

# Ethephon as an Alternative Ripening Agent for 'Cardaba' Banana (*Musa acuminata* x *M. balbisiana*)

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## Abstract

*Ethephon and bioethylene from Gliricidia sepium were evaluated as potential alternatives to the unsafe calcium carbide (CaC<sub>2</sub>) in ripening 'Cardaba' banana. In Experiment 1, bananas were treated with CaC<sub>2</sub> (1 and 2 g kg<sup>-1</sup>), G. sepium leaves (30% and 40% w/w), or ethephon (200 and 500 µL L<sup>-1</sup>). In Experiment 2, fruit were applied with CaC<sub>2</sub> (2.5 or 5 g kg<sup>-1</sup>) or ethephon (200 or 500 µL L<sup>-1</sup>). Results showed that the use of low CaC<sub>2</sub> concentration (1 or 2 g kg<sup>-1</sup>) or G. sepium leaves for 48 h was not sufficient to effectively ripen banana fruit (Experiment 1). The fruit dipped in 500 µL L<sup>-1</sup> ethephon for 3 min exhibited similar effect as the 5 g kg<sup>-1</sup> CaC<sub>2</sub> for 72 h, which allowed the fruit to ripen faster than the lower concentrations of CaC<sub>2</sub> (2.5 g kg<sup>-1</sup>) and ethephon (200 µL L<sup>-1</sup>), and the control (Experiment 2). The higher concentrations of ethephon and CaC<sub>2</sub> resulted in bananas with higher total soluble solids, least firmness, most advanced peel color after 2-3 days and least green (a\*) color that was towards the yellow hue angle. Ethephon-treated fruit had better visual quality than those treated with CaC<sub>2</sub> and reached the full yellow stage the earliest (4.5 to 5.8 days) compared with the other treatments (7 to 11 days). As ripening took place earlier, fruit dipped in 500 µL L<sup>-1</sup> ethephon solution had the least visual quality rating after seven days resulting in shorter shelf life of eight days compared with the control at 11 days.*

**Keywords:** calcium carbide, 'Cardaba' banana, ethephon, *Gliricidia sepium*, ripening

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## 1. Introduction

Banana (*Musa* spp.) is one of the widely grown and commonly eaten fruit globally (Maduwanthi and Marapana, 2019). It is the top traded fruit globally with the Philippines as a major producer (Department of Agriculture, 2018).

In 2018, the overall production of bananas in the Philippines reached 9.2 million MT with an estimated annual growth rate of 2% (Philippine Statistics Authority, 2019). While ‘Cavendish’ banana is a widely exported variety that comprises 53% of the total banana production, other varieties are also produced including ‘Saba’ (*Musa*, BBB group) and ‘Cardaba’ (*Musa*, ABB group) bananas which comprise 28% of the production. ‘Cardaba’ is a cooking banana variety containing high starch, fiber and potassium (Ayo-Omogie *et al.*, 2010). A good portion of ‘Cardaba’ banana production is also processed into banana chips for export or utilized as a snack food for domestic consumption.

Banana, a climacteric fruit, is often harvested in the mature unripe stage. It can be artificially ripened to attain the desired ripeness stage to enhance its color uniformity and commercial value. Ripening agents are chemical substances that speed up the ripening process such as ethylene gas, ethephon, ethylene glycol, bioethylene sources and calcium carbide ( $\text{CaC}_2$ ) (Maduwanthi and Marapana, 2019; Singal *et al.*, 2012, Lacap *et al.*, 2019).  $\text{CaC}_2$  releases acetylene ( $\text{C}_2\text{H}_2$ ) gas in the presence of moisture. Acetylene acts as an analog of ethylene ( $\text{C}_2\text{H}_4$ ), which effectively starts the natural ripening process in the fruit (Medlicott *et al.*, 1987). However,  $\text{CaC}_2$  powder contains traces of arsenic and phosphorus which releases toxic residues namely arsine ( $\text{AsH}_3$ ) and phosphine ( $\text{PH}_3$ ) upon a chemical reaction that could contaminate the fruit upon artificial ripening (Chandel *et al.*, 2017). When inhaled or ingested, these residues are harmful to humans which pose high health risks to individuals involved in fruit ripening and handling. Therefore, there is a need to assess other ripening agents as a substitute for  $\text{CaC}_2$ .

Ethephon (2-chloroethylphosphonic acid) is an artificial hormone in a liquid form that regulates plant growth (Win *et al.*, 2018). It breaks down to release ethylene within the fruit and is commonly used to stimulate fruit ripening (Tan *et al.*, 2014). It has been used in ripening different varieties of banana including *Musa paradisiaca* ‘Phee-gyan-hnget-pyaw’ (Win *et al.*, 2018), *M. acuminata* ‘Grand Naine’ (Mahajan *et al.*, 2010), *M. sapientum* ‘Latundan’ (Tan *et al.*, 2014), and *M. acuminata* (AA Group) ‘Lacatan’ (Politud and Nacaya, 2016). These studies reported that bananas treated with ethephon ripened faster than the untreated fruit.

An even safer option is to make use of bioethylene or ethylene produced from biological sources such as plants. Bioethylene from ‘kakawate’ (*Gliricidia sepium* Steud) leaves was reported to ripen ‘Saba’ banana (*Musa*, BBB group)

(Acedo and Bautista, 1991) and tomato fruit (Tirtsoekotjo and Bautista, 1984). Other sources of bioethylene which hastened the ripening of bananas include apple fruit (Singal *et al.*, 2012) and indigenous plants such as *Adhatoda vesica* 'Asuro' and *Bauhinia variagata* 'Koiralo' (Ram *et al.*, 2009).

