キャベンディッシュバナナの追熟抑制に対する1-MCPの複数回処理と外生エチレンの影響
Influence of Multiple Applications and Exogenous Ethylene on the Efficacy of 1-MCP in Delaying Ripening of ‘Cavendish’ Bananas

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Ethylene-free, mature-green ‘Cavendish’ bananas (Musa sp., AAA type) were subjected to multiple (3 X) applications of an ethylene antagonist 1-methylcyclopropene (1-MCP) for 24 hrs at 20°C to investigate the ripening response. By performing application of 1-MCP three times, it was found that a distinct ethylene evolution in treated bananas appeared at 27 days after treatment (DAT) whereas a ethylene evolution in untreated bananas appeared at 8 DAT. Single treatment was less effective in delaying ripening than repeated applications but the concentrations of 100 and 500 nE / L of 1-MCP affected the retardation to the same extent. Regardless of ripening period, the fruit firmness and breakdown of starch to sugar at the ripe stage were almost at the same level in both treated and untreated bananas, although some bananas changed abnormally in peel color. It is often observed that the untreated bananas demonstrated ethylene evolution at 4 DAT, much earlier than expected, thus we investigated the 1-MCP response of the bananas possibly contaminated by ethylene during transit or storage. The simultaneous presence of ethylene affected the effectiveness of 1-MCP treatment. At ≤ 0.5 nE / L ethylene, 20 nE / L 1-MCP was sufficient to obtain almost maximum delay in ripening. However, at 1.0 nE / L ethylene, 100 nE / L 1-MCP was necessary to obtain the maximum delay. These indicate a competitive relationship between ethylene and 1-MCP, the effect of exposure to ethylene during transit or storage could be canceled out by using higher concentrations of 1-MCP.

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Banana is the main fruit in international trade, ranking number one in value as an exported fruit. It is a highly perishable crop, with only a few days of shelf life once ripening has started. Refrigeration at 14°C remains the most popular way to transport and store bananas until they are ready for market distribution. However, such a storage facility may not always be available.

A novel compound called 1-methylcyclopropene (1-MCP) has been found to delay ripening of many fruits including bananas®-®. At the moment, the only workable option to maximize the utility of 1-MCP on banana fruits seems to be the application of 1-MCP at the mature green stage not yet induced to ripen with exogenous ethylene. Considering that fruits may quickly resume ethylene sensitivity in as early as three days after 1-MCP treatment®-®, a single application might be inadequate to fully curb ripening of green mature fruit until needed. In many other crops, multiple applications of 1-MCP have been reported to enhance the effect over a single application. However, the effects of 1-MCP application on fruit attributes have been varied including improvement in the acidity component of the flavor®-®, impairment of softening process®, and simply extended ripening®-®. In the case of bananas, multiple applications have not been tried yet. As it is beneficial to delay the onset of ripening, multiple applications could be useful in minimizing losses due to premature ripening during transit and storage.

In addition, during shipping or storage bananas

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are often exposed to exogenous ethylene produced from mechanical stresses, pathogen infections, other ripening fruits, and residual ethylene contamination in warehouses. Being antagonistic with 1-MCP at the binding site, the presence of such minute amounts of ethylene could possibly influence the effectiveness of 1-MCP. The objectives of this study were to: ① characterize and evaluate the effectiveness of multiple applications of 1-MCP on ripening of green mature ‘Cavendish’ bananas, and ② to determine whether or not the simultaneous presence of low concentrations of exogenous ethylene at the time of treatment affects the effectiveness of 1-MCP to delay the ripening of the treated banana.

Materials and Methods

1. Fruit preparation
Mature green Philippine-grown ‘Cavendish’ banana fruits untreated with ethylene were obtained from a local fruit distributor, Yoshioka Banana (Miyazaki, Japan), and sorted for defect-free fruit. The fingers were separated, randomly mixed, sterilized by soaking in 400mg/l thiaobendazole for 4 min, and then air-dried.

2. Overview of the experiments
Three experiments were conducted in this study. In experiment 1, mature-green bananas without exogenous ethylene application were subjected to triple-ℓ applications (3 X) of 100 nℓ / ℓ 1-MCP at 3-day intervals (0, 3 and 6 days) at 20°C. Then, single (1 X) and triple applications (3 X) of 100 and 500 nℓ / ℓ 1-MCP were performed in experiment 2. In the third experiment, 0, 20 and 100 nℓ / ℓ 1-MCP were applied to evaluate the efficiency of 1-MCP in the presence of 0, 0.1, 0.5, and 1.0μℓ / ℓ ethylene. The efficiency of 1-MCP was evaluated on the basis of the time of appearance of color index 6 of banana peel, firmness as well as the appearance of the climacteric respiratory and ethylene peaks. Time was measured from the day after the first treatment of 1-MCP (DAT). The experiments were terminated before the appearance of brown-black spots on the skin.

