Appendix 5.
RIPESTUFF™ RESEARCH REPORTS

Summary of research findings from UQ and UPMin

prepared for
ACIAR Project HORT/2012/098

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Executive summary

Ripestuff™ is an encapsulated form of ethylene that offers a promising and safe alternative to calcium carbide for batch-ripening of fruit in the Philippines. However, testing of the product on mango fruit in newspaper-lined woven bamboo baskets realised only inconsistent ripening responses to date. This was unexpected as Ripestuff™ had already been shown successful for ripening mango fruit during shipment in refrigerated road containers. In this work we used 'model' chambers to characterise Ripestuff™ ethylene release under varying humidity and air pressure conditions from the same prototype small container delivery system used in both the batch-ripening and in-transit studies; viz., 70 mL specimen container with four 0.5 mm ∅ holes in the lid. Test results were used to improve the delivery system design and test its efficacy to ripen fruit in 5 kg capacity woven bamboo baskets as scaled-down versions of the 20-22 kg capacity baskets used in Philippine wet markets. Ten experiments were conducted at UPMin, Philippines and seven at UQ, Australia. They revealed that less than successful early attempts to batch-ripen fruit in baskets were evidently due to overloading the delivery system with Ripestuff™. Ethylene release from Ripestuff™ powder requires more-or-less direct exposure to moisture vapour. The rate of moisture diffusion into the original prototype small container delivery system from the high humidity basket environment proved insufficient to activate significant ethylene release from relatively large Ripestuff™ quantities. On the other hand, direct addition of water to the delivery system generated rapid and substantial ethylene release to successfully ripen 'Carabao' mango, 'Solo' papaya and 'Cavendish' banana fruit in baskets. Nonetheless, eliminating the need to add water into the small container delivery system is desirable from a practical viewpoint to simplify the process for fruit wholesalers and retailers. Relatively minor modifications to delivery system design in terms of the amount of Ripestuff™ and the surface area available for moisture ingress into the container (viz. number of holes in the lid) enabled fruit ripening without water inclusion. A delivery system comprising 1 g Ripestuff™ in a container with 64 holes in the lid was duly found to ripen mango fruit in baskets at a rate similar to conventional calcium carbide and notably faster than for untreated control fruit in baskets. Overall, the study provided proof-of-concept in an appropriate technology context to enable further refinement and commercialisation of a Ripestuff™ delivery system to replace calcium carbide in a developing country wet market situation. Based on the key learnings, recommendations for future research include: (1) pilot testing of the improved delivery system by mango wholesalers and retailers in wet markets in the Philippines and other south Asia and Pacific countries; (2) testing the delivery system on other climacteric fruit crops of economic significance, including vegetable fruit like tomato; (3) developing simpler, cheaper delivery system alternatives, such as polymer film sachets; and, (4) characterising conditions encountered by fruit consignments in road containers in-transit to better understand Ripestuff™ ethylene release during transport from production centres to consumer markets.

Contents

1 Background

Small-scale mango producers in developing countries rely on batch ripening with calcium carbide to achieve marketable fruit of uniform quality within a shorter time than natural ripening. Around 22 kg green mature fruit is generally packed into an open-weave bamboo basket lined with newspaper and containing a newspaper-wrapped bundle of calcium carbide at a rate of 8 to 10 $g.kg^{-1}$ of fruit in the base (Figure 1). Newspaper is wrapped over the top of the basket and held in place with string to fully enclose the fruit. Ripening is triggered when acetylene released from calcium carbide binds with ethylene receptors in the fruit. However, calcium carbide contains carcinogenic impurities (Asif, 2012) and can be explosive if improperly handled (Abat, 2013). Individuals involved in ripening practices are most at risk, but poor packing of calcium carbide during ripening may expose consumers to arsenic residues on fruit surfaces (Chandel et al., 2018). These health and safety concerns have led to calcium carbide being banned for fruit ripening use in several countries (Islam et al., 2016; Vasdev, 2001; Vikram, 2015). Furthermore, anecdotal evidence suggests that calcium carbide produces uneven fruit ripening in that treated fruit appear visually ripe in terms of peel colour, but tend to have firm unripened flesh (Hossain et al., 2015). Despite these issues, calcium carbide is still widely used because it is cheaper compared to other recommended ripening practices, such as ethephon or compressed ethylene gas (Islam et al., 2016).

Figure 1. Traditional mango ripening in the Philippines uses calcium carbide and newspaper-lined woven bamboo baskets (left). Preparation of ~22 kg mango fruit for batch-ripening with calcium carbide in a basket (right). Photo credits: Angelyn Lacap.

Ripestuff™ is an encapsulated form of ethylene that offers a promising and safe alternative to calcium carbide for batch-ripening of fruit in newspaper-lined baskets, as is the current commercial practice in the Philippines (Ekman et al., 2018). However, limited knowledge of Ripestuff™ ethylene release kinetics under commercial conditions has hindered development of a suitable small scale appropriate technology delivery system. Nevertheless and prior to this study, Ripestuff™ in a prototype delivery

system consisting of 70 mL specimen containers each with four 0.5 mm ∅ holes in their lid was used to successfully ripen mango fruit in-transit during shipment to markets in refrigerated road containers (Duong et al., 2017). However, the same system initially did not reliably ripen mango fruit in a wet market basket configuration at ambient conditions (Ekman et al., 2018).

It had been established that ethylene release from Ripestuff™ is triggered by exposure to moisture vapour (Ho et al., 2011). In this regard, fruit transpire and respire to produce water vapour during postharvest ripening and thereby increase the relative humidity of surrounding air in enclosed environments. Moisture flux from basket headspace into the small container Ripestuff™ delivery system was relatively uncharacterised at the inception of this study. Moreover, ethylene concentration profiles over time in the headspace in baskets were unknown for Ripestuff™ treatments. Understanding these parameters was thought essential to help understand inconsistent ripening responses observed in past tests and inform optimisation of an effective and reliable appropriate technology delivery system.

