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Research article

Ethylene regulates peel spotting in fruit of cv. Sucrier banana (*Musa acuminata*, AA Group): Dependence on ripening stage

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Abstract

Importance of the work: Ethylene can induce banana ripening. Therefore, ethylene may be involved in the development of peel spotting in bananas.

Objectives: To test the effect of ethylene on peel spotting in bananas (cv. Sucrier) in two stages of development.

Materials & Methods: In the first study, fruits ripened to stage 3 (light green-yellow) were placed in airtight plastic containers and manipulated using carbon dioxide scrubber or ethylene absorbent, or both. In the second and third studies, fruit ripened to either stage 1 (green) or stage 3 were exposed to ethylene or 1-methylcyclopropene (1-MCP). Changes in spotting were monitored.

Results: Removal of carbon dioxide or ethylene, or both had no significant effects on peel spotting of fruit in stage 3 (light green-yellow skin color) whereas spotting was reduced by ethylene during stage 1 (green skin color) but fruit in stage 3 was not affected. The 1-MCP treatment had no effect on peel spotting of fruit in stage 3, but in stage 1 fruit, the 1-MCP treatment clearly delayed the early development of the disorder.

Main finding: The development of peel spotting in the early stage of fruit ripening in this cultivar was regulated differently by endogenous and exogenous ethylene. In contrast, the development of peel spotting in stage 3 or later was independent of endogenous ethylene.

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Introduction

Senescent spotting or peel spotting is an important physiological disorder of banana and is sometimes misunderstood as being a pathological disease (Meredith, 1961). However, the microorganisms causing diseases that can be found in the spotted areas are the result of secondary infections (Ketsa, 1995) and senescent spotting should be classified as a non-pathogenic disease or disorder (Wardlaw, 1961). Senescent spotting is widespread in both local and export markets so that this problem can become a major constraint for banana growers and traders, and for export development (Ketsa and Wisutiamonkul, 2022). The development of senescent spotting is strongly influenced by cultivar (Ketsa and Wisutiamonkul, 2022). For example, in cv. Sucrier (*Musa acuminata*, AA Group), peel spotting starts to develop at an early stage of ripening, initially as small brown spots that subsequently become enlarged and prominent during the late ripening stages. Other cultivars with the same genome composition and AA ploidy as cv. Sucrier (also known as 'Kluai Khai' in Thailand (Valmayor et al., 2000), commonly have peel spotting which becomes severe in overripe fruit (Ketsa and Wisutiamonkul, 2022). Peel spotting can also be found in the overripe fruit of other cultivars, such as Grande Naine (*Musa acuminata*, AAA Group, Cavendish type) (Liu, 1976), and Gros Michel (*Musa acuminata*, AAA Group, non-Cavendish type); however, it is seldom present and is never as intense as that in cv. Sucrier (Ketsa, 2000).

Ethylene may be directly or indirectly involved in the development of peel spotting in banana fruit, similar to its involvement in the brown spotting found in iceberg lettuce (Ke and Saltveit, 1988). Ethylene was the cause of increased phenylalanine ammonia lyase and peroxidase activity, both thought to regulate the rate of browning (Ke and Saltveit, 1988). Furthermore, ethylene enhanced the accumulation of many free phenolic compounds, which are the substrates of browning reactions (Ke and Saltveit, 1988). Liu (1976) reported that ethylene inhibited the development of senescent spots in banana cv. Valery fruit (*Musa* spp. AAA, Cavendish subgroup). The compound 1-methylcyclopropene (1-MCP), is an effective antagonist of ethylene action (Blankenship and Dole, 2003). It has been shown to delay the ripening processes of several climacteric fruits (Adkins et al., 2005; Bron and Jacomino, 2009; Piriyavinit et al., 2011; Bulens et al., 2012; Wisutiamonkul et al., 2017) including banana (Jiang et al., 1999; Golding et al., 1998; Ketsa et al., 2013). 1-MCP not only blocks the ethylene receptor, but also inhibits ethylene production if this production is autocatalytic (Watkins, 2006).

Therefore, ethylene may be involved in the formation of peel spotting in cv. Sucrier and these responses may be dependent on the stage of fruit development. The current research was undertaken to specifically test the effect of ethylene on peel spotting in cv. Sucrier at two stages of fruit development. The different treatments were: 1) the removal of ethylene; 2) the application of ethylene at varying concentrations; and 3) the application of 1-MCP.