While the use of  $\text{CaC}_2$  is prohibited in several places in the world, it is continued to be used in some countries such as the Philippines because a similarly cheap and efficient alternative ripening agent is not available (Lacap *et al.*, 2019). The acetylene that it produces is not a natural plant hormone like ethylene and is likely to give off traces of arsenic and phosphorus which are both extremely toxic to humans (Lakade *et al.*, 2018). With this, a safer alternative to  $\text{CaC}_2$  must be harnessed. Thus, this research was done to evaluate the ripening agents (ethephon and bioethylene from *G. sepium*) as potential alternatives to  $\text{CaC}_2$  in ripening 'Cardaba' banana.

## 2. Methodology

### 2.1 Plant Material Preparation and Treatment

Two experiments were conducted in this study. Commercially mature green 'Cardaba' banana fruit (36.6% dry matter) with good quality and uniform size were harvested from banana orchards in Los Amigos (7° 08' 09.5" N, 125° 28' 50.5" E (Experiment 1) and Biao Guianga (7°09' 14.2" N, 125° 29' 51.3" E (Experiment 2), Davao City, Philippines. Both orchards practice organic farming. Twenty banana bunches were harvested for each experiment and transferred to the Postharvest Biology Laboratory in the University of the Philippines Mindanao through an air-conditioned vehicle. Only the first four hands from each bunch were used for the experiments. Banana fingers at the opposite ends of each hand were removed. Each hand was divided into four finger clusters using a sharp curved knife. The fruit were rinsed with tap water to remove dirt and debris, then sanitized with 200  $\mu\text{L L}^{-1}$  NaOCl solution for 5 min, then air-dried. After drying, the clustered fruit from the first four bunches were evenly distributed across treatments with replicates having a representative cluster from hands 1 to 4.

In Experiment 1, the bananas were applied with  $\text{CaC}_2$  (1 and 2 g  $\text{kg}^{-1}$ ), *G. sepium* leaves (30% and 40% w/w), and ethephon (48% active ingredient). The application of  $\text{CaC}_2$  was based on the common practice of ripening in the local market. One or 2 g  $\text{kg}^{-1}$  of  $\text{CaC}_2$  were carefully wrapped in two layers of

newspaper and positioned at the bottom-center section of bamboo baskets (~5-7 kg capacity) that were lined with newspaper sheets. The treatment of *G. sepium* leaves was based on the methods of Acedo and Bautista (1991) with slight modifications. The rate of 30% w/w was benchmarked from the mango study (Lacap et al., 2019) which tested the use of 20% w/w *G. sepium* leaves but was found ineffective in ripening 'Carabao' mango. Thus, the present study increased the rate to 30% and 40% w/w. The amounts of *G. sepium* leaves (30% and 40% w/w) were calculated based on the total weight of the fruit samples in a basket. The *G. sepium* leaves were placed at the bottom-center of the basket together with the bananas. Two newspaper sheets were placed on top of the baskets and tied securely to the edges of the basket with polypropylene twine. The CaC<sub>2</sub>, *G. sepium* and the control treatments were placed in baskets for 48 h. Thereafter, fruit were held in plastic crates and stored in ambient conditions. The ethephon treatment consisted of dipping the fruit in ethephon solution (200 and 500  $\mu\text{L L}^{-1}$ ) for 3 min followed by air-drying and holding in plastic trays in ambient conditions.

In Experiment 2, the bananas were dipped in ethephon (200, 500  $\mu\text{L L}^{-1}$ ) for 3 min, while fruit assigned the CaC<sub>2</sub> (2.5, 5 g kg<sup>-1</sup>) treatments were packed with the wrapped chemical inside bamboo baskets for 72 h. Untreated fruit clusters inside bamboo baskets served as a control for each of the experiments. A data logger was placed inside each of the baskets together with the fruit to record hourly temperature and relative humidity (RH) (Tinytag Ultra 2, Gemini Data Loggers Ltd., United Kingdom). All baskets were randomly distributed in the laboratory ensuring that these were exposed to uniform ambient conditions (28.5±0.9 °C, 79.7±4.1% RH). Fruit quality was evaluated at zero, two, seven and 12 days after treatment for Experiment 1, and zero, three, seven and 12 days for Experiment 2.

## 2.2 Quality Evaluation

Weight loss (%) was calculated as the difference of the final weight of the fruit from the initial and expressed in percentage. Firmness (N) was measured using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, United States). For the total soluble solids (TSS), the pulp (10 g) was homogenized for 1 min with 30-mL distilled water using a blender and filtered through a cheesecloth. The filtrate was dropped onto the refractometer prism to obtain TSS reading (% Brix). The value was multiplied by a dilution factor of 3. Necessary temperature corrections were applied.

Peel color was quantitatively measured as lightness ( $L^*$ ), greenness ( $a^*$ ), yellowness ( $b^*$ ), chroma, hue angle ( $^\circ$ ) and color difference ( $\Delta E^*$ ) using a color meter (Nix Pro Color Sensor, Nix Sensor Ltd., Canada) and qualitatively using a peel color index (PCI) (1 = green; 2 = light green breaking towards yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow with green tips; 6 = fully yellow; and 7 = yellow flecked with brown) (Oliveira *et al.*, 2016). Visual quality was assessed using a subjective index (5 = excellent; 4 = good; 3 = fair, limit of saleability; 2 = poor; 1 = very poor) (Nunes *et al.*, 2006). Degree of decay was measured using the scale (1 = no decay; 2 = 1-5% of the surface decayed; 3 = 6-10%; 4 = 11-25%; and 5 = >26%) while shelf life was ended at the visual quality rating of 3 and/or a decay rating of 2 (Ekman *et al.*, 2019).