3. Application of 1-MCP and ethylene
1-Methylcyclopropene was applied in powder form as Smartfresh® (Rohm and Haas, Co.) according to the manufacturer’s specification of 1.6 g powder plus 25mℓ water to generate 1,000 nℓ / ℓ 1-MCP gas per cubic meter space. The first experiment, performed in duplicate, used 28-ℓ plastic incubation containers containing 50mℓ saturated potassium hydroxide with a pre-measured amount of Smartfresh® powder calculated to generate 100 nℓ / ℓ 1-MCP. After incubation for 24 hrs at 20°C, banana fruits were stored in open trays at >85% RH at 20°C. For 3 X application of 1-MCP, the treatments were repeated 3 days apart (0, 3 and 6 days) based on the findings that 1-MCP treated tissues resume ethylene sensitivity starting on the third day after treatment®. Treatments were replicated four times.

For the second experiment, three to four banana fingers were incubated inside an 8-ℓ jar together with the treatment and 30mℓ saturated potassium hydroxide to absorb CO₂ for 24h at 20°C. The banana fingers were subjected to a single or 3 X application of 100 or 500 nℓ / ℓ 1-MCP. The temperature and incubation period were the same as in the first trial.

The third experiment was also performed in duplicate and aimed at determining the efficiency of 1-MCP in the simultaneous presence of small amounts of ethylene. For this study, both 1-MCP and ethylene were injected immediately one after another into 8-ℓ jars containing four to five banana fingers and 30 mℓ saturated potassium hydroxide solution. Since this experiment involved very small quantities of Smartfresh® powder and minute amounts of ethylene, 1-ℓ stocks of each of 1-MCP and ethylene gases (175μℓ / ℓ and 10μℓ / ℓ, respectively) were initially prepared. The 1-MCP stock was prepared according to the manufacturer’s specification. The ethylene stock was formulated from pure ethylene gas in a cylinder and confirmed chromatographically. From these stocks, volumes of gas calculated to generate 20 and 100 nℓ / ℓ 1-MCP and 0.1, 0.5, and 1.0μℓ / ℓ ethylene were withdrawn and injected into the sealed incubation jars through a rubber septum. Prior to each withdrawal of gas from the stock bottle, an equal volume of water was first injected into the stock vessel to ensure maintenance of normal atmospheric pressure. The treatment duration and incubation temperature were the same as in the previous experiments.

4. Color evaluation
Peel color was evaluated using the 8-point color index (CI) where 1 = dark green, 2 = green with tinge of yellow, 3 = more green than yellow, 4 = more yellow than green, 5 = yellow with green tips, 6 = full yellow, 7 = yellow with spots, 8 = full ripe
stage with increasing number of spots. Objective color was measured in terms of hue angle at various intervals from the dorsal equatorial region of the finger using a Minolta Chromameter MC-2002. The L value was used to indicate peel browning. The ripening period was defined as the number of days after first treatment (DAT) until the fruits reached CI 6.

5. Respiration and ethylene evolution

Using the method of Kader (1992), carbon dioxide (CO₂) production and ethylene production were measured by gas chromatograph (GC) analysis of a 1 mL sample of the headspace gas accumulated over 1 hr or 2 hr in 3-l jars. For CO₂ determinations, the GC was equipped with a thermal conductivity detector and a stainless column packed with 60/80 silica gel. The temperature was set at 80°C, with He as the carrier gas supplied at 50 ml/min. For ethylene evolution, the GC was fitted with a flame ionization detector and a Gaskuro Pack 54 (80/100) steel column using N₂ as the carrier gas. The column temperature was also set at 80°C.

6. Physicochemical measurement

Whole fruit firmness was measured by puncturing at five equidistant points (proximal end, equatorial region, distal end and points in-between) with a hand-held penetrometer. Tissue from the equatorial region of the pulp was then sampled for carbohydrate analysis were frozen in a -80°C deep freezer until needed. The pH was measured from previously frozen pulp tissue homogenized using a homogenizer (Nihonseiki AM-3) at 2°C with 40 mL of cold 80% ethanol for 10 min at 10,000 rpm in an ice-water bath, then centrifuged at 4°C for 30 min at 3,000 g. Tissue residue was washed two more times with ethanol, dried in vacuo at ambient temperature, and was used for starch analysis. The ethanol supernatant were concentrated in vacuo at 40°C and reconstituted with 50 mL distilled water for use in glucose and sucrose content assays. Glucose content was assayed using a glucose assay kit (Wako Pure Chemical Industries, Japan). For sucrose content analysis, the sample was first hydrolyzed with an equal volume of 100:1 invertase liquid concentrate from yeast (4 g/mL/30 min saccharose activity at 20°C), pH 4.0 (Wako Pure Chemicals Industries, Japan), and then incubated at 25°C for 10 min. Glucose content was then determined using the glucose assay kit. Starch content analysis was performed following the AOAC method with slight modifications. Amylase from Bacillus amyloliquefaciens and glucoamylase from Rhizopus sp. reagents were obtained from Sigma Aldrich and Seikagaku Corporation, Japan, respectively.