Materials and Methods

Plant material

Fruit of the banana (*Musa acuminata*, AA Group) cv. Sucrier, locally known as 'Kluai Khai' (Valmayor et al., 2000), were harvested at commercial maturity (green color stage 1) from a commercial plantation. Individual banana fingers were approximately 12 cm in length and 4 cm in diameter. Banana bunches were transported in a refrigerated truck (25 °C) to the laboratory within 2 h of harvest. After selection for uniformity in size and color, the banana bunches were rinsed in 200 µg/L Clorox and dried at 29–30 °C and relative humidity (RH) of 70–75%, with light conditions of 12 h/d daylight to develop to color stage 3 (approximately 2–3 d). The stages used to define banana ripening color were stage 1: green; stage 2: light green (breaking color to yellow); stage 3: light green-yellow; stage 4: light yellow (more yellow than green); stage 5: yellow with green tips; stage 6: yellow; and stage 7: yellow, flecked with brown (Anon, 1972). Fruit in stages 1–3 do not show peel spotting in this cultivar (Ketsa and Wistiamonkul, 2022). Either three or six replications, each consisting of two hands per container, were used in the various treatments (see above). Ten fingers from two rows of the individual hands were sampled to determine the color, firmness, SS concentration and TA, while individual hands were evaluated for peel spotting.

Removal of ethylene and carbon dioxide

Two hands of bananas at development stage 3 were placed in airtight, 15 L plastic containers maintained at 25 °C. The containers were connected to an air supply (100% RH), using a flow rate of 125 mL/min. An ethylene absorbent was made by soaking pieces of chalk (7 mm diameter, 5 mm long) in saturated potassium permanganate solution. About 50 g of

the dried chalk material was placed in a perforated polyethylene sachet (Ketsa et al., 2000). One sachet of absorbent was placed inside each plastic container and was renewed daily. In other treatments, excess carbon dioxide was removed by placement of 50 mL 1N KOH in each 15 L container. The solution of KOH was not changed daily. Combined ethylene absorbent (EA) and carbon dioxide scrubber (CS) were also used to complete the range of treatments. The concentrations of ethylene and carbon dioxide were measured regularly in each container (see below). Each treatment had six replications (containers). The experiments were conducted in 12 h/d daylight.

Ethylene treatment

Hands of banana fruit at either stage 1 or stage 3 were placed separately in the plastic chambers (37 cm × 47 cm × 35 cm) with a continuous flow of air containing 0 µL/ L ethylene gas (control), 5 µL/ L ethylene gas or 10 µL/ L ethylene gas. The temperature in the chambers was 25 °C and RH was 70–75%. All banana fruits were kept continuously in these containers for up to 7 d. Each treatment had three replications (containers) and there were two hands in each container.

1-methylcyclopropene treatment

Hands of banana fruit in stage 1 were placed in a sealed plastic chamber (37 cm × 47 cm × 35 cm) containing 1-MCP powder (EthylBlock®) in a small glass bottle taped to the inside chamber wall. The chamber lid was sealed and 1 mL of water was injected into the glass bottle containing the EthylBlock® using a hypodermic needle through a septum in the lid. A substantial amount of the total 1-MCP content was released immediately after the addition of the water to the powder. The chambers were used to expose the bananas to concentrations of 0 nL/L 1-MCP or 1,000 nL/L 1-MCP. The chambers remained sealed for 4 h at 25 °C. Then, the bananas were removed from the chambers and were individually placed in plastic baskets at 25 °C (70–75% RH) for further examination. Each treatment had three replications and there were two hands in each replication. The experiments were conducted in 12 h/d daylight.

Determination of ethylene, carbon dioxide and oxygen concentration

Concentrations of ethylene, carbon dioxide and oxygen within the sealed plastic containers containing the banana hands were measured using a syringe to secure an air sample through a sampling port. The ethylene, carbon dioxide, and oxygen concentrations were measured using the method of Uthaichay et al. (2005). The samples were injected into a gas chromatograph equipped with a flame ionization detector (Shimadzu; Tokyo, Japan) for ethylene analysis and with a thermal conductivity detector (Shimadzu; Kyoto, Japan) for determining the carbon dioxide and oxygen concentrations. The gas concentrations of the carbon dioxide and oxygen were expressed in kilopascals, while the ethylene was expressed in microliters per liter. Samples were taken every day at 10.00 a.m. from each of the containers.

Determination of color, firmness, soluble solids concentration and titratable acidity

Five individual fingers from each hand were randomly sampled for determination of peel and pulp color, firmness, soluble solids (SS) concentration and titratable acidity (TA). The peel and pulp color were determined using a colorimeter (Model CR-300, Minolta, Osaka, Japan) to record the L* and b* values (Hunter scale). Banana firmness with and without peel was determined using an Effegi firmness tester (Alfonsine, Italy) with a spherical 1.1 cm diameter plunger. The plunger was inserted to a depth of 5 mm and the necessary force was recorded in newtons. The total soluble solids (TSS) concentration of the pulp was measured using a hand refractometer (Atago; Japan) and the TA in the pulp was determined based on titration with 0.0998 N NaOH, using phenolphthalein as the indicator and expressed as the percentage of citric acid.