2 Objectives

This research contributed to the overarching project objective *'to reduce losses and improve quality through development of effective postharvest intervention strategies'*. The specific objective as stated in the HORT/2012/098 project variation was to:

> *Conduct trials on replacements for calcium carbide, a potentially carcinogenic, contaminating and explosive product currently used to ripen mangoes. Products to be tested include the Ripestuff™, a form of encapsulated ethylene. A method of applying this will be developed which is consistent with normal supply chains and their limitations.*

3 Methodology

Collaborative research was undertaken by project team members at UPMin in the Philippines and at UQ in Australia. A total of 10 experiments were conducted between March 2018 and June 2019 at UPMin. They focused on fruit ripening responses, particularly of 'Carabao' mango, following exposure to prototype Ripestuff™ delivery systems (Figure 2) in a woven bamboo basket configuration (Figure 3). The baskets were a scaled-down version of those used commercially; viz., 5 kg as opposed to 20-22 kg fruit capacity. Selection of appropriate wet market delivery systems was guided by findings of seven complementary experiments conducted at UQ. These focused on Ripestuff™ ethylene release kinetics under controlled conditions using a 'model' chamber configuration (Figure 4). The detailed findings from all Ripestuff™ experiments are presented in a series of reports listed in Appendix A. The reports themselves form Appendices B to H, in separate documents to this report.

An alternative ripening procedure involving sealed lengths of ethylene- or acetylene-filled silicone tubing was also investigated. Preliminary experiments monitored ethylene or acetylene release from tubing in either model chambers or bamboo baskets. These experiments were conducted by Khamla Mott during her PhD candidature and have been presented as an excerpt from her draft thesis (Appendix I, a separate document to this report).

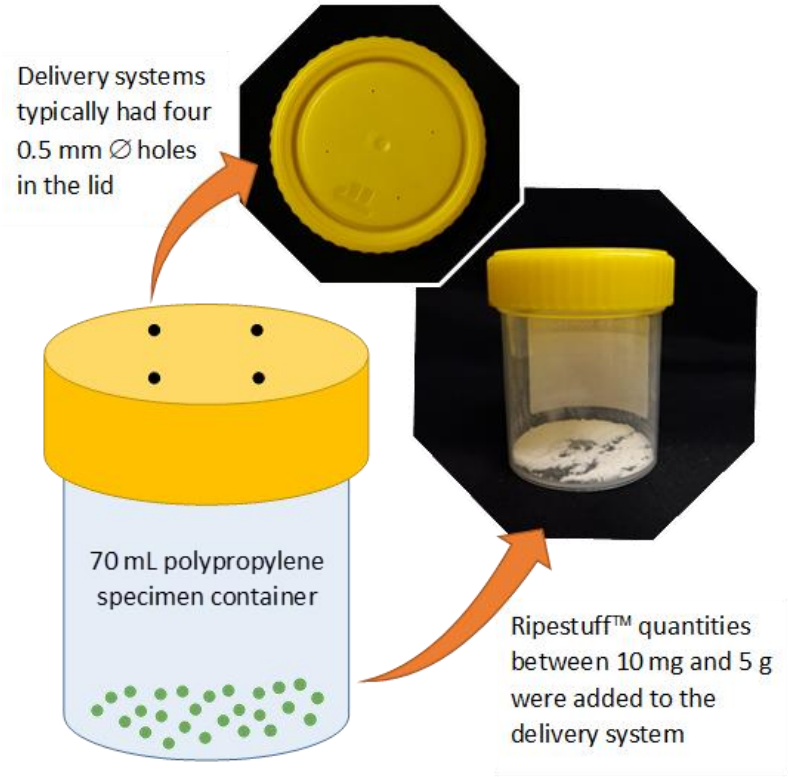

Figure 2. A typical prototype Ripestuff™ delivery system was comprised of a 70 mL polypropylene specimen container with a polyethylene screw-top lid into which four 0.5 mm ∅ holes had been pierced. Delivery systems with 1, 2, 16, 32 or 64 holes in the lid were also tested. Ripestuff™ quantities added to the system ranged from 10 mg to 5 g, either in powder form or as an aqueous solution.

Figure 3. Example wet market basket configuration used in UPMin experiments to test the efficacy of Ripestuff™ on mango ripening. Each woven bamboo basket contained ~5 kg fruit (left) wrapped in newspaper secured with polypropylene twine (right).

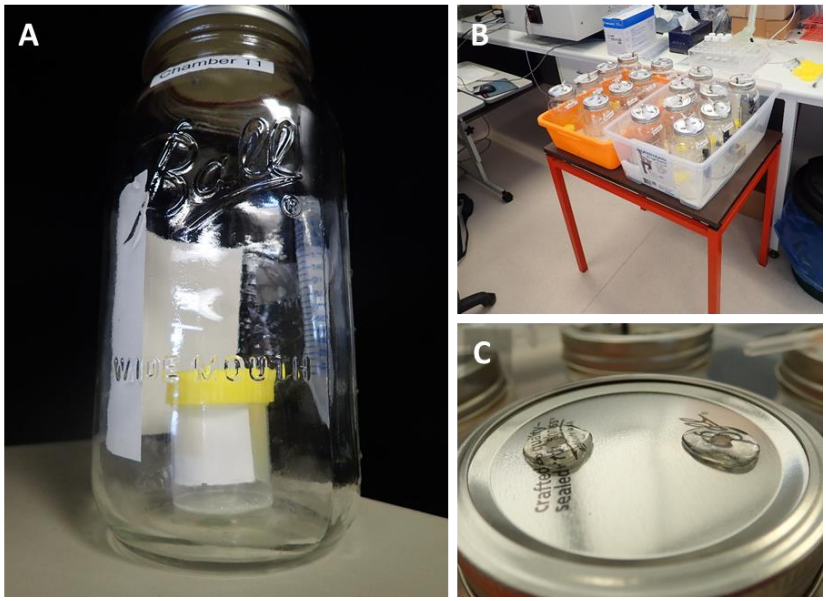

Figure 4. Example 'model' chamber configuration employed in experiments conducted at UQ showing (A) a Ripestuff™ delivery system within a 2 L chamber, (B) the laboratory work environment in which chambers were maintained during each experiment, and (C) a chamber lid with two sampling ports, each comprising a 4 mm ∅ hole filled with clear silicone sealant.

Knowledge transfer between Filipino and Australian team members was facilitated by regular email contact and five Skype teleconferences. Additionally, UPMin team member Angelyn Lacap spent 1 week at UQ in December 2018 meeting with UQ team members, undertaking training and research in headspace gas chromatography for ethylene analysis (Figure 5) and planning subsequent experiments. Research activities undertaken during this project variation extension are listed in Appendix J. Early findings were presented at the UPMin College of Science and Mathematics Research Colloquium in December 2018 and slides of this presentation have been included in Appendix K, a separate document to this report.

Figure 5. UPMin team member Angelyn Lacap undertaking headspace sampling (left), sample injection into a gas chromatograph (top right), and subsequent quantification of ethylene (bottom right) during her visit to UQ in December 2018.

