP (Table 3).

Table 3. Percentage of the cut surface area affected by internal blackening and core rots, in 'African Pride' custard apple fruit either untreated, or treated with 25 µL 1-MCP/L for 14 h at 20°C; fruit were ripened at 20°C

Means followed by the same letter in each column are not significantly different ($P = 0.05$) ($n = 40$)
Data are angular transformed, with back-transformed means in parentheses

Treatment	Internal black	Core rot
Nil 1-MCP	6.2a (1.2)	5.7a (1.0)
1-MCP	7.1b (1.5)	6.4b (1.2)
l.s.d. ($P = 0.05$)	0.6	0.5

Fruit treated only with ethylene had lower °Brix and TA compared with untreated fruit, but the difference in flesh °Brix and TA between 1-MCP-treated and untreated fruit was small or absent (Table 2).

Mango

Ethylene alone reduced the DTR by 41% and 1-MCP alone increased the DTR by 37%, compared with untreated fruit (Table 4). 1-Methylcyclopropene applied before ethylene doubled the DTR compared with fruit treated with ethylene alone. There was no significant difference in the DTR between 1-MCP and 1-MCP + ethylene. Ethylene alone increased the percentage weight loss/day compared with the other treatments.

Ethylene treatment alone reduced the severity of stem rots compared with untreated fruit (Table 4). However, 1-MCP treatment alone significantly increased the rot severity, and 1-MCP + ethylene increased severity even more compared with untreated fruit.

There was no effect of ethylene treatment on the L^* value (lightness) of the ripe skin, but 1-MCP significantly reduced the L^* value from 26.9 (without 1-MCP) to 25.7 (with 1-MCP) (data not shown). There were no treatment effects on

Table 2. Days to reach the eating soft stage (days to ripe at 20°C), percentage weight loss per day, percentage of the fruit surface area with black tips, percentage of the cut surface area of the flesh affected by grey flesh, and °Brix and titratable acidity (TA) of 'African Pride' custard apple fruit treated with 25 µL 1-MCP/L for 14 h at 20°C followed by exposure to 100 µL ethylene/L for 24 h at 20°C and finally ripening at 20°C

Means within each column followed by the same letter are not significantly different at $P = 0.05$ ($n = 20$)

Treatment	Days to ripe	Weight loss/day (%)	Black tips	Grey flesh	°Brix	TA
Untreated	5.9b	1.3b	2.6b	1.6a	28.0c	0.2b
Ethylene	3.4a	1.5c	2.0a	2.1b	24.4a	0.1a
1-MCP	9.3c	1.2b	3.2c	2.1b	26.6b	0.2b
1-MCP + ethylene	9.1c	1.1a	3.2c	2.1b	27.2bc	0.2b
l.s.d. ($P = 0.05$)	0.6	0.08	0.3	0.2	1.0	0.02

Table 4. Days for fruit to reach the eating soft stage (days to ripe at 20°C), percentage weight loss per day and percentage of fruit surface area affected by stem rots in 'Kensington Pride' mango fruit treated with 25 µL 1-MCP/L for 14 h at 20°C followed by exposure to 100 µL ethylene/L for 24 h at 20°C and finally ripening at 20°C

Means within each column followed by the same letter are not significantly different at $P = 0.05$ ($n = 20$)
Stem rots data are angular transformed, with back-transformed means in parentheses

Treatment	Days to ripe	Weight loss/day (%)	Stem rots
Untreated	13.6b	0.3a	9.6b (2.8)
Ethylene	7.9a	0.4b	1.3a (0.1)
1-MCP	18.7c	0.3a	18.0c (9.6)
1-MCP + ethylene	18.2c	0.3a	25.8d (18.9)
l.s.d. ($P = 0.05$)	0.4	0.03	7.2

the percentage of the skin with green colour at ripe, *C* and h° values of ripe skin, body rots, °Brix and TA (data not shown).