Assessment of peel spotting

Individual fingers were rated for overall visual symptoms of peel spotting. The spotting was estimated using a scale of 1–8, where 1 was no spotting and 8 was severe spotting, based on the method of Maneenuam et al. (2007) where score 1 = peel is yellow without spots; score 2 = the surface is a little darker yellow, some brownish spots occur, small as the point of a needle; score 3 = spots found all over the surface, their number, intensity of browning, and size (now up to 0.5–1 mm) increasing; score 4 = most spots still separated but increasing in size; score 5 = size of spots enlarged with sometimes overlap; score 6 = size and darkness of the spots has further increased; score 7 = spots sometimes overlap into larger patches; and score 8 = spots are darker, or are even black and form sunken pits on the surface.

Statistical analysis

Analysis of variance was performed and followed by mean comparisons using Duncan's multiple range test or least significant difference using the SPSS Statistics version 23.0 software (IBM Corp.; New York, NY, USA). The tests were significant at $p < 0.05$.

Results

Color, firmness, total soluble solids concentration and titratable acidity

In fruit treated in either stages 1 or 3 with 0 µL/L ethylene, 5 µL/L ethylene, or 10 µL/L ethylene, the a values of the pulp, L values of the peel and pulp, the peel firmness and the TA of the pulp were not significant different (Table 1), the b values of the peel,the pulp firmness and the TSS of the pulp were significantly different between the control and the ethylene treatments (Tables 2, 3 and 4). Respective mean values for peel, pulp and TA parameters in columns on day 0 (CI 1 and CI 3) were 65.25, 80.99, -18.22, -6.59, 33.82, 4.36 N and 0.53 (%), respectively (Table 1).

Fruit in development stages 1 and 3, treated with concentrations of 0 nL/L 1-MCP and 1,000 nL/L 1-MCP for 4 h, there was no effect on the color of the peel or pulp (data not shown). In contrast, the 1-MCP treatment resulted in higher firmness of the peel on both days 4 and 8 (Table 5), while 1-MCP treatment did not affect the firmness of the pulp on day 8 (Table 5). Fruit in the 1-MCP treatment had a significant reduction in soluble solids concentration on day 4 (Table 6).

Table 2 Values of b* of peel banana cv. Sucrier with and without ethylene treatments, where onset of treatment started when fruit had reached color index (CI) 1 and 3

Color index	Ethylene treatment (µL/L)	b* value		
		Initial time (unripe)	Day 0	Day 6
CI 1	0	30.40±0.8	34.00±0.7	29.93±4.3[a]
	5			28.43±5.6[a]
	10			27.00±1.1[a]
CI 3	0		34.93±0.3	18.33±3.5[b]
	5			15.87±4.3[b]
	10			19.43±1.7[b]
	F test		0.103	0.002

Mean±SD values in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 1 Values of L* of peel, L* of pulp, a* of peel, a* of pulp, peel firmness and titratable acidity (TA) of banana cv. Sucrier with and without ethylene treatments on day 6, where onset of treatment (day 0) started when fruit had reached color index (CI) 1 and 3

Color index	Ethylene treatment (µL/L)	L* Peel	L* Pulp	a* Peel	a* Pulp	b* Pulp	Peel firmness (N)	TA (%)
CI 1	0	54.57	71.90	4.23	-4.60	33.83	0.42	0.40
	5	55.07	73.50	4.07	-4.73	34.57	0.32	0.38
	10	55.23	72.90	3.67	-4.97	33.83	0.26	0.40
CI 3	0	52.83	71.37	4.47	-4.50	32.73	0.38	0.35
	5	51.90	72.13	3.97	-4.47	32.80	0.31	0.35
	10	54.80	72.97	3.80	-4.43	32.07	0.40	0.37
	F-test	0.76	0.69	0.74	0.39	0.51	0.12	0.44

Table 3 Total soluble solids (TSS) of banana cv. Sucrier with and without ethylene treatments, where onset of treatment started when fruit had reached color index (CI) 1 and 3,

Color index	Ethylene treatment (µL/L)	TSS (%)[1]		
		Initial time (unripe)	Day 0	Day 6
CI 1	0	0.73±0.1	2.07±0.1	5.80±0.0[a]
	5			5.20±0.0[c]
	10			5.47±0.1[abc]
CI 3	0		2.40±0.2	5.60±0.3[ab]
	5			5.20±0.2[c]
	10			5.40±0.2[bc]
	F test		0.067	0.014

Mean±SD values in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 4 Titratable acidity (TA) banana cv. Sucrier without and with ethylene treatments, where onset of treatment started when fruit had reached color index (CI) 1 and 3, results are means ± SD from three replicates and each replicate had 10 bananas

Color index	Ethylene treatment (µL/L)	TA (%)[1]		
		Initial time (unripe)	Day 0	Day 6
CI 1	0	0.28±0.03	0.53±0.10	0.40±0.05
	5			0.38±0.03
	10			0.40±0.05
CI 3	0		0.52±0.10	0.35±0.05
	5			0.35±0.00
	10			0.37±0.03
	F test		0.854	0.440