### 2.3 Statistical Analysis

There were four sample fruit in a cluster (replication) and a total of three replications per treatment. The experiments were arranged in a completely randomized design (CRD). Data expressed in percentage were transformed using arcsine transformation. All data were analyzed using analysis of variance (ANOVA). Where there was a significant difference in ANOVA, the means were separated using Fisher's least significant differences (LSD) at  $p \leq 0.05$ .

## 3. Results and Discussion

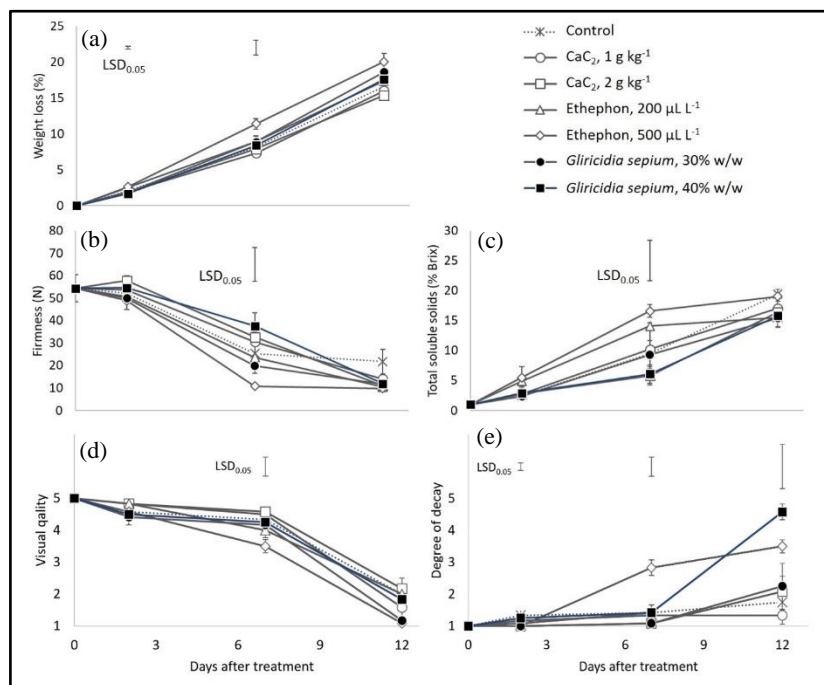
### 3.1 Experiment I

#### 3.1.1 Weight Loss

Fruit weight loss followed an increasing trend over time (Figure 1a). After two days, bananas treated with ethephon (200 or 500  $\mu\text{L L}^{-1}$ ) had elevated weight losses than those applied with  $\text{CaCl}_2$ , *G. sepium* leaves, or the control. After seven days, bananas dipped in 500  $\mu\text{L L}^{-1}$  ethephon solution had the highest weight loss at 11.4% while the rest of the treated and untreated fruit were lower at 7.3-8.9%. Weight loss among fruit samples did not vary at 12 days of storage.

### 3.1.2 Firmness

The firmness of the fruit decreased with storage (Figure 1b). A significant difference in fruit firmness was found on the seventh day of storage where bananas treated with 500  $\mu\text{L L}^{-1}$  ethephon were the least firm indicating that fruit ripened faster than the others. All fruit exhibited similar firmness at 12 days.



LSD bar represents the difference in means at  $p \leq 0.05$ .  
Error bar represents the standard error of the mean ( $n = 3$ ).

Figure 1. Weight loss (a), firmness (b), TSS (c), visual quality (d) and degree of decay (e) of 'Cardaba' bananas applied with different concentrations of  $\text{CaC}_2$  (1 or 2  $\text{g kg}^{-1}$ ), ethephon (200 or 500  $\mu\text{L L}^{-1}$ ), or *G. sepium* leaves (30 or 40% w/w) then stored in ambient room conditions ( $28.5 \pm 0.9$  °C,  $79.7 \pm 4.1$  RH) for 12 days (Experiment 1)

### 3.1.3 TSS

Total soluble solids increased as the storage time progressed (Figure 1c). Similar to fruit firmness, a significant difference in fruit sweetness was detected only on the seventh day after treatment. Bananas treated with 500  $\mu\text{L}$

L<sup>-1</sup> ethephon had the highest TSS (16.6% Brix) while the TSS of other samples ranged from 5.8 to 9.5% Brix. All fruit had similar TSS values at 12 days.