Papaya

There were no significant interactions between 1-MCP and ethylene for any of the parameters measured nor any significant effect of ethylene, thus only the main effects for 1-MCP are presented.

1-Methylcyclopropene increased the DTR by 325% (Table 5), with some of the treated fruit taking up to 25 days to reach the ripe stage. The severity of external blemishes and rots increased in association with 1-MCP treatment. There was a significant but small increase in °Brix with 1-MCP treatment.

Table 5. Days for fruit to reach the eating soft stage (days to ripe at 20°C), percentage weight loss per day during ripening, the percentage of the fruit surface area with blemishes, stem and body black rots and anthracnose, and the °Brix, of 'Solo' papaya fruit treated with 25 µL 1-MCP/L for 14 h at 20°C followed by exposure to 100 µL ethylene/L for 24 h at 20°C and finally ripening at 20°C

Means within each row followed by the same letter are not significantly different at $P = 0.05$ ($n = 40$)
Where appropriate, data are angular transformed, with back-transformed means in parentheses

Quality	Nil 1-MCP	1-MCP	l.s.d. ($P = 0.05$)
Days to ripe	4.8a	20.4b	1.6
External blemishes	1.1a	3.1b	0.3
Stem black rots	5.8a (1.0)	6.9b (1.4)	0.4
Body black rots	5.7a (1.0)	6.4b (1.2)	0.4
Anthracnose	5.9a (1.1)	7.0b (1.5)	0.7
°Brix	11.09a	11.47b	0.35

Discussion

Exposure of fruit to ethylene can stimulate ripening and senescence (Burg and Burg 1962). 1-Methylcyclopropene has been shown to counteract this effect in apple, banana and tomato (Sisler *et al.* 1996; Fan *et al.* 1999; Macnish *et al.* 2000*a*). The present results confirm similar responses in avocado, custard apple and mango. 1-Methylcyclopropene can also delay senescence in ornamentals (Porat *et al.* 1995; Newman *et al.* 1998; Macnish *et al.* 2000*b*) and broccoli (Ku and Wills 1999).

The ability of 1-MCP to inhibit ethylene action is thought to be due to irreversible binding to ethylene receptors in the tissue (Sisler and Serek 1997). Ripening is triggered again when a critical concentration of ethylene receptors is reached due to continued synthesis (Jiang *et al.* 1999*b*). Presumably, the greater the delay in ripening, the slower the rate of accumulation of new ethylene receptors.

Sisler and Serek (1997) found that banana fruit ripening was delayed by 1-MCP concentrations as low as 0.7 nL/L for 24 h, and Macnish *et al.* (2000*a*) and Fan *et al.* (1999) observed that inhibition of banana and apple ripening reached a maximum with 1 µL 1-MCP/L for 12–20 h at about 20°C, respectively. The 1-MCP concentration used in the present experiment (25 µL/L) was similar to those used by Macnish *et al.* (2000*a*), but considerably lower than the relatively high concentrations (45–450 µL 1-MCP/L for 1–6 h) used by Golding *et al.* (1998). These studies indicated that the extent of ripening inhibition of banana fruit is related to 1-MCP treatment concentration, duration and temperature, while the rate of receptor turnover is also an important factor. It is assumed that these different treatment conditions influence the percentage of receptor sites inactivated, thereby affecting the time required for sufficient sites to become available for an ethylene response to be elicited (Sisler and Serek 1997). The greater effect of 1-MCP in delaying papaya ripening may indicate a slow turnover of receptors in this fruit. Thus, effective 1-MCP treatment concentrations might be considerably lower than those used in the present study.

The ability of 1-MCP to extend DTR in the absence of exogenous ethylene implies that it also has the ability to prevent the action of endogenous ethylene, as well as negating the effects of exogenous ethylene. Similar results were obtained by Macnish *et al.* (2000*a*) with banana.