Mean±SD values in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 5 Firmness of cv. Sucrier peel and pulp in stages 1 and 3, where bananas had been subjected to 1-MCP treatment

Stage	1-MCP treatment	Peel firmness (N)			Pulp firmness (N)		
	(nL/L)	Day 0	Day 4	Day 8	Day 0	Day 4	Day 8
1	0	20.66±1.1	10.79±1.1^{c}	10.32±0.9^{b}	10.87±0.8	9.25±0.9^{a}	7.77±0.7
	1,000	-	15.80±1.2^{a}	14.78±1.0^{a}	-	9.70±0.8^{a}	8.00±0.9
3	0	19.12±1.2	12.02±0.9^{c}	9.96±0.8^{b}	10.32±0.9	8.39±0.7^{b}	7.28±0.8
	1,000	-	13.99±0.9^{b}	10.89±0.8^{b}	-	8.44±0.7^{b}	8.59±0.9
	F test	-	**	**	-	**	ns

Mean±SD values in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 6 Total soluble solids (TSS) concentration of cv. Sucrier pulp in stages 1 and 3 where hands had been subjected to 1-MCP treatment. Results are means ± SD from three replicates and each replicate had 10 fingers

Stage	1-MCP treatment	TSS (%)[1]		
	(nL/L)	Day 0	Day 4	Day 8
1	0	15.00±1.1	20.69±1.2^{a}	21.60±1.1
	1,000	-	18.50±1.3^{b}	21.50±0.9
3	0	14.90±1.0	20.00±1.0^{a}	23.00±1.0
	1,000	-	18.20±1.1^{b}	21.90±0.9
	F test	-	**	ns

Mean±SD values in the same column superscripted with different lowercase letters are significantly ($p < 0.05$) different

Peel spotting development with ethylene removal

Peel spotting of the fruit in stage 3 increased steadily from day 1 onward (Table 7). No significant differences in peel spotting were found between fruit in the control containers with air and the containers with EA (Table 7). Similarly, no effect was found in those treatments where excess carbon dioxide was removed. The oxygen concentrations in the containers were in the range 18–19% in all treatments from day 2 onward (Fig. 1A). In contrast, the carbon dioxide concentrations tended to be lower in the treatments that included the scrubber for carbon dioxide (CS); however, the differences among the treatments were small (Fig. 1B). The ethylene concentrations in the control were higher than those in the containers with EA, CS or the combined EA and CS treatments. There were no significant differences among the latter treatments (Fig. 1C).

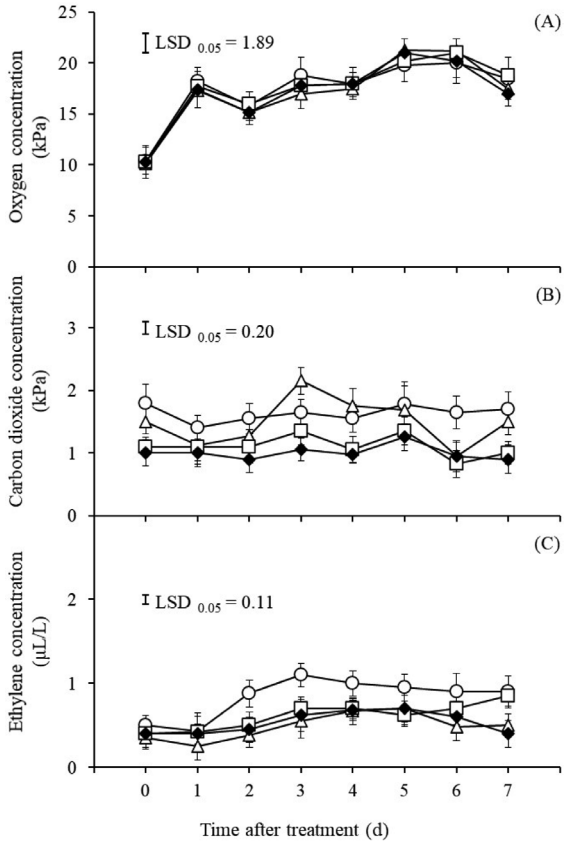

Fig. 1 Concentrations of oxygen (A), carbon dioxide (B) and ethylene (C) in plastic containers holding cv. Sucrier bananas in stage 3, where plastic containers had either no carbon dioxide scrubber (CS) or ethylene absorbent (EA) (○), or included the EA (Δ), CS (□) or the EA + CS (♦). Error bars represent SD; Different lowercase letters above bars indicate significant ($p < 0.05$) difference within same days.