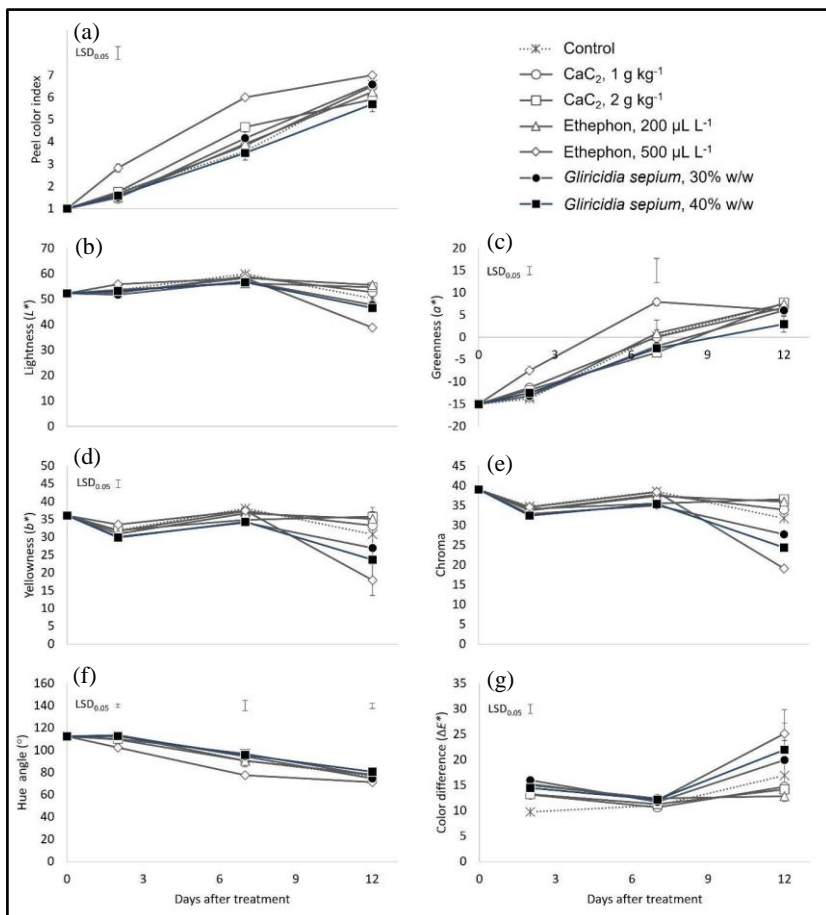
### 3.1.4 Visual Quality, Degree of Decay and Shelf Life

The visual quality of fruit did not vary among treatments except after seven days of storage (Figure 1d). Bananas treated with 500  $\mu\text{L L}^{-1}$  ethephon had a visual quality that deteriorated faster than the rest of the treatments. However, the degree of decay of those fruit dipped in 500  $\mu\text{L L}^{-1}$  ethephon was lesser than the untreated fruit as well as those treated with either 1 or 2 g kg<sup>-1</sup> CaC<sub>2</sub> (Figure 1e). Consequently, it also resulted in the shortest shelf life of 8.2 days which varied from the rest of the treatments (9.6 to 11.8 days).

### 3.1.5 Peel Color

The ‘Cardaba’ banana peel color turned from green to full yellow with flecks of brown over 12 days (Figure 2a). A significant difference in peel color was already observed in bananas treated with 500  $\mu\text{L L}^{-1}$  ethephon two days after treatment (Figure 2a). At this time, ethephon-treated banana fruit (500  $\mu\text{L L}^{-1}$ ) already turned light green breaking towards yellow while the others were still green. This was further confirmed by the quantitative measurement of greenness ( $a^*$ ), yellowness ( $b^*$ ) and hue angle wherein bananas dipped in 500  $\mu\text{L L}^{-1}$  ethephon solution had higher  $a^*$ ,  $b^*$  and hue angle suggesting advanced development of yellow color on the peel starting from two days after treatment (Figures 2c, 2d and 2f). Lightness ( $L^*$ ) and chroma did not vary (Figures 2b and 2e). The color difference was higher in treated bananas compared with the untreated ones (Figure 2g). Bananas soaked in 500  $\mu\text{L L}^{-1}$  ethephon also achieved the full yellow stage sooner after 4.5 days while the others ripened only after 9.3 to 11.3 days.

Overall, the banana fruit dipped in 500  $\mu\text{L L}^{-1}$  ethephon ripened faster than the rest of the treated and untreated bananas as evidenced by higher weight loss (Figure 1a), least firmness (Figure 1b), and highest TSS (Figure 1c) after seven days and most advanced peel color (i.e., more green than yellow) after two days compared with the others with still green to light green color (Figure 2a). The peel of banana treated with 500  $\mu\text{L L}^{-1}$  ethephon also had the least green color component (Figure 2b) with color towards the yellow hue. The skin lightness ( $L^*$ ) and chroma were not affected by the treatments (Figures 2b and 2e).



LSD bar represents a difference in means at  $p \leq 0.05$ .  
Error bar represents the standard error of the mean ( $n = 3$ ).

Figure 2. Peel color index (a), lightness ( $L^*$ ) (b), greenness ( $a^*$ ) (c), yellowness ( $b^*$ ) (d), chroma (e), hue angle ( $^\circ$ ) (f), and color difference ( $\Delta E^*$ ) (g) of 'Cardaba' banana applied with different concentrations of calcium carbide ( $\text{CaC}_2$ , 1 or 2 g kg<sup>-1</sup>), ethephon (200 or 500 µL L<sup>-1</sup>), or *G. sepium* leaves (30% or 40% w/w) then stored in ambient room conditions ( $28.5 \pm 0.9$  °C,  $79.7 \pm 4.1$  RH) for 12 days (Experiment 1)