4 Key results and discussion

Most experiments were conducted using a prototype Ripestuff™ delivery system comprising a 60-70 mL specimen container with four 0.5 mm ∅ holes in the lid. Experiments 2.1, 2.2, and 3 conducted at UPMin (Appendix B) showed an inconsistent mango ripening response to Ripestuff™ exposure in a basket configuration. Improved ripening was only observed in Experiment 2.1, which used untreated bench-ripened fruit as the control treatment. Fruit ripened on the bench were exposed to relatively large fluctuations in temperature and relative humidity. Hence, it was not known whether the difference in ripening response was caused by Ripestuff™ or the basket environment in which the fruit were treated. When compared with untreated fruit ripened in baskets, as in Experiments 2.2 and 3, Ripestuff™-treated fruit exhibited no difference in ripening response.

These results were addressed in the series of experiments at UQ which examined Ripestuff™ ethylene release from the same delivery system as used in the UPMin experiments, but under controlled conditions in a 'model' 2 L chamber without fruit (Appendices C and D). These experiments showed that although ethylene could readily diffuse out of the delivery system, moisture diffusion into the delivery system was evidently a factor limiting ethylene release from Ripestuff™.

Inclusion of water in the delivery system was one way to overcome this problem. Subsequent experiments showed that inclusion of water generated sufficient ethylene from Ripestuff™ to promote ripening of 'Carabao' mango (Appendix B, Experiments 8 and 9), 'Cavendish' banana (Appendix E), and 'Solo' papaya (Appendix B, Section 11) in baskets (Figure 6). In these situations, Ripestuff™ induced faster ripening than the control treatment and similar ripening to calcium carbide, where tested. For mango fruit, inclusion of 0.5 g Ripestuff™ in the delivery system produced a faster

ripening response than 0.25 g in terms of days to saleability (Appendix B, Experiment 8), whereas Ripestuff™ quantities below 0.1 g did not generate sufficient ethylene to initiate ripening (Appendix B, Experiment 7).

Figure 6. Ripening response of 'Carabao' mango (top), 'Solo' papaya (middle), and 'Cavendish' banana (bottom) after 72 h in newspaper-lined baskets containing no Ripestuff™ (left) or Ripestuff™ in a delivery system with added water (right).

However, from a practical viewpoint, eliminating the need to add water to the delivery system was considered desirable so as to simplify the procedure for fruit wholesalers and retailers. Further work at UQ using model chambers revealed that such a process was possible provided that the amount of Ripestuff™ in the delivery system was 'matched' to the number of holes in the lid. It was demonstrated that Ripestuff™ quantities previously trialled in baskets (1.25 to 5 g) were overloading the delivery system and restricting ethylene release (Appendix F). By reducing the amount of Ripestuff™ in the delivery system and/or increasing the number of holes in the lid to increase the rate of moisture diffusion into the delivery system, it was shown that ~90% ethylene release could be achieved without the inclusion of water and within the 72 h timeframe required to ripen fruit (Appendix G). Furthermore, the ratio of Ripestuff™ quantity to hole number was a strong predictor of ethylene release. Ethylene release profiles for 12 delivery system variations were modelled using the Avrami equation. It was postulated that a combination of multiple delivery systems with differing ethylene release characteristics could sustain high ethylene levels in the basket headspace throughout the treatment period.

In the final UPMin experiment (Appendix B, Experiment 10), a combination of five different delivery systems comprising a total of 3 g Ripestuff™ was used to ripen mango fruit in baskets. Details of the five systems are as follows:

- 1.0 g Ripestuff™, 4 holes in lid
- 0.5 g Ripestuff™, 4 holes in lid
- 0.5 g Ripestuff™, 16 holes in lid
- 0.5 g Ripestuff™, 64 holes in lid
- 0.5 g Ripestuff™, 4 holes in lid, water added

This 'combination' treatment did not sustain the expected level of ethylene in the basket headspace over a long period. Instead, it generated a peak concentration of 20 $\mu L.L^{-1}$ at 1 h followed by a decline to baseline levels by 24 h. However, this ethylene dosage was enough to trigger uniform ripening of mango fruit and the ripening rate was comparable to that induced by calcium carbide and faster than that observed in untreated control fruit (Figure 7). The experiment also investigated the effect of a single delivery system containing 1 g Ripestuff™ and 64 holes in the lid. This last treatment produced a similar ripening response to the 'combination' treatment despite generating a much lower peak ethylene concentration of 5 $\mu L.L^{-1}$ in the basket headspace.

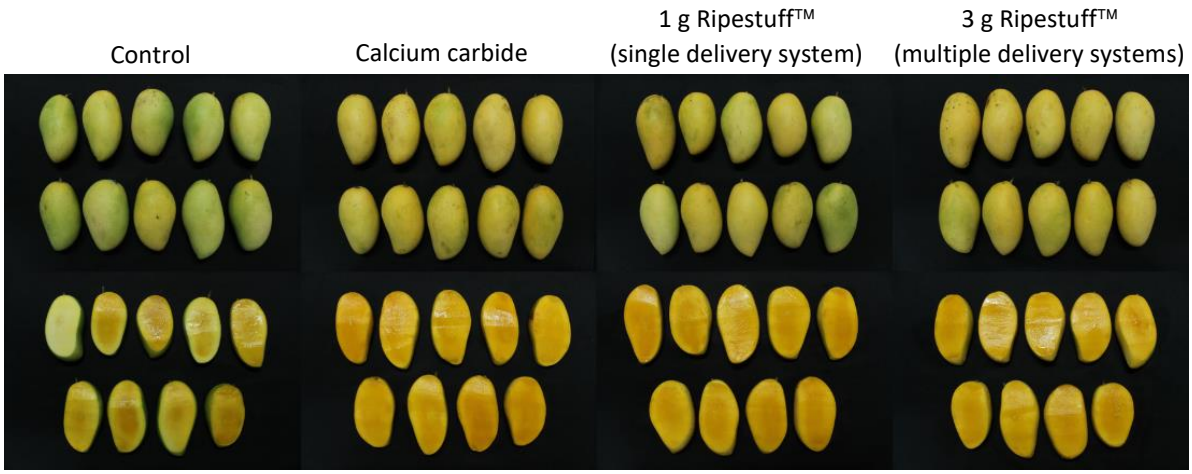

Figure 7. UPMin Experiment 10 showed 'Carabao' mango had a similar ripening response after 72 h exposure to either calcium carbide, 1 g Ripestuff™ in a single delivery system with 64 holes in the lid or 3 g Ripestuff™ in multiple delivery systems with differing ethylene release characteristics.