Table 7 Peel spotting scores of cv. Sucrier banana stage 3 without and with ethylene, carbon dioxide scrubber (CS) and ethylene absorbent (EA)

Treatment	Spotting score							
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	1±0.1	1.2±0.2	2.0±0.2	3.3±0.2	4.5±0.2	5.5±0.2	6.2±0.3	6.7±0.3
EA	1±0.1	1±0.1	1.8±0.1	3.5±0.3	4.3±0.3	5.3±0.2	6.3±0.2	6.8±0.3
CS	1±0.2	1±0.1	2.0±0.2	3.3±0.3	4.7±0.2	5.5±0.3	6.5±0.2	6.8±0.2
EA + CS	1±0.2	1±0.2	2.7±0.2	3.7±0.2	5.0±0.3	5.5±0.2	6.3±0.3	6.7±0.2
F test	ns	ns	ns	ns	ns	ns	ns	ns

EA = ethylene absorbent; CS = carbon dioxide scrubber

Impact of ethylene on peel spotting development

At the onset of the treatments, irrespective of the developmental stage, individual fruit showed no peel spotting. Fruit in either stage 1 or stage 3 that were continuously exposed to ethylene at 5 µl/L or 10 µl/L (at 25 °C) showed slight spotting for the first 3 d of treatment. Then, peel spotting on fruit in both developmental stages increased steadily from day 4 onward (Fig. 2). Ethylene treatment significantly inhibited peel spotting in fruit in developmental stage 1 on days 3, 4 and 5 but not on day 6, although the effects in absolute terms were small. In contrast, no significant differences were found for fruit exposed to the two ethylene concentrations in development stage 3 (Fig. 2).

Impact of 1-MCP treatment on peel spotting

Fruit treated with 1,000 nL/L 1-MCP at 25 °C for 4 h resulted in a significant delay in peel spotting in development stage 1 on days 3–7 but not on day 8 (Fig. 3). In contrast, when fruit in development stage 3 was treated with 1-MCP, there was no effect on peel spotting (Fig. 3).

Discussion

Removal of the ethylene produced by banana fruit inside the treatment containers had no effect on the incidence of peel spotting when the fruit were in development stage 3. Equally, treatment of bananas in stage 3 with ethylene did not affect peel spotting. However, ethylene treatment with either 5 µL/L or 10 µL/L in stage 1 did delay the development of the disorder, albeit by only a small amount, especially by day 6. Treatment with a range of 1-MCP concentrations was also ineffective in changing peel spotting where fruit had reached developmental stage 3. These data strongly suggested that ethylene was not involved in inducing peel spotting but may be implicated in reducing it. The 1-MCP treatment of fruit in stage 1 (green fruit) resulted in a delay in the development of peel spotting until at least day 7. These data suggested that by stage 3, peel spotting had become initiated, was irreversible and could not be delayed by reducing any ethylene effects. In contrast, when fruit was in stage 1, the peel spotting could be delayed by an inhibitor of ethylene action, indicating that peel spotting was regulated by endogenous ethylene in this developmental stage.

Excess carbon dioxide can reduce the effects of ethylene (de Wild et al., 1999, 2005). Therefore, care should be taken to remove all excess carbon dioxide before concluding that ethylene has no effect, as the absence of an effect might be due to the presence of a high carbon dioxide concentration. In the present study, a flow-through system was used that prevented the accumulation of carbon dioxide, while potassium hydroxide was used to remove any excess carbon dioxide. This resulted in a reduced level of carbon dioxide compared to the control which did not have the potassium hydroxide treatment.

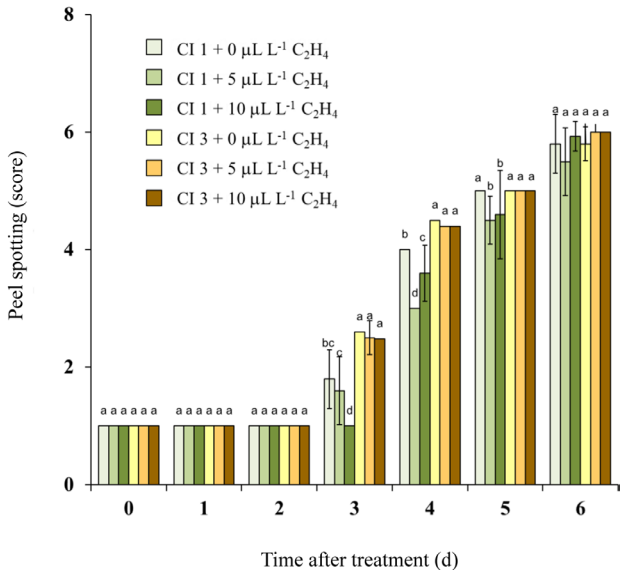

Fig. 2 Peel spotting of cv. Sucrier bananas without and with ethylene treatment, where fruit in stage 1 (green color: ■, ■, ■) or 3 (yellow color: ■, ■, ■) were continuously treated with 0 µL/L (■, ■), 5 (■, ■) or 10 µL/L (■, ■) ethylene at 25°C for 6 d. Error bars represent SD; Different lowercase letters above bars indicate significant ($p < 0.05$) difference within same days.