$\text{CaC}_2$  was not able to ripen the fruit effectively due to its low concentration (1 and 2 g per kg of banana fruit) in a newspaper-lined bamboo basket setup. Traditionally, ripeners in the Philippine local market use  $\text{CaC}_2$  at a concentration of 8 to 10 g kg<sup>-1</sup> of fruit but in 'Carabao' mangoes, Lacap *et al.* (2019) showed that a lower concentration can be as effective. The amount of  $\text{CaC}_2$  might not have been adequate to produce the required amount of acetylene to trigger the ripening process in 'Cardaba' banana. Acetylene,



shown to have a lower biological activity than ethylene (Burg and Burg, 1967) and produced from  $\text{CaC}_2$  enhanced ripening in bananas (Hartshorn, 1931). The minimum effective concentration of ethylene in bananas was reported to be  $0.1$  to  $1 \mu\text{L L}^{-1}$  (Burg and Burg, 1962) and acetylene requires  $270$ - $2,700 \mu\text{L L}^{-1}$  to match this effect (Saltveit, 1999). Furthermore, Maduwanthi and Marapana (2019) confirmed that the concentration of acetylene should be at  $2,800 \mu\text{L L}^{-1}$  to enhance ripening in bananas.

On one hand, the use of 30 and 40% *G. sepium* leaves also did not affect the ripening of the 'Cardaba' bananas inside the bamboo baskets. Although newspaper linings were supposed to serve as barriers against gas diffusion, regular paper is deemed to have low barrier ability against water vapor and gas because of its hydrophilic and porous structure (Ferrer *et al.*, 2017; Kopacic *et al.*, 2018). Hence, the ethylene produced by *G. sepium* leaves might have rapidly diffused outside leaving insufficient ethylene in the basket to ripen the fruit. Further, *G. sepium* leaves emitted a relatively low amount of ethylene (i.e.,  $0.3$  and  $1 \mu\text{L L}^{-1}$  after 6 and 12 h, respectively) which could be insufficient to trigger ripening in 'Cardaba' banana compared with ethephon (Acedo and Bautista, 1991).

In this experiment, it was only  $500 \mu\text{L L}^{-1}$  ethephon that effectively ripened the 'Cardaba' banana relative to the other treatments and the control. The lower concentration of ethephon ( $200 \mu\text{L L}^{-1}$ ) could have also been insufficient to trigger ripening in the fruit. It has been reported that the efficacy of ethephon in ripening fruit is dependent on its concentration (i.e., the fruit ripens quickly with increasing concentration) (Dhall and Singh, 2013; Maduwanthi and Marapana, 2019). In mango, the use of  $500 \mu\text{L L}^{-1}$  ethephon did not reduce the firmness of the fruit after three days of ripening treatment indicating that this concentration may not be sufficient to ripen mango rapidly (Lacap *et al.*, 2019); however, it worked in the present study. Ethephon penetrates the fruit and breaks down to ethylene (Singal *et al.*, 2012) and has been shown to speed up the ripening of banana and other fruits including apple, citrus, tomatoes, guava, mango and peaches (Maduwanthi and Marapana, 2019).

## 3.2 Experiment 2

### 3.2.1 Weight Loss

Weight loss increased over time with significant differences in means observed after three to seven days of storage (Figure 3a). Bananas dipped in

500  $\mu\text{L L}^{-1}$  ethephon had the highest weight loss (4.5%) which varied from those treated with 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  and the control. After seven days, bananas soaked in 500  $\mu\text{L L}^{-1}$  ethephon still had the highest weight loss (12.2%) compared with the other treatments (7.6 to 10.7%). Weight loss no longer differed among treatments on the 12<sup>th</sup> day of storage.

### 3.2.2 Firmness

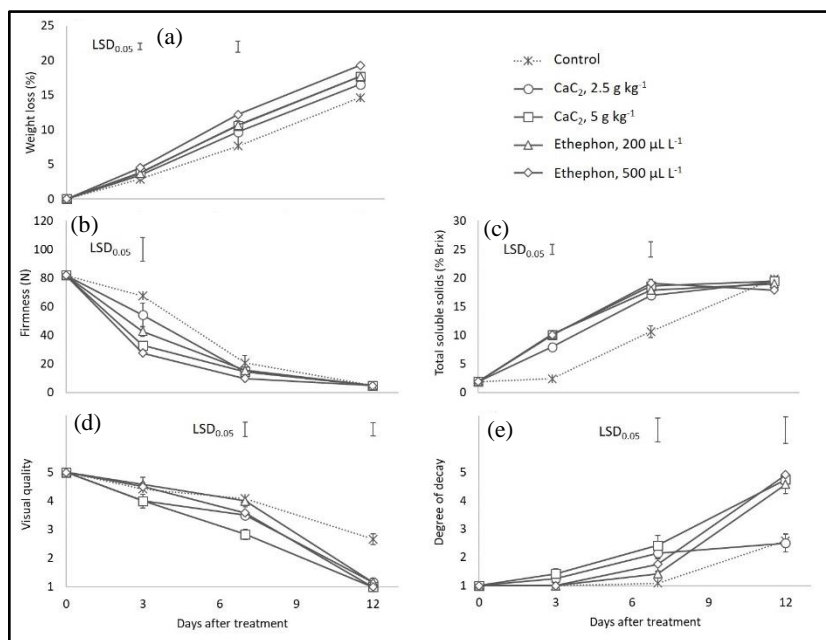
Fruit firmness decreased towards the end of storage (Figure 3b). Significant differences in firmness were noted three days after treatment. Bananas dipped in 200 or 500  $\mu\text{L L}^{-1}$  ethephon solution and those applied with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  were less firm (27.5 to 42.7 N) than those treated with 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  (54.2 N) or the control (67.6 N). Fruit firmness did not differ among treatments at days seven and 12.