The misalignment between predicted and actual ethylene concentrations in the basket headspace showed that whilst model chambers are useful for characterising Ripestuff™ ethylene release under controlled conditions, they do not accurately reflect the basket environment. Newspaper-lined baskets of fruit exhibit substantial ethylene leakage (Appendix B, Experiment 9) unlike the hermetically sealed chambers. This continual loss to the atmosphere would likely create a concentration gradient to drive ethylene diffusion from the delivery system to the basket headspace, resulting in faster ethylene release. Furthermore, fruit ripening experiments at UPMin were conducted at higher temperatures than the 23°C used in the model chamber experiments at UQ. Sharper and earlier ethylene release rate peaks would be expected to occur as a result of both of these factors. A comparison of the ethylene release profiles for the same delivery system (1 g Ripestuff™,

64 holes in lid) in the model chamber (Appendix G) and basket (Appendix B, Experiment 10) shows this to be the case. Ethylene release spanned periods of >72 and 24 h, respectively, and release rates peaked at 28 and 9 h, respectively. Thus, ethylene release occurred around three times faster in the baskets than the chambers.

Another question investigated was the successful application of a purportedly overloaded Ripestuff™ delivery system for in-transit ripening of 'Honey Gold' mango. Previous researchers showed that peak ethylene release occurred at 6 h when delivery systems containing 12 g Ripestuff™ powder in a specimen container with 4 holes in the lid were placed amongst a consignment of fruit in refrigerated road containers maintained at 14-23°C (Duong et al., 2017). An experiment employing the model chamber configuration revealed that oscillations in air pressure promote moisture diffusion into the delivery system (Appendix H). The interior of a moving vehicle encounters frequent, small magnitude pressure oscillations known as infrasound. These are believed to result from flexing of the vehicle body, external turbulence, vehicle acceleration, and/or altitude changes (Vanderkooy, 2014). Calculations based on heavy vehicle infrasound levels reported in the literature suggested that such in-transit pressure changes may account for the rapid Ripestuff™ ethylene release reported by Duong et al. (2017).

In addition to the research on Ripestuff™, preliminary investigations were made into the potential use of an either ethylene or acetylene gas-filled sealed length of silicone tubing to ripen fruit in baskets (Appendix I). When tested in static chambers, ethylene-filled tubes produced a rapid increase in headspace ethylene concentration in the first 24 h, followed by a more gradual increase spanning a total period of 22 d. The final concentrations were low ($\leq$ 15.3 $\mu L.L^{-1}$) in comparison to those obtained from only 10 mg Ripestuff™ in a similar-sized chamber configuration ($\sim$70 $\mu L.L^{-1}$; Appendices B and C). Furthermore, acetylene-filled tubes tested in a basket configuration produced no detectable change in headspace acetylene levels. However, refinements to the gas-filled tube system such as increasing its holding capacity, increasing its surface area to volume ratio, and/or altering its thickness or composition to improve ethylene and/or acetylene diffusivity may generate greater ethylene or acetylene concentrations capable of inducing fruit ripening. Hence, further research in this alternative to Ripestuff™ approach is warranted.

5 Conclusions

This study demonstrated that exposure to ethylene released from Ripestuff™ achieves uniform ripening of 'Carabao' mango fruit under simulated supply chain conditions such as are typically encountered in the Philippines; viz., newspaper-lined baskets at ambient temperature. Efficacious ripening is dependent on either inclusion of water in the delivery system or careful matching of Ripestuff™ quantity with the number of holes in the lid of the delivery system to ensure adequate moisture diffusion to the inclusion complex for ethylene release. For a delivery system without added water, 1 g Ripestuff™ coupled with 64 holes in the lid was sufficient to trigger mango ripening and offers the simplest approach for wholesalers and retailers to implement. Use of a combination of multiple delivery systems containing different Ripestuff™ quantities and/or number of holes in the lid may further enhance fruit ripening by maintaining elevated ethylene levels in the basket headspace over a longer period. However, these would ultimately need to be incorporated into a single system such as a multi-chambered cartridge for ease of application by commercial operators. Overall, the findings provide proof-of-concept to justify further refinement and commercialisation of an appropriate technology Ripestuff™-based delivery ethylene system to replace calcium carbide.

6 Recommendations

1. With a view to gaining widespread adoption, engage with fruit ripeners in the Philippines and other calcium carbide using countries through pilot scale testing of the prototype Ripestuff™ delivery system and obtain feedback on performance, ease of use and limitations.

2. With a view to commercialisation, apply new knowledge to further refine current Ripestuff™ delivery system design for mango ripening and/or develop manufacturable simple lower cost alternatives; e.g., sachets made of ethylene- and moisture-permeable flexible polymer films.

3. With a view to broader application, investigate the efficacy of Ripestuff™ for batch-ripening other climacteric fruit crops of economic significance; e.g. avocado, tomato. Widespread adoption of Ripestuff™ in place of calcium carbide or various relatively ineffective treatments for fruit ripening would benefit the health of workers in the Philippines and other countries where the practice is legally or illegally employed.

4. With a view to fine-tuning function, characterise infrasound levels in fruit consignments during road transportation in developed and developing countries and verify their contribution to Ripestuff™ ethylene release from a prototype delivery system. This knowledge could subsequently be used to optimise delivery system design for in-transit ripening applications globally.

7 Acknowledgements

The authors thank ACIAR for funding this research and specifically Richard Markham and Irene Kernot for their support. We also thank Jenny Ekman and Adam Goldwater from Applied Horticultural Research for administering this extension to the project. UniQuest, and Deon Goosen in particular, is gratefully acknowledged for providing access to the Ripestuff™ material used in the experiments. We also acknowledge the assistance of Marina Isabel A. Tac-an, Leizel B. Secretaria, and Christine Diana S. Lubaton from UPMin in setting up experiments, collecting data, and reviewing the report for work undertaken in the Philippines. The assistance provided by Sohail Mazhar, Khamla Mott, Peter Isherwood, Kath Raymont, and Lachlan Fowler from UQ for work undertaken in Australia is similarly acknowledged.