Porat *et al.* (1999) found that 1-MCP treatment had no significant effect on orange fruit total soluble solids or acid content, and Golding *et al.* (1998) reported that sugar composition in ripe banana pulp was generally unaffected by 1-MCP treatment. These results are similar to the present small or nil effects of 1-MCP on °Brix and TA in the flesh of ripe custard apple, mango and papaya fruits, suggesting that it is likely to have little significant effect on these components of eating quality.

Recent studies have shown that 1-MCP treatment can reduce scald and core flush in apple during cold storage (Fan and Mattheis 1999; Watkins *et al.* 2000). However, the present results showed that 1-MCP may be associated with external blemishes in custard apple and papaya. There are also differing results in relation to the effects of 1-MCP on diseases in ripe fruit. For example, Mullins *et al.* (2000) found no effect of 1-MCP on *Penicillium digitatum* severity in grapefruit, while Porat *et al.* (1999) found that 1-MCP treatment of 'Shamouti' oranges increased the development of mould rots caused by *P. digitatum* and *P. italicum*, and stem-end rot caused by *Diplodia natalensis*. Likewise, Ku *et al.* (1999) reported that treatment of strawberry fruit with 50 nL 1-MCP/L and above accelerated the development of rots compared with fruit treated with lower 1-MCP concentrations. Both Porat *et al.* (1999) and Ku *et al.* (1999) concluded that either low endogenous ethylene concentrations produced by these fruit were required to maintain their natural resistance against pathogens, or that 1-MCP was imposing a toxic, yet unknown effect on fruit tissue.

The effects of 1-MCP on diseases in this study may be associated with changes in natural antifungal compounds in ripening fruit (Prusky and Keen 1993). Treatments that delay ripening but do not maintain antifungal concentrations will result in antifungal concentrations falling below the critical levels required to prevent disease development before the fruit are ripe. In the current experiments, it is feasible that the decrease in antifungal concentrations during fruit ripening (as observed in other studies; Prusky and Keen 1993) was not affected by 1-MCP treatment but that fruit ripening was delayed, resulting in fruit close to the full ripe stage having lower antifungal concentrations, and potentially more disease. Such a mechanism is also supported by the fact that avocado fruit which ripen more slowly, generally have more disease when ripe (Hopkirk *et al.* 1994).

The increased disease severity in mango after 1-MCP and ethylene treatment may be associated with the added stress to the fruit induced by ethylene treatment. Similar mechanisms may be involved in the increased severity of anthracnose in citrus following ethylene treatment for degreening (Wild 1990).

Joyce *et al.* (1999) and Macnish *et al.* (2000*a*) showed that there is considerable potential to manipulate ripening behaviour of banana by varying the concentration, duration and temperature of 1-MCP treatment, and altering the relative timing of ethylene and 1-MCP application. Hence, 1-MCP could be used to prevent undesirable ripening during storage or transport, followed by ethylene treatment to induce ripening when required. Alternatively, first treating with ethylene to allow partial ripening, then with 1-MCP could delay further ripening of partially ripened fruit. It may be possible to use these treatments to achieve considerable control over ripening of climacteric fruits. Such treatments would be particularly beneficial for climacteric subtropical and tropical fruit because of their rapid ripening.

In summary, this study has demonstrated the potential of 1-MCP to delay ripening in a number of subtropical and tropical climacteric fruits, which is similar to the responses observed with other fruits. Quality was only slightly compromised in most instances by small increases in disease severity. The interaction between 1-MCP, ethylene and disease needs to be confirmed with fruit from other locations and seasons, but it is possible that additional disease control measures, and/or fruit from healthier orchards are required if delayed ripening by 1-MCP treatment is to be considered.

Acknowledgments

We thank Jenny Barker for technical assistance and Matt Adkins for assistance with the paper presentation. We also thank the Department of Primary Industries, Queensland, and the Australian Centre for International Agricultural Research for financial assistance.

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Received 25 September 2000, accepted 23 January 2001