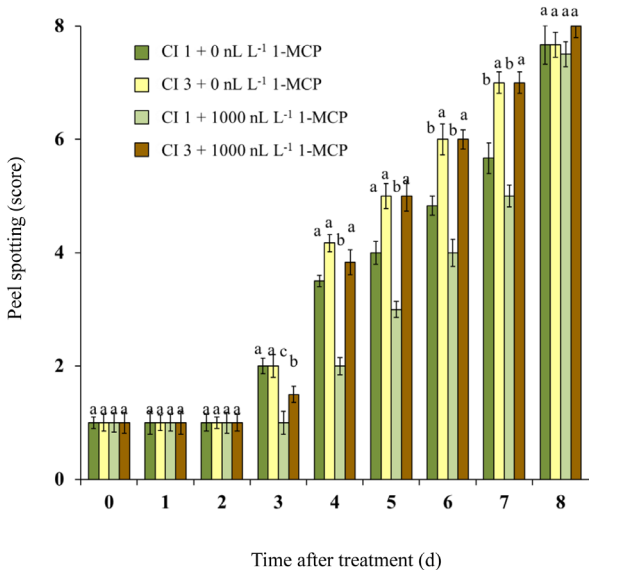

Fig. 3 Peel spotting of cv. Sucrier bananas, in stage 1 (■, ■) or 3 (■, ■) and treated with 0 nL/L 1-MCP (■, ■) or 1,000 nL/L 1-MCP (■, ■) at 25°C for 4 h, prior to shelf life at 25°C, Error bars represent SD; Different lowercase letters above bars indicate significant ($p < 0.05$) difference within same days.

The ethylene concentration in the control treatment was greater than that in other treatments because the control treatment did not include an EA which could lower the ethylene concentration (Ketsa et al., 2000). Carbon dioxide concentrations in the CS treatments were detectable throughout the experiment. These levels of carbon dioxide, although low, might have been able to partially inhibit ethylene production (Quazi and Freebairn, 1970). Another possibility was that ethylene could be dissolved in potassium hydroxide solution, similar to carbon dioxide but less soluble, thus lowering the ethylene concentrations in containers.

1-MCP delayed ripening (indicated by peel firmness) when applied in fruit stage 1 (Golding et al., 1998; Jiang et al., 1999; Ketsa et al., 2013), while it did not delay ripening when applied in stage 3. This suggested that by developmental stage 3, ripening had been irreversibly initiated and could not be delayed by reducing the effects of ethylene (Prasanna et al., 2007). However, the data did not show that peel spotting was causally connected to ripening. Peel spotting development in stage 1 (green) and stage 3 (light green-yellow) from day 0 to day 2 (Figs. 2 and 3) was not significant. Peel spotting development, especially in stage 3 with more advanced ripening than in stage 1 could not be detected visually. However, peel spotting development on fruit in stage 3 was more advanced than that in stage 1 from day 3 onward until the end of experiment because fruit in stage 3 had more advanced ripening. When fruit became overripe, fruit spotting in either stage 1 or stage 3 was not significantly different (Figs. 2 and 3).

It may have been that peel spotting had already been initiated in the fruit used in the present study that had ripened to stage 3. Had this been the case, then that could explain why neither ethylene nor 1-MCP had no effect. However, other studies have shown that several methods can effectively reduce peel spotting in cv. Sucrier that had ripened to stages 3 and 4 either by modified atmosphere (Choehom et al., 2004), heat treatment (Trakulnaleumsai et al., 2006) or surface coating (Promyou et al., 2007). Apparently, these treatments inhibited peel spotting development after its initiation by endogenous ethylene and their inhibitory effects may be different from that of 1-MCP.

Liu (1976) reported that 10 mL/L ethylene in air, which was applied continuously, inhibited the development of senescent spotting in ripe cv. Valery banana (*Musa acuminata*, AAA, Cavendish subgroup) which had yellow and green tips (stages 4–5). In the present study, continuous ethylene application of 5 mL/L or 10 mL/L in air to cv. Sucrier banana fruit in development stage 1 had a significant but small inhibitory effect on peel spotting, but no significant effect on peel spotting and ripening of banana fruit when applied in stage 3. The cv. Sucrier belongs to the AA group which might explain the different result reported by Liu (1976), as differences in peel spotting between cultivars have been identified (Ketsa, 2000).

Both the 1-MCP and ethylene treatments did not inhibit peel spotting of banana fruit in stage 3, whereas 1-MCP inhibited peel spotting in developmental stage 1 but only in the first 2 days and not thereafter. The 1-MCP treatment resulted in a temporary delay of senescent spotting of banana in stage 1. One of the reasons suggested for this temporary effect of 1-MCP was the ongoing synthesis of new receptor protein, which will bind with ethylene. This binding would mean that ethylene would not be left to compete with 1-MCP (Jiang et al., 1999). Therefore, it was likely that the peel spotting process had already been initiated in the banana fruit by stage 3 and had become independent of the impacts of ethylene. This suggested that endogenous and exogenous ethylene may impact differently in the early development of peel spotting.