### 3.2.3 TSS

Total soluble solids increased over time which varied at three and seven days of storage (Figure 3c). Three days after the application of treatments, bananas treated with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  and ethephon (200 or 500  $\mu\text{L L}^{-1}$ ) exhibited a higher TSS than those treated with 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  (8.0% Brix) and the control (2.4% Brix). On day seven, all treated fruit had higher TSS (17.0 to 19.1% Brix) than the untreated bananas (10.7% Brix). The TSS did not vary at day 12.

### 3.2.4 Visual Quality

Fruit visual quality deteriorated as time progressed with significant differences noticed among treatments on days seven and 12 of storage (Figure 3d). The visual quality of all the fruit was “excellent” at day 0 then down to “good” three days after the application of the treatments (Figure 4). Differences in visual quality rating were observed on the seventh day of storage wherein treated fruit had lower visual quality rating (3.6 “fair” to 2.8 “poor”) than those dipped in 200  $\mu\text{L L}^{-1}$  ethephon and the control (4.0 to 4.1 “good”). On the 12<sup>th</sup> day of storage, all treated fruit had lower visual quality (1.0 to 1.2 “very poor”) than the untreated fruit (2.7 “poor”). In terms of shelf life, bananas treated with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  resulted in the shortest shelf life (6.8 days) followed by those treated with 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  (8.3 days), 500  $\mu\text{L L}^{-1}$  ethephon (8.8 days), or 200  $\mu\text{L L}^{-1}$  ethephon (9.4 days). The control fruit had the longest shelf life because it ripened the slowest (11.6 days).



LSD bar represents the difference in means at  $p \leq 0.05$ .  
Error bar represents the standard error of the mean ( $n = 3$ ).

Figure 3. Weight loss (a), firmness (b), TSS (c), visual quality (d) and degree of decay (e) of 'Cardaba' banana treated with different concentrations of CaC<sub>2</sub> (2.5 or 5 g kg<sup>-1</sup>) or ethephon (200 or 500 µL L<sup>-1</sup>) then stored in ambient room conditions (28.5±0.9 °C, 79.7±4.1% RH) for 12 days (Experiment 2)

### 3.2.5 Degree of Decay

The degree of decay increased as storage time progressed (Figure 3e). Decay started to show in CaC<sub>2</sub>-treated bananas although not statistically different from the other treatments. After seven days, the degree of decay was higher in bananas treated with 2.5 or 5 g kg<sup>-1</sup> CaC<sub>2</sub> (6 to 10% surface decayed) than those dipped in 200 or 500 µL L<sup>-1</sup> ethephon solution and the control (1 to 5% surface decayed). After 12 days, bananas treated with CaC<sub>2</sub> (5 g kg<sup>-1</sup>) and ethephon (200 and 500 µL L<sup>-1</sup>) exhibited a higher degree of decay (11 to 25% surface decayed) than those treated with 2.5 g kg<sup>-1</sup> CaC<sub>2</sub> (6 to 10% surface decayed) and the untreated bananas which suggest that these treatments promoted the earlier onset of senescence.

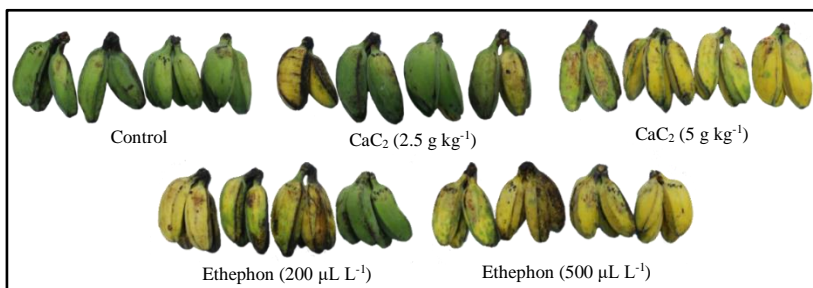
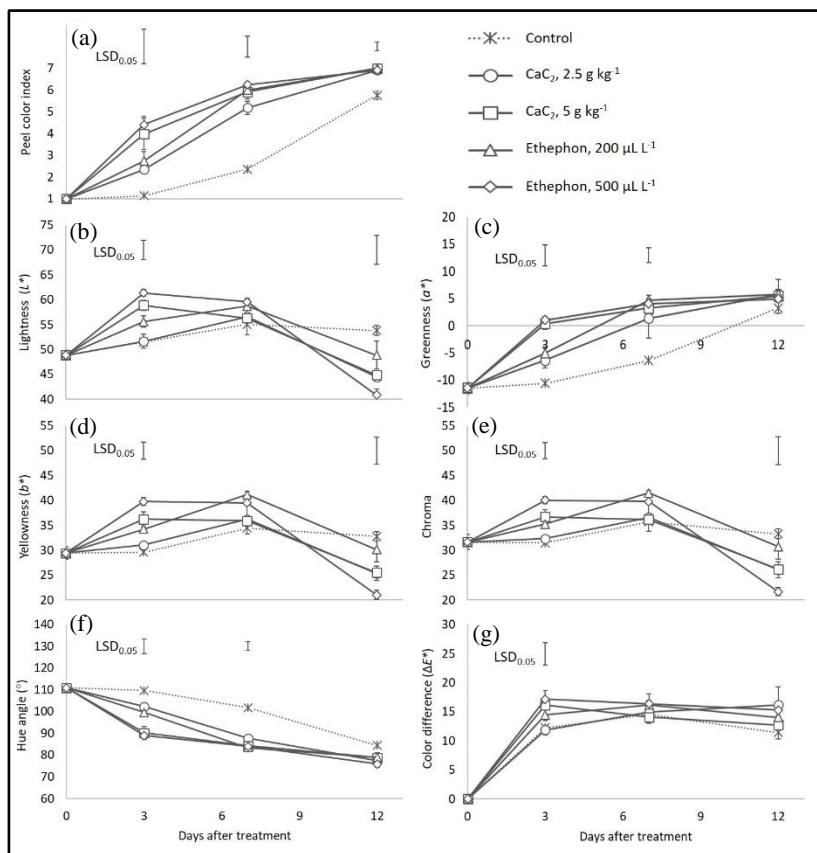