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Appendix A. Ripestuff™ research reports arising from the HORT/2012/098 project variation

Title	Description
APPENDIX B **UPMIN RESEARCH REPORT** Application of Ripestuff™ for safer ripening of 'Carabao' mango in the Philippines	Presents **ten** experiments on: • Mango ripening in 96 L chambers (Experiments 1, 4 & 6) • Mango ripening in baskets (Experiments 2, 3, 7, 8, 9 & 10) • Ethylene release in 1.7 L chambers (Experiment 5)
APPENDIX C **UQ RESEARCH REPORT 1** Characterisation of Ripestuff™ ethylene release in a 'model' static chamber configuration	Presents **one** experiment on ethylene release and moisture diffusion in 2 L chambers
APPENDIX D **UQ RESEARCH REPORT 2** Relative humidity effects on Ripestuff™ ethylene release from a prototype delivery system	Presents **two** experiments on ethylene release and moisture diffusion in 2 L chambers
APPENDIX E **UQ RESEARCH REPORT 3** Efficacy of a prototype Ripestuff™ delivery system for batch-ripening of 'Cavendish' banana fruit	Presents **one** experiment on banana ripening, ethylene release and moisture diffusion in baskets
APPENDIX F **UQ RESEARCH REPORT 4** Overloading inhibits ethylene release from a prototype Ripestuff™ delivery system	Presents **one** experiment on ethylene release and moisture diffusion in 2 L chambers
APPENDIX G **UQ RESEARCH REPORT 5** Development of a prototype Ripestuff™ delivery system capable of sustained ethylene release	Presents **one** experiment on ethylene release and moisture diffusion in 2 L chambers
APPENDIX H **UQ RESEARCH REPORT 6** Air pressure oscillation effects on Ripestuff™ ethylene release from a prototype delivery system	Presents **one** experiment on ethylene release and moisture diffusion in 2 L chambers

Appendix B
APPLICATION OF RIPESTUFF™ FOR SAFER RIPENING OF 'CARABAO' MANGO IN THE PHILIPPINES

A research report prepared for
ACIAR Project HORT/2012/098

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Summary

The Ripestuff™ research is an extension to the ACIAR HORT 2012/098 “Improved Postharvest Management of Fruit and Vegetables in the Southern Philippines and Australia”. The research aimed to better understand the regulating influences on ethylene release kinetics from Ripestuff™ (University of Queensland (UQ)), and refine in a proof-of-concept context on the use of Ripestuff™ to ripen ‘Carabao’ mango fruit under laboratory and simulated conditions (University of the Philippines Mindanao (UP Min)).

The study conducted in UP Min was composed of 10 experiments on ‘Carabao’ mango and two for other crops. Experiment 1 tested the effect of environmental influences such as increased airflow (through fan) and relative humidity (through addition of water in the surrounding) and the combination of both to release ethylene from Ripestuff™ and for it to ripen ‘Carabao’ mango inside enclosed chambers. Experiment 2 tested different amounts of Ripestuff™ in proportion to fruit weight in the basket. In Experiment 3, the relative humidity inside the basket was increased by adding water in the surrounding environment of Ripestuff™ in combination with different amounts of Ripestuff™ powder. These three experiments failed to ripen ‘Carabao’ mango because ethylene was not effectively released from the dry Ripestuff™ powder with four holes in the lid of its container. The results of the release kinetics experiment conducted in UQ paved the way to optimize the release of ethylene from Ripestuff™ in the Philippine setting. Experiment 4 applied the best treatment from UQ experiment through the addition of water in its vessel which led to the fastest release of ethylene from Ripestuff™. The ethylene gas was not contained inside the enclosed chamber due to leakage. Although ethylene from Ripestuff™ was able to trigger ripening in ‘Carabao’ mango, it did not turn the fruit into its readily saleable stage after 72 h of treatment. Experiments 5 and 6 probed into other factors that could possibly influence the release of ethylene (e.g., number of holes in the lid of the container, and airflow) and the response of the fruit (e.g., maturity) to Ripestuff™. Results showed that ethylene release was faster when there were more holes in the lid of the container. It also eliminated the effect of airflow as a factor that influences the release of ethylene from Ripestuff™. Moreover, fruit maturity did not affect the ripe attributes of the mango fruit except that sinkers (i.e., mature mangoes) were sweeter when it ripened. Experiment 7 (in basket) applied the concept used in Experiment 4 (in chamber) and increased the target ethylene concentration by doubling the dose of Ripestuff™ up to four times. The application of Ripestuff™ based on calculated maximum ethylene release of 30 µL L^{-1} in a static chamber did not yield similar results if applied in a basket configuration due to low Ripestuff™ mass accompanied by leakage in the basket. Experiment 8 addressed this problem by increasing the mass of Ripestuff™ to 250 or 500 mg per 5 kg of fruit in the basket. Doubling the amount of Ripestuff™ to 500 mg resulted in better ripening in mangoes which was on par with the effect of calcium carbide, however, color development was uneven due to unsustained ethylene concentration in the basket during the treatment period. Experiment 9 was conducted to determine how ‘leaky’ the baskets were. The experiment confirmed that the basket configuration for the treatment of Ripestuff™ in mangoes resulted in high leakage of gas. To address the leakage issue, collaborators in UQ conducted an experiment and modelled a prototype treatment that would sustain ethylene concentration in the basket through a combination of various Ripestuff™ treatments with differing release rates. Experiment 10 tested the effect of ‘low’ sustained ethylene release on the ripening of ‘Carabao’ mango inside bamboo baskets. Results showed that the prototype was able to sustain a relatively higher concentration of ethylene in the basket for the entire treatment period, despite the basket’s leaky behaviour. Furthermore, the ‘low’ sustained ethylene release was able to achieve a more uniform ripening in ‘Carabao’ mango that was comparable to the effect of calcium carbide and 1000 mg dry Ripestuff™ contained in a vessel with 64 holes on the lid. The latter treatment is deemed as a more convenient solution to Ripestuff™ application which will not need water, fewer containers, and less Ripestuff™ mass.

Ripestuff™ also showed promising results with regards to its application to other tropical crops such as ‘Solo’ papaya and ‘Lakatan’ banana.

Contents

General introduction

Mango has an increasing commercial importance all over the world. In the Philippines, it is considered as a high value crop, ranking third among the most important fruit crops, next to banana and pineapple (Rodeo, 2016). In 2016, its production reached 814,055 MT where 14,343 MT of it was utilized for export (CountrySTAT Philippines, 2018). 'Carabao' mango, internationally known as "Philippine Super Mango" is the country's economically important variety and one of the world's best varieties due to its unique taste and aroma. Thus, it has a great demand both in local and international markets.

Figure 1. Traditional ripening method in the Philippines uses calcium carbide and bamboo baskets.