7. Experimental design and statistical analysis

The first and second experiments were laid out in completely randomized design (CRD). Data from the first experiment were analyzed using analysis of variance (ANOVA) and the means separated by Student-Newman-Keuls (SNK) Test at 5% level of confidence. A t-test was used in the second experiment. The third experiment was laid out in duplicate for a factorial experiment of three 1-MCP concentrations (0, 10, and 100 nL/L) and four levels of ethylene (0, 0.1, 0.5, and 1.0 mL/L). The Least Significant Difference (LSD) at P < 0.05 was used to separate means.

Results and Discussion

1. Effect of 1-MCP multiple application on the climacteric rise of the treated banana

1-Methylcyclopropene (1-MCP) is a recently developed compound that inhibits ethylene action by irreversibly binding to ethylene receptors. Since banana fruits may recover quickly by regenerating ethylene receptors after 1-MCP treatment, more than one 1-MCP subsequent application may be necessary to block new ethylene receptors.

In the first experiment, green bananas without exogenous ethylene application were subjected to triple applications (3 X) of 100 nL/L 1-MCP for 24 hrs at 20°C and stored at 20°C to check the effectiveness of the treatment. A distinct ethylene peak for treated bananas appeared at 27 DAT, which was delayed by 19 days (Fig. 1 A) compared with the untreated bananas at 8 DAT. A delay of 19 days in ethylene production was almost coincident with a delay of 20 days in climacteric CO₂ (Fig. 1 A and 1 B). It was observed that the
started to lose firmness at 22 DAT and softened to the firmness level of the untreated bananas eight days later. The slow softening rate implies that 3 X-treated bananas could still be relatively firm by the end of their yellow stage while the untreated could have extremely softened by then. The yellow stage (CI 6) was achieved by the 3 X-treated bananas at 26 DAT while the untreated fruits similarly yellowed at 10 DAT.

Then, the second experiment was performed to investigate whether or not multiple application is more effective than a single one when either using 100 or 500 nR / R 1-MCP. In Table 1, untreated bananas reached CI 6 in 8.3 DAT whereas those X-treated either with 100 or 500 nR / R 1-MCP followed later at 18 DAT. When 3 X-treated, color change was further delayed for up to 25-26 DAT, regardless of concentration. Similarly, the onset of the climacteric rises in ethylene was also significantly delayed by 14 days in the case of 1 X-treatment and was delayed by more than 20 days on 3 X-treatment irrespective of concentration. One hundred and 500 nR / R 1-MCP were almost equally effective in delaying the onset of the climacteric rises and color change, whether applied 1 X or 3 X. Regardless of ripening period, however, the treated bananas had statistically comparable levels of pH and SSC to the untreated bananas at the full yellow stage although abnormal peel browning, as indicated by a lower $L^*$ value, was often observed among 3 X-treated bananas. GOLDING et al. (1998) and HARRIS et al. (2000) also reported peel color abnormalities but only indicated uneven coloration and "unacceptable" peel color.