Figure 4. Visual quality of 'Cardaba' banana fruit treated with  $\text{CaC}_2$  (2.5 or 5  $\text{g kg}^{-1}$ ) or ethephon (200 or 500  $\mu\text{L L}^{-1}$ ) and stored for three days in ambient room conditions ( $28.5 \pm 0.9$  °C,  $79.7 \pm 4.1\%$  RH)

### 3.2.6 Peel Color

The peel color of banana changed from green to yellow over time with differences in peel color index (PCI) found on days three, seven and 12 of storage (Figure 5a). Bananas dipped in 500  $\mu\text{L L}^{-1}$  ethephon solution showed the fastest color development as it already changed from green to more yellow than green (PCI 4.4) three days after treatment. It was followed by those treated with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  with more green than yellow peel color (PCI 3.9); 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  and 200  $\mu\text{L L}^{-1}$  ethephon with light green peel color (PCI 2.4-2.8); and lastly, by the untreated fruit with green peel color (PCI 1.2). A similar trend was noticed after seven days of storage. Upon the end of storage (day 12), all treated fruit had turned full yellow with some flecks of brown (PCI 6.9 to 7.0). Bananas dipped in 500  $\mu\text{L L}^{-1}$  ethephon solution was the fastest to reach the full yellow stage in 5.8 days followed by those treated with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  with 6.7 days, 200  $\mu\text{L L}^{-1}$  ethephon with 7.2 days, 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  with 8.5 days, and lastly, by the untreated fruit with 11.7 days.

In terms of perceptual lightness of the peel color (Figure 5b), bananas dipped in 500  $\mu\text{L L}^{-1}$  ethephon had the highest  $L^*$  (61.4) together with those treated with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  ( $L^* = 58.9$ ). Those treated with 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$  and the untreated fruit had the lowest  $L^*$  (51.5). Bananas soaked in 500  $\mu\text{L L}^{-1}$  ethephon or 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  had the highest  $a^*$  which meant a lesser green component in the peel color (Figure 5c). Further, bananas soaked in 500  $\mu\text{L L}^{-1}$  ethephon had the highest  $b^*$  and chroma or a higher yellow component in the peel color – appearing more vivid than the rest of the treated and untreated fruit (Figures 5d-5e).



LSD bar represents the difference in means at  $p \leq 0.05$ .  
Error bar represents the standard error of the mean ( $n = 3$ ).

Figure 5. Peel color index (a), lightness (b), greenness (c), yellowness (d), chroma (e), and hue angle (f), and color difference (g) of 'Cardaba' banana treated with different concentrations of  $\text{CaC}_2$  (2.5 or 5  $\text{g kg}^{-1}$ ) or ethephon (200 or 500  $\mu\text{L L}^{-1}$ ) then stored in ambient room conditions ( $28.5 \pm 0.9^\circ\text{C}$ ,  $79.7 \pm 4.1\%$  RH) for 12 days (Experiment 2)

The treatment of 500  $\mu\text{L L}^{-1}$  ethephon or 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  resulted in bananas with the lowest hue angle ( $88.9$  to  $90.2^\circ$ ) heading towards yellow followed by those soaked in 200  $\mu\text{L L}^{-1}$  ethephon ( $99.5^\circ$ ) or 5  $\text{g kg}^{-1}$   $\text{CaC}_2$  ( $102.2^\circ$ ) or a greener hue (Figure 5f). The untreated fruit had the highest hue angle at  $109.6^\circ$  showing a slower transition from green to a yellow hue. The color difference ( $\Delta E$ ) from initial until three days after treatment was significantly higher in bananas soaked in either 200 or 500  $\mu\text{L L}^{-1}$  ethephon and those applied with 5  $\text{g kg}^{-1}$   $\text{CaC}_2$ , compared with the untreated and those treated with 2.5  $\text{g kg}^{-1}$   $\text{CaC}_2$ , suggesting a greater color change for the former treatments (Figure 5g).

Since the low concentration of  $\text{CaC}_2$  (1 and 2 g  $\text{kg}^{-1}$ ) in Experiment 1 was unable to effectively ripen the 'Cardaba' banana, its concentration was increased to 2.5 and 5 g  $\text{kg}^{-1}$  in Experiment 2 to determine its comparative efficacy with ethephon. In this experiment, the effect of 5 g  $\text{kg}^{-1}$   $\text{CaC}_2$  was comparable with that of 500  $\mu\text{L L}^{-1}$  ethephon in ripening 'Cardaba' banana fruit (Figure 4). Bananas dipped in 500  $\mu\text{L L}^{-1}$  ethephon had the highest weight loss until seven days (Figure 3a). Fruit treated with ethephon (200 or 500  $\mu\text{L L}^{-1}$ ) or 5 g  $\text{kg}^{-1}$   $\text{CaC}_2$  were the least firm (Figure 3b) and higher TSS (Figure 3c) due to advanced ripening. As a consequence, the degree of decay was also higher (Figure 3e) than those treated with the lower concentration of  $\text{CaC}_2$  (2.5 g  $\text{kg}^{-1}$ ) or the control. The visual quality of bananas treated with 5 g  $\text{kg}^{-1}$   $\text{CaC}_2$  deteriorated faster than the rest of the treatments (Figure 3d).