Being a climacteric fruit, mango is generally harvested at mature green stage and subsequently ripened through artificial methods. When ripened, the fruit goes through various physical, physiological and biochemical changes, and gradually becomes sweet, yellow, less firm, and palatable. Natural ripening in tropical ambient conditions usually takes 6 to 10 days from harvest to reach full ripening of mango fruit. A traditional method of ripening in the Philippines uses calcium carbide which releases acetylene gas in the presence of moisture and activates fruit ripening like ethylene (Medlicott et al., 1987). Calcium carbide is used by wholesalers and retailers at a rate of 8 to 10 g kg^{-1} of fruit to hasten ripening. However, if not packed properly, calcium carbide could leave residues of heavy metals such as arsenic and phosphorus on the fruit surface which are potentially carcinogenic (Sy and Wainwright, 1990). Exposure of the skin to calcium carbide may result in dermatitis and burns, while ingestion and inhalation could cause difficulty in breathing, nausea, and vomiting (US National Library of Medicine, 2014). Moreover, the individuals involved in fruit ripening have the highest health risk. Chronic exposure to inorganic arsenic results in higher chances of suffering from lung, skin, bladder, liver, kidney, and prostate cancers (Asif, 2012). Disposal of used calcium carbide is also a problem since it could pollute the environment. Its toxic components such as calcium hydroxide, strontium, and polycyclic aromatic hydrocarbons were reported to be toxic to marine animals and microorganisms leading to decreased biodiversity in the environment (Ihejirika et al., 2014; Semikolennykh et al., 2012). Despite these hazards, calcium carbide is still widely used because it is cheaper compared to other recommended ripening practices such as ethephon or compressed ethylene gas (Islam et al., 2016).

Ethylene, a plant hormone, is the major signaling molecule that controls most aspects of ripening in climacteric fruit (Pech et al., 2011). Stimulatory quantities of ethylene, such as 0.1 µL L^{-1} for mangoes, are required to accumulate prior to the onset of the climacteric rise in respiration (Burg and Burg, 1962). The fruit is then able to undergo ripening through an autocatalytic process in which ethylene stimulates its own biosynthesis (McMurchie et al., 1972).

Ripestuff™ (ethylene-α-cyclodextrin inclusion complex) powder is an alternative source of ethylene for fruit ripening (Ho et al., 2016). It contains 0.92-1.03 mole ethylene/mole cyclodextrin and can be fully released from the Ripestuff™ powder within 14 days at 93.6% RH (Ho et al., 2013; 2016). The controlled release of ethylene gas from ethylene-α-cyclodextrin inclusion complex powder with deliquescent salts increased the RH that accelerated the complete release of ethylene within 24 h (Ho et al., 2013; 2015; 2016). Their results further confirmed that the ethylene-α-cyclodextrin inclusion complex powder is an effective ethylene release system and can produce similar mango ripening effects like those

associated with ethylene from conventional sources. Experiments conducted by Ekman et al. (2018) on ripening of 'Carabao' mango using various ripening agents showed that Ripestuff™ had comparable effects with different concentrations of calcium carbide or ethephon (Ekman et al., 2018). However, non-optimized conditions resulting to uncontrolled release of ethylene from Ripestuff™ caused some undesirable effects on 'Carabao' mango during the later stage of storage. Therefore, there is a need to optimize the conditions for controlled release of ethylene with respect to delivery device, Ripestuff™ mass, and treatment conditions.

Compared with calcium carbide, Ripestuff™ is not classified as a dangerous material and can therefore be a safer alternative to other forms of fruit ripening (UniQuest, 2014). To achieve the full potential of Ripestuff™ without undesirable effects on fruit quality, this research aimed to (1) optimize the conditions that influence the release of ethylene from Ripestuff™, and (2) characterize the efficacy of Ripestuff™ in ripening 'Carabao' mangoes. Furthermore, this research aimed to define a 'proof-of-concept' context on the use of Ripestuff™ to ripen 'Carabao' mango fruit under laboratory and simulated conditions.

1 Experiment 1- Effect of Ripestuff™ facilitated by increased RH and airflow on the ripening of 'Carabao' mango inside enclosed chambers

1.1 Introduction

Ho et al. (2013; 2015; 2016) reported that the release of ethylene gas from the cyclodextrin inclusion complex (Ripestuff™) powder accelerates in concurrence to increasing relative humidity (RH). Their results also confirmed that Ripestuff™ powder is an effective ethylene release system and can produce similar mango ripening effects like those associated with ethylene from conventional sources. Prior research conducted in UP Mindanao showed that the use of Ripestuff™ powder alone at a rate of 0.75 g kg^{-1} (3.75 g total mass) contained in a vessel with four holes in the lid was not effective in ripening 'Carabao' mangoes compared to those treated with calcium carbide or ethephon (Ekman et al., 2018). This experiment aimed to optimize the conditions for faster release of ethylene from the Ripestuff™ powder by increasing the RH through the presence of moisture and airflow in the surrounding environment.

1.2 Materials and Methods

Freshly harvested mature green 'Carabao' mango at 107 days after flower induction were harvested from a farm in San Isidro, Island Garden City of Samal, Davao del Norte in April 2018. Seventy-five mangoes with uniform size and excellent quality were sanitized with 200 µL L^{-1} NaOCl for 3 min then air-dried. Ripestuff™ powder (obtained from UQ in July 2016) at a rate of 0.75 g kg^{-1} of mango fruit (i.e., 3.75 g total mass) was placed inside a specimen container with lid pierced four times with Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). Mangoes were treated for 72 h inside sealed chambers (V= 95.57 L) with the following treatments: Ripestuff™ alone, Ripestuff™ with fan, Ripestuff™ with water, or Ripestuff™ with fan and water (Figure 2). Each chamber contained a data logger (Tinytag Ultra 2 TGU-4500, Gemini Data Loggers Ltd., England) to monitor the temperature and RH during treatment. Untreated mangoes were held in ambient room conditions and served as control. The ethylene and CO_2 concentrations were monitored after 24, 48 and 72 h of treatment using Kitagawa ethylene and CO_2 detector tubes (Kitagawa Precision Gas Detector Tubes, Komyo Rikagaku Kogyo, Japan) and aspirating pump (Kitagawa AP-20 Aspirating Pump, Komyo Rikagaku Kogyo, Japan). After 72 h, the chambers were opened and mango fruit were held in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH). Mango fruit quality was evaluated at 3 (after 72 h of treatment), 4, 7 and 10 days after harvest (DAH). The effect of the treatments were determined using the following parameters: weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel color index (Appendix 14.2.1), peel and flesh color measurements (*L*, a*, b**, and color difference (ΔE)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease).The experiment was arranged in a Completely

Randomized Design (CRD) with three replicates each treatment. Each replicate had 10 fruit samples. Data were analyzed using Analysis of Variance (ANOVA) and differences in means were compared using Fisher's Least Significant Difference (LSD) at 5% level of significance.

Figure 2. Treatment of 'Carabao' mango with Ripestuff™ through a chamber with or without fan and/or water (A). Specimen container with 3.75 g Ripestuff™ powder (0.75 g kg^{-1}) placed on a weighing boat containing 5 mL distilled water (B). The fan was located overhead.