Fig. 2 shows time courses of the breakdown of starch in both the untreated and 3 X-treated bananas in the experiment 1, which seemed to be concomitant with the accumulation of glucose and sucrose. Despite the differences in the time of onset of starch degradation and accompanying rise in glucose and sucrose, the residual levels of these carbohydrates at CI 6 were statistically the same in both the untreated and 3 X-treated bananas ($P < 0.05$), indicating that the starch degradation process nevertheless resumed normally and was completed despite the lengthy inhibition of ripening. These results indicated that multiple applications of 1-MCP on 'Cavendish' bananas can be effective in delaying the ripening process, the breakdown of starch and the accompanying accumulation of glucose and
Table 1 Some physiological and chemical characteristics of ‘Cavendish’ bananas treated once or three times with 1-MCP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of days to CI 6 $^a$</th>
<th>$L^*$ value at CI 6</th>
<th>pH $^b$</th>
<th>Soluble solid content ($^\text{Bx}$) $^c$</th>
<th>Respiration $^d$</th>
<th>Ethylene production $^e$</th>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>Rate (mgCO$_2$·kg$^{-1}$·h$^{-1}$)</td>
<td>Peak appearance (DAT) $^f$</td>
</tr>
<tr>
<td>Untreated</td>
<td>8.3±0.9 $^g$</td>
<td>59.4±0.8 $^g$</td>
<td>5.1±0.1</td>
<td>16.2±0.8 $^h$</td>
<td>72.5±4.0 $^i$</td>
<td>6.0±0.5 $^j$</td>
</tr>
<tr>
<td>100μl·l$^{-1}$, 1x</td>
<td>17.8±1.7 $^m$</td>
<td>56.1±1.5 $^m$</td>
<td>5.1±0.5</td>
<td>16.4±0.6 $^m$</td>
<td>102.7±3.5 $^i$</td>
<td>17.0±0.0 $^j$</td>
</tr>
<tr>
<td>100μl·l$^{-1}$, 3x</td>
<td>24.9±1.2 $^m$</td>
<td>49.9±1.4 $^m$</td>
<td>5.1±0.9</td>
<td>17.3±0.6 $^m$</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>500μl·l$^{-1}$, 1x</td>
<td>17.9±0.2 $^m$</td>
<td>57.5±1.6 $^m$</td>
<td>5.0±0.4</td>
<td>14.9±0.9 $^m$</td>
<td>92.3±9.1 $^i$</td>
<td>17.0±0.0 $^j$</td>
</tr>
<tr>
<td>500μl·l$^{-1}$, 3x</td>
<td>26.2±1.6 $^m$</td>
<td>59.0±0.8 $^m$</td>
<td>5.1±0.7</td>
<td>15.9±0.4 $^m$</td>
<td>n.d.</td>
<td>n.d.</td>
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</tbody>
</table>

$^a$means in a column with common letters are not significantly different at $P > 0.05$. Each data shows the average of three replicates $±$ standard deviation; $^b$days after treatment (DAT); $^c$sampled at CI 6; $^d$peak production rate. # No appearances of distinct peaks.

Consistent with the results from the many trials on 1-MCP effect on green bananas, this study has demonstrated that 1-MCP treatment of bananas with repeated applications or single application, can, no doubt, delay the ripening process in general and extend the post-harvest green life of the fruit.

The above results suggest that repeated application of 1-MCP can be an added option in managing ripening during transit or storage at the warehouse. A single treatment of 1-MCP concentration may not suffice as fruits may recover quickly by regenerating ethylene receptors as early as three days after 1-MCP treatment. Thus, if further extension of green life of bananas is desired, one or two 1-MCP subsequent exposures may be necessary to block new ethylene receptors. The ripening process is said to be triggered once the fruit has accumulated a certain threshold level of ethylene exposure. To delay ripening, therefore, is to interfere with this process by blocking ethylene receptors as soon as they are synthesized and before ethylene molecules can bind to them. A periodic application of a fresh dose of 1-MCP, the most potent ethylene receptor blocker known to date, could best serve this purpose.

Repeating the application enhanced the effect of single application in agreement with other studies on other crops, without again affecting the physico-chemical characteristics at the ripe stage. Since the balance between acidity and soluble solids roughly indicates the taste of the fruit, these results imply that a single or repeated 1-MCP applications on fruit could delay the ripening without incurring a large side effect on the sugar accumulation at the ripe stage. Other fruits had different responses to repeated exposures. A repeated application impaired the softening of avocado fruits, maintained the firmness of apples while prevention of loss of acidity was reported for tomatoes and apples.

2. Efficiency of 1-MCP in the simultaneous presence of exogenous ethylene

In the above experiment 2, untreated banana without exogenous ethylene application was found to give the climacteric peak at 4 DAT much earlier than expected. Fluctuations in ripening rates among lots of commercial banana commonly occur. Palayo et al. (2003) reported the variability in 1-MCP response, which probably depends on preharvest factors and postharvest handling. As for preharvest factors, difference in the maturation period of the
banana harvest was reported to bring a different green life\textsuperscript{26}. From harvesting to shipment at the port of call until the arrival at the warehouse, the bananas could be exposed to exogenous ethylene production from pathogenic infections, mechanical wounding, ripening of other fruits, or possibly from the residual ethylene in ripening rooms.