As the bananas ripened, it also led to weight loss and quality deterioration. Physiological weight loss is an indication of water loss due to transpiration (Larotonda *et al.*, 2008). The elevated temperature in the 5 g  $\text{kg}^{-1}$   $\text{CaC}_2$  treatment ( $28.0 \pm 1.2$  °C) could have also contributed to the ripening of 'Cardaba' bananas. The basket with 'Cardaba' bananas treated with 5 g  $\text{kg}^{-1}$   $\text{CaC}_2$  had a higher temperature than those with only 2.5 g  $\text{kg}^{-1}$  ( $27.4 \pm 0.8$  °C) and the untreated basket ( $26.3 \pm 0.5$  °C). High temperatures accelerate fruit ripening but can also reduce quality and increase disease (Ahmad *et al.*, 2001).

Higher concentrations of  $\text{CaC}_2$  and ethephon reduced banana fruit firmness as early as three days from treatment. Ethylene released from ethephon has a signaling pathway that includes various transcription factors responsible for different ripening responses including that of firmness (Tucker *et al.*, 2017). As the fruit ripens, the elasticity and viscosity of the fruit diminish which then affects the coordinated breakdown of biopolymers such as cell wall polysaccharides and starch that are responsible for fruit firmness (Kojima *et al.*, 1994). These changes in cell wall structure and composition are catalyzed by various enzymes including polygalacturonase and pectinesterase (Mohd Ali *et al.*, 2004). TSS increases as ripening proceeds with the hydrolysis of starch to sugars. In some studies, chemically-treated fruit produced more sugars due to the increase in soluble pectin, organic acids, and hydrolysis of starch to soluble sugars (Win *et al.*, 2018).

Peel color is one of the most important indicators of banana ripening. Both the ethephon and  $\text{CaC}_2$  treatments advanced the 'Cardaba' fruit color development compared with the control (Figure 5a). Specifically, the treatment of 500  $\mu\text{L L}^{-1}$  ethephon or 5 g  $\text{kg}^{-1}$   $\text{CaC}_2$  resulted in banana fruit with a lighter color (Figure

5b), less green (Figure 5c), more yellow (Figure 5d), vivid (i.e., higher chroma (Figure 5e), and lower hue angle (i.e., towards yellow hue) (Figure 5f), and higher color difference (Figure 5g) compared with those applied with lower concentrations and the control. Some modifications during ripening include the differences in peel color and pulp texture, starch conversion into sugar, polyphenol reduction and aromatic compounds synthesis (Larotonda *et al.*, 2008).

Quick ripening rate prompts faster color changes. When the banana fruit comes in contact with ethylene or its structural analog, acetylene, chlorophyll (green pigments) in the peel breaks down and gets replaced by a yellow pigment (carotenoid), which also signals that ripening is taking place in the fruit. Ripe bananas produce a relatively high amount of ethylene gas which causes the yellow carotenoid pigments to turn brown due to enzymatic browning (Quevedo *et al.*, 2009). Browning in a banana is closely associated with decay and consequently affects its saleability; thus, limiting shelf life and its commercial value. In the current study, the onset of decay occurred the earliest in bananas treated with 5 g kg<sup>-1</sup> CaC<sub>2</sub> (5.8 days) or 500 µL L<sup>-1</sup> ethephon (7.6 days).

The higher concentration of ethephon (500 µL L<sup>-1</sup>) effectively advanced the ripening of the ‘Cardaba’ banana similar to the traditional ripening agent (CaC<sub>2</sub>). Ethephon releases ethylene upon breakdown which coordinates most aspects of ripening in climacteric fruit (Pech *et al.*, 2012). Ethephon is deemed to be the most effective non-gaseous ethylene-releasing chemical (Win *et al.*, 2018). It is categorized as non-carcinogenic to humans by the Hazardous Substances Data Bank (National Library of Medicine, 2021), thus it could be a safer alternative to the unsafe CaC<sub>2</sub>. However, ethephon has the potential to cause severe skin and eye irritation. Thus, personal protective equipment, which includes chemical-resistant gloves, as well as protective eyewear, is required for handlers (United States Environmental Protection Agency, 1995).

## 4. Conclusion

This study showed that ethephon is an equally effective and safer alternative to CaC<sub>2</sub> in ripening ‘Cardaba’ bananas. The higher concentration of ethephon (500 µL L<sup>-1</sup>) was at par with the effect of 5 g kg<sup>-1</sup> CaC<sub>2</sub>. The use of a lower concentration of ethephon at 200 µL L<sup>-1</sup> was able to initiate ripening although not as fast as 500 µL L<sup>-1</sup> ethephon. Treatments with *G. sepium* leaves and

lower concentrations of  $\text{CaC}_2$  (1 or 2 g  $\text{kg}^{-1}$ ) were unable to quickly trigger ripening in 'Cardaba' banana.

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