1.3 Results and Discussion

The maximum concentration of ethylene, 10 µL L^{-1}, released by 3.75 g (0.75 g kg^{-1}) Ripestuff™ was easily attained within 24 h when treated with addition of water in the surrounding environment (Figure 3A). Ripestuff™ alone, or when treated together with fan, released 10 µL L^{-1} of ethylene only after 72 h of treatment. There was no ethylene detected in the control chamber. CO_2 production was consistently highest in chamber containing Ripestuff™ with water and fan indicating an increased respiration rate probably due to ripening of the mangoes (Figure 3B). The control chamber with untreated mangoes had the lowest concentration of CO_2.

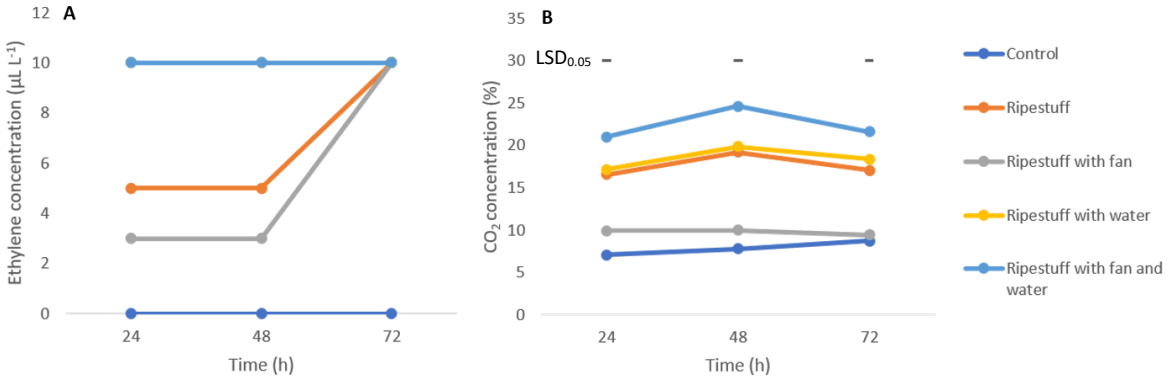

Figure 3. Ethylene (A) and CO_2 (B) concentrations inside chambers during treatment of 'Carabao' mango with Ripestuff™ in combination with fan and/or water.

After 72 h of treatment, mangoes treated with Ripestuff™, whether with fan and/or water in the surrounding, had more advanced peel and flesh color than the untreated mangoes (Figures 4-6). At 3 DAH, mangoes treated with Ripestuff™ and fan already showed yellow with trace of green peel color while the rest of the Ripestuff™ treatments had mangoes still at the turning stage while control mangoes where still green. At 7 DAH, all the Ripestuff™-treated mangoes had uniform yellow color. The differences (ΔE) between initial and final peel and flesh color did not vary in Ripestuff™-treated mangoes which suggests that ripening happened regardless of the presence of water and/or fan in chambers.

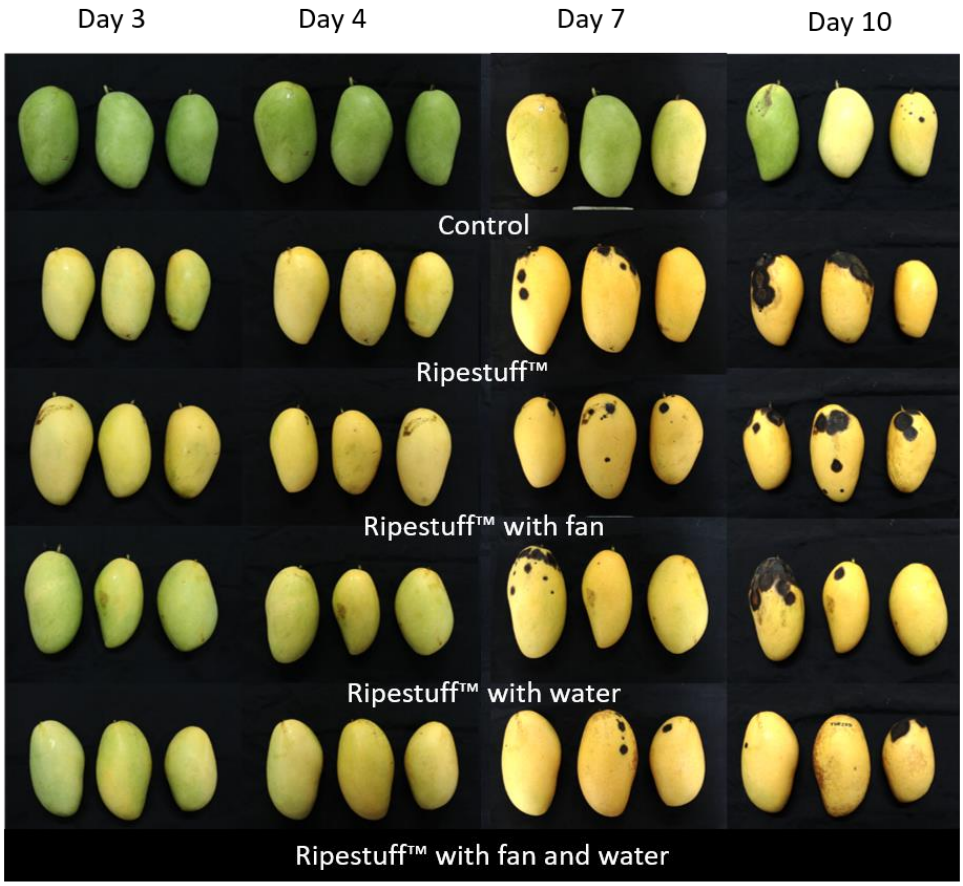

Figure 4. Appearance of 'Carabao' mango treated with Ripestuff™ in combination with fan and/or water, then stored in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH).