Ma et al. (2017) reported that exogenous ethylene inhibited peel browning in pear, where ethylene treatment significantly increased the activity of catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and radical scavenging activity but inhibited the activities of polyphenol oxidase (PPO) and peroxidase. The development of peel spotting in cv. Sucrier has been reported to be related to the presence of free radicals (Chotikakham et al., 2019, 2020). Therefore, the inhibition of peel spotting in cv. Sucrier by ethylene may have inhibited peel spotting of fruit in stage 1 through improving the activities of antioxidant enzymes (CAT, APX and SOD) and by reducing the activity of PPO (Ma et al., 2017).

The incidence of peel spotting in Cavendish banana (*Musa acuminata*, AAA Group) was delayed and diminished in fruit treated with 1-MCP either alone or in combination with a chitosan-based edible coating (Baez-Sañudo et al., 2009). Similarly, 1-MCP in combination with heat treatment significantly inhibited peel spotting in cv. Sucrier banana (Linh and Joomwong, 2011).

In conclusion, ethylene stimulus was required at an early stage of fruit development (stage 1, green) for the induction of peel spotting, while in stage 3 (light green-yellow) or later, the peel spotting process was independent of endogenous ethylene.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Adkins, M.F, Hofman, P.J., Stubbings, B.A., Macnish, A.J. 2005. Manipulating avocado fruit ripening with 1-methylcyclopropene. Postharvest Biol. Technol. 35: 33–42. doi.org/10.1016/j.postharvbio.2004.05.021

Anon. 1972. Banana Ripening Guide. Commonwealth Scientific and Industrial Research Organization (CSIRO). Division of Food Research Circular 8. Melbourne, VIC Australia.

Baez-Sañudo, M., Siller-Cepeda, J., Muy-Rangel, D., Heredia, J.B. 2009. Extending the shelf-life of bananas with 1-methylcyclopropene and a chitosan-based edible coating. J. Sci. Food Agric. 89: 2343–2349. doi.org/10.1002/jsfa.3715

Blankenship, S.M., Dole, J.M. 2003. 1-Methylcyclopropene: A review. Postharvest Biol. Technol. 18: 1–25. doi.org/10.1016/S0925-5214(02)00246-6

Bron, I.U., Jacomino, A.P. 2009. Ethylene action blockade and cold storage affect ripening of 'Golden' papaya fruit. Acta Physiol. Plant. 31: 1165. doi.org/10.1007/s11738-009-0336-x

Bulens, I., van de Poel, B., Hertog, M.L.A.T.M., de Proft, M.P., Geeraerd, A.H., Nicolai, B.M. 2012. Influence of harvest time and 1-MCP application on postharvest ripening and ethylene biosynthesis of 'Jonagold' apple. Postharvest Biol. Technol. 72: 11–19.

Choehom, R., Ketsa, S., van Doorn, W.G. 2004. Senescent spotting of banana peel is inhibited by modified atmosphere packaging. Postharvest Biol. Technol. 31: 167–175. doi.org/10.1016/j.postharvbio.2003.07.001

Chotikakham, S., Faiyue, B., Uthaibutra, J., Saengnil, K. 2019. Effects of methyl salicylate on senescent spotting and hydrogen peroxide concentration in ripening 'Sucrier' bananas. Acta Hortic. 1245: 115–125. doi: 10.17660/ActaHortic.2019.1245.17

Chotikakham, S., Faiyue, B., Uthaibutra, J., Saengnil, K. 2020. Exogenous methyl salicylate alleviates senescent spotting by enhancing the activity of antioxidative ascorbate-glutathione cycle in harvested 'Sucrier' bananas. Sci. Hortic. 267: 109324. doi.org/10.1016/j.scienta.2020.109324

de Wild, H.P.J., Balk, P.A., Fernandes, E.C.A., Peppelenbos, H.W. 2005. The action site of carbon dioxide in relation to inhibition of ethylene production in tomato fruit. Postharvest. Biol. Technol. 36: 273–280. doi.org/10.1016/j.postharvbio.2005.02.004

de Wild, H.P.J., Woltering, E.J., Peppelenbos, H.W. 1999. Carbon dioxide and 1-MCP inhibit ethylene production and respiration of pear fruit by different mechanisms. J. Exp. Bot. 50: 837–844. doi.org/10.1093/jxb/50.335.837

Golding, J.B., Shearer, D., Wyllie, S.G., McGlasson, W.B. 1998. Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. Postharvest Biol. Technol. 14: 87–98. doi.org/10.1016/S0925-5214(98)00032-5

Jiang, Y., Joyce, D.C., Macnish, J.D. 1999. Responses of banana fruit to treatment with 1-methylcyclopropene. Plant Growth Reg. 28: 77–82. doi.org/10.1023/A:1006222631666

Ke, D., Saltveit, M.E.Jr. 1988. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. Plant Physiol. 88: 1136–1140.