Thus we designed the third experiment to investigate the 1-MCP response of the banana possibly exposed to ethylene contamination. Since ripening could be initiated by as low as 0.1 $\mu $l / l ethylene and that pre-climacteric bananas could produce as much as 0.2 $\mu $l / l ethylene\textsuperscript{27}, the concentration of ethylene gas applied was set at 0 ~ 1.0 $\mu $l / l ethylene in the present study. In the third experiment, 0, 20, and 100 nl / l 1-MCP were applied to evaluate the efficiency of 1-MCP in the presence of 0, 0.1, 0.5 and 1.0 $\mu $l / l ethylene.

Fig. 3 shows the relative time of appearance of the climacteric peaks of respiration (Fig. 3 A) and ethylene (Fig. 3 B) at various combinations of ethylene and 1-MCP concentrations. Without 1-MCP application, the untreated bananas ripened naturally in the absence of ethylene and reached the climacteric peak at 15 DAT (Fig. 3 B). The prolonged absence of a detectable ethylene peak even throughout the unripe stage in untreated bananas guarantees that the bananas are free from the exogenously exposed ethylene gas during shipping and storage. Since the respiratory peak is often seen as a consequence of ethylene climacteric appearance\textsuperscript{3}, only Fig. 3 B illustrating ethylene production shall be used in the following discussion.

The efficiency of 1-MCP to delay ripening was very significantly affected by the amount of ethylene present ($p<0.001$). The application of 0.10 ~ 1.0 $\mu $l / l ethylene significantly advanced the appearance of the peak from 15 to 3 DAT depending on ethylene concentration. The higher the concentration of ethylene, the sooner the peak appeared. Thus, bananas treated with 1.0 $\mu $l / l ethylene ripened the earliest at 3 DAT whereas those exposed to 0.5 $\mu $l / l ethylene ripened at 9 DAT. The 0.1 $\mu $l / l ethylene effect was negligible and statistically similar as no ethylene at all ($p<0.05$).

When ethylene and 1-MCP were simultaneously applied, the ability of 1-MCP to delay the climacteric peak was influenced by the level of ethylene present in a concentration-dependent response. For instance, 20 nl / l 1-MCP was sufficient to maximize delay of climacteric ethylene appearance at 17.8 DAT in the presence of $\leq 0.5 $\mu $l / l ethylene. This is considerably close to the saturating concentrations of 10 nl / l\textsuperscript{29} and 50 nl / l 1-MCP\textsuperscript{29} reported for bananas. In contrast, when 1.0 $\mu $l / l ethylene was present, 20 nl / l 1-MCP was insufficient to cancel the effect of ethylene. Thus, 100 nl / l 1-MCP must be used to achieve the maximum delay at 18 DAT. The result suggests a competitive relationship between 1-MCP and ethylene when present at the same time. Ethylene and 1-MCP are said to saturate half of the binding sites, respectively, at 0.3 $\mu $l / l\textsuperscript{30} and at 17 nl / l\textsuperscript{30}. JIANG et al. (1999)\textsuperscript{30} investigated the response of ethylene-treated banana to subsequent treatment with 1-MCP at 20°C. The treatment of 1-MCP
delayed the ripening of ethylene pretreated fruits when applied 1 day after the pretreatment, and the relative response was increased with the 1-MCP concentrations (10 \~ 1,000 n\text{R}/\text{R}) . However the subsequent treatment was ineffective when applied at 3 or 5 days. Zhong et al. (2001) \textsuperscript{10} similarly found a competition between 0 \~ 160 n\text{R}/\text{R} 1-MCP and 0 \~ 20 \mu\text{R}/\text{R} ethylene when applied simultaneously in citrus fruits. As ethylene concentration increased, higher 1-MCP concentrations were required to inhibit ethylene-induced ripening. This is said to be typical of cyclopropene compounds (e.g. 1-MCP) which are found to be competitive if added with ethylene but do not appear to if the inhibitor is applied at 30 hours after ethylene treatment. It is typical of cyclopropene compounds (e.g. 1-MCP) which are found to be competitive if added with ethylene but do not appear to if the inhibitor is allowed to bind first \textsuperscript{10}.

In view of the competitive relationship between ethylene and 1-MCP, the prevalence of ethylene in shipping containers or at warehouses, and possibly the residual ethylene coming from commercial ripening operations, it is practical to apply 1-MCP at sufficiently high concentrations for maximum efficiency. One hundred n\text{R}/\text{R} 1-MCP seems to be effective for this purpose as it was not only demonstrated in the first and second experiments to be effective in delaying ripening, but was also shown in the third experiment to be effective in overcoming an ethylene level (1.0 \mu\text{R}/\text{R}) capable of accelerating ripening in three days. This may also suggest that for the maximum 1-MCP efficiency, it is important to remove or diminish residual ethylene from the storage and treatment rooms to prevent interference with 1-MCP treatment.

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