Ketsa, S. 1995. A study on mechanism of senescent spotting and its control in ripe Kluai Khai. Final project report. National Research Council of Thailand. Bangkok, Thailand.

Ketsa, S. 2000. Development and control of senescent spotting in banana. Food Preserv. Sci. 2: 173–178.

Ketsa, S., Wisutiamonkul, A. 2022. Postharvest physiological disorders of banana fruit: Finger drop, senescent spotting, and chilling injury. Hortic. Rev. 49: 971–69. doi.org/10.1002/9781119851981.ch3

Ketsa, S., Wongs-agee, C., Klein, J.D. 2000. Storage life and quality of 'Kluai Khai' banana fruit affected by modified atmosphere using bulk packaging. Thai J. Agric. Sci. 33:37–44.

Ketsa, S., Wisutiamonkul, A., van Doorn, W.G. 2013. Apparent synergism between the positive effects of 1-MCP and modified atmosphere on storage life of banana fruit. Postharvest Biol. Technol. 85: 173–178. doi.org/10.1016/j.postharvbio.2013.05.009

Linh, C.N., Joomwong, A. 2011. Effect of 1-MCP in combination with heat treatment on preservative quality of banana (cv. Kluai Khai) fruit. Agricultural Sci. J. 42: 341–344.

Liu, F.W. 1976. Ethylene inhibition of senescent spots on ripe bananas. J. Amer. Soc. Hort. Sci. 101: 684–686.

Ma, Y., Yang, M., Wang, J., Jiang, C.-Z., Wang, Q. 2017. Application of exogenous ethylene inhibits postharvest peel browning of 'Huangguan' pear. Front. Plant Sci. 7: 2029. doi.org/10.3389/fpls.2016.02029

Maneenuam, T., Ketsa, S., van Doorn, W.G. 2007. High oxygen levels promote peel spotting in banana fruit. Postharvest Biol. Technol. 43: 128–132. doi.org/10.1016/j.postharvbio.2006.08.007

Meredith, D.S. 1961. Fruit-spot ('speckle') of Jamaican bananas caused by *Deightoniella torulosa* (Syd.) Ellis: I. Symptoms of disease and studies on pathogenicity. Trans. Brit. Mycol. Soc. 44: 95–104. doi.org/10.1016/S0007-1536(61)80012-0

Piriyavinit, P., Ketsa, S., van Doorn, W.G. 2011. 1-MCP extends the storage and shelf life of mangosteen (*Garcinia mangostana* L.) fruit. Postharvest Biol. Technol. 61: 15–20. doi.org/10.1016/j.postharvbio.2011.02.007

Prasanna, V., Prabha, T.N., Tharanathan, R.N. 2007. Fruit ripening phenomena–An over view. Crit. Rev. Food Sci. Nutr. 47: 1–19. doi.org/10.1080/10408390600976841

Promyou, S., Ketsa S., van Doorn, W.G. 2007. Effect of surface coating on ripening and early peel spotting in 'Sucrier' banana (*Musa acuminata*). N.Z.J. Crop Hortic. Sci. New Zeal. J. Crop Hort. 35: 259–265.

Quazi, M.H., Freebairn, H.T. 1970. The influence of ethylene, oxygen, and carbon dioxide on ripening of bananas. Bot. Gaz. 131: 5–14.

Trakulnaleumsai, C., Ketsa, S., van Doorn, W.G. 2006. Temperature effects on peel spotting in 'Sucrier' banana fruit. Postharvest Biol. Technol. 39: 285–290. doi.org/10.1016/j.postharvbio.2005.10.015

Uthaichay, N., Ketsa, S., van Doorn, W.G. 2005. Effect of gas composition and relative humidity on senescent spotting in banana (*Musa acuminata* AA Group) fruit cv. Sucrier. Thai J. Agric. Sci. 38: 123–127.

Valmayor, R.V., Jamaluddin, S.H., Silayoi, B., Kusumo, S., Danh, L.D., Pascua, O.C., Espino, R.R.C. 2000. Banana Cultivar Names and Synonyms in Southeast Asia. International Network for the Improvement of Banana and Plantain. Los Banos, the Phillippines.

Wardlaw, C.W. 1961. Banana Diseases, Including Plantains and Abaca. John Wiley and Sons Inc., New York, NY, USA.

Watkins, C.B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24: 389–409. doi.org/10.1016/j.biotechadv.2006.01.005

Wisutiamonkul, A., Ampomah-Dwamena, C., Allan, A.C., Ketsa, S. 2017. Carotenoid accumulation in durian (*Durio zibethinus*) fruit is affected by ethylene via modulation of carotenoid pathway gene expression. Plant Physiol. Biochem. 115: 308–319. doi.org/10.1016/j.plaphy.2017.03.021