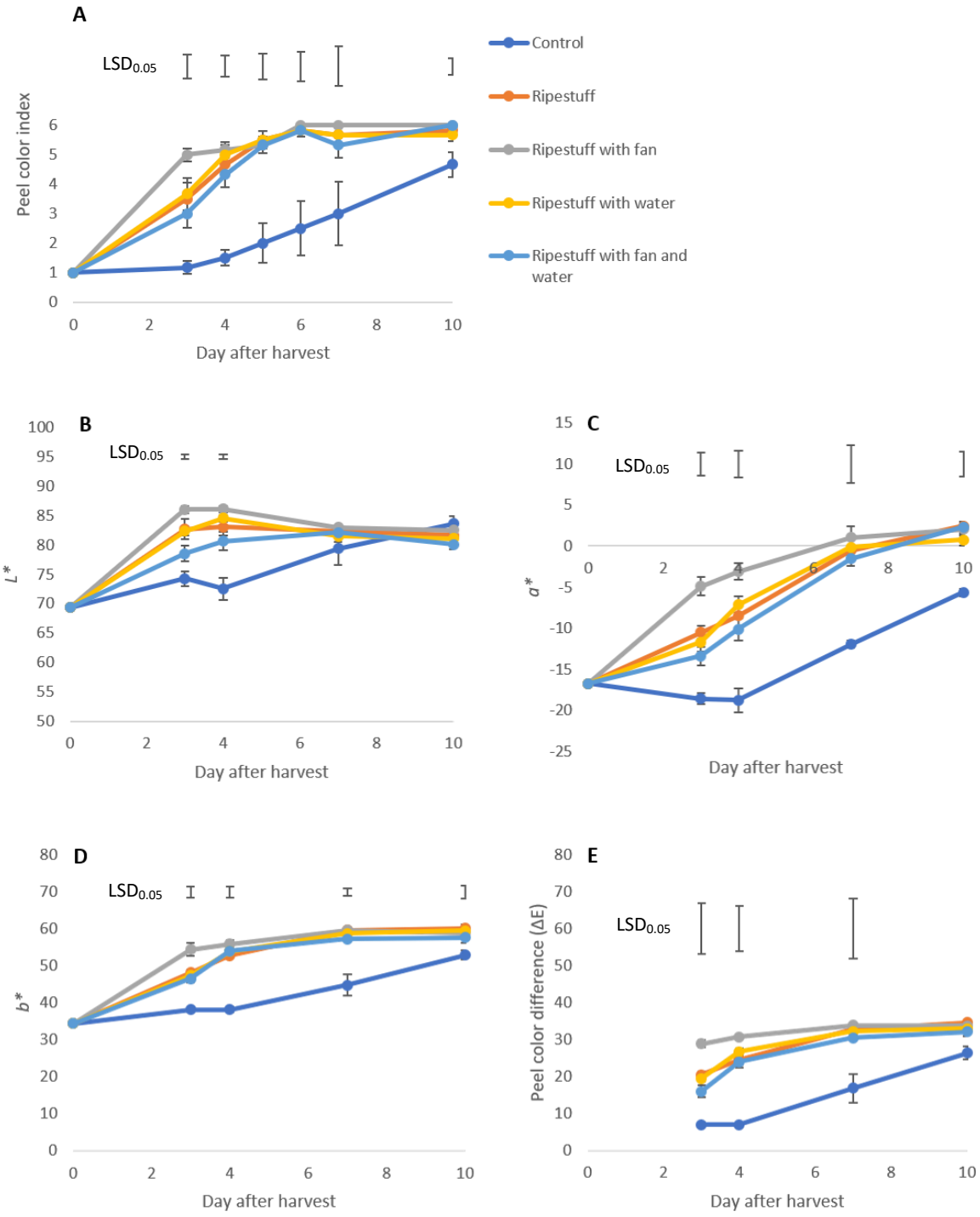

Figure 5. Peel color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) in mangoes treated with Ripestuff™ in combination with fan and/or water, and stored in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH). Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

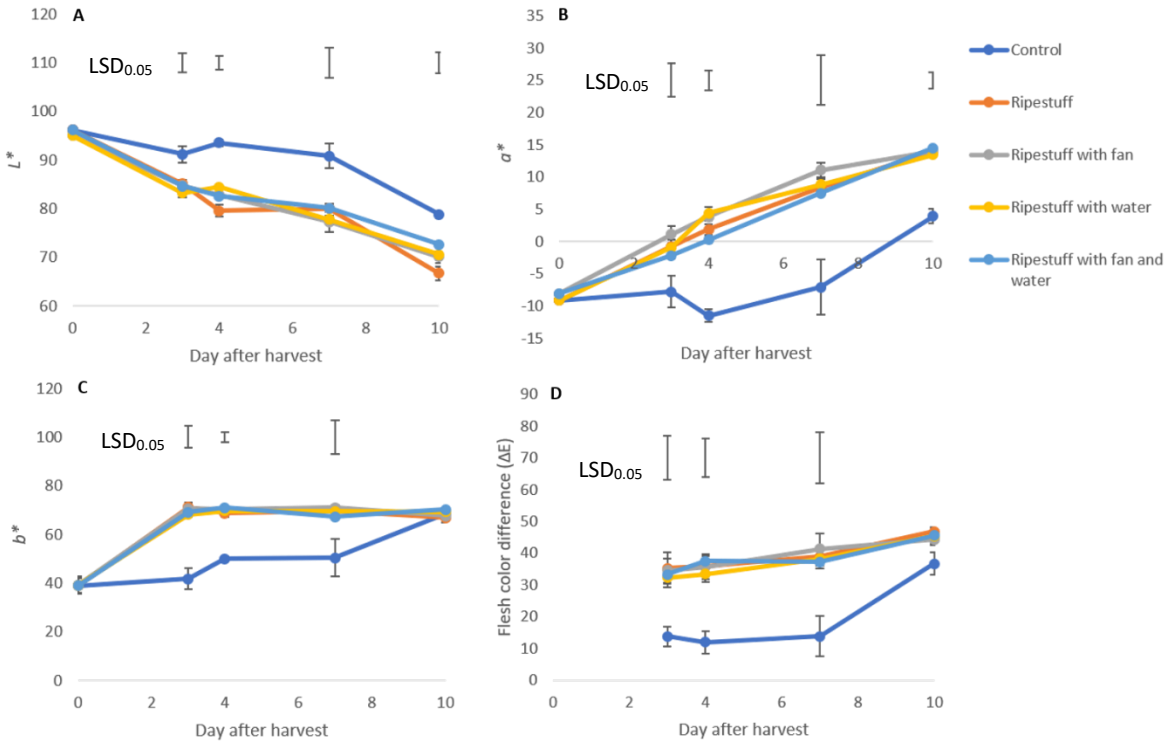

Figure 6. Flesh color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) in mangoes treated with Ripestuff™ in combination with fan and/or water, and stored in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH). Flesh color index: 1= white yellow; 2= light yellow; 3= bright yellow; 4= yellow-orange; 5= orange. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Ripestuff™-treated mangoes had higher weight loss, sweeter, and less firm than the control fruit (Figure 7). These indicate that mangoes treated with Ripestuff™ ripened regardless of the presence of fan and/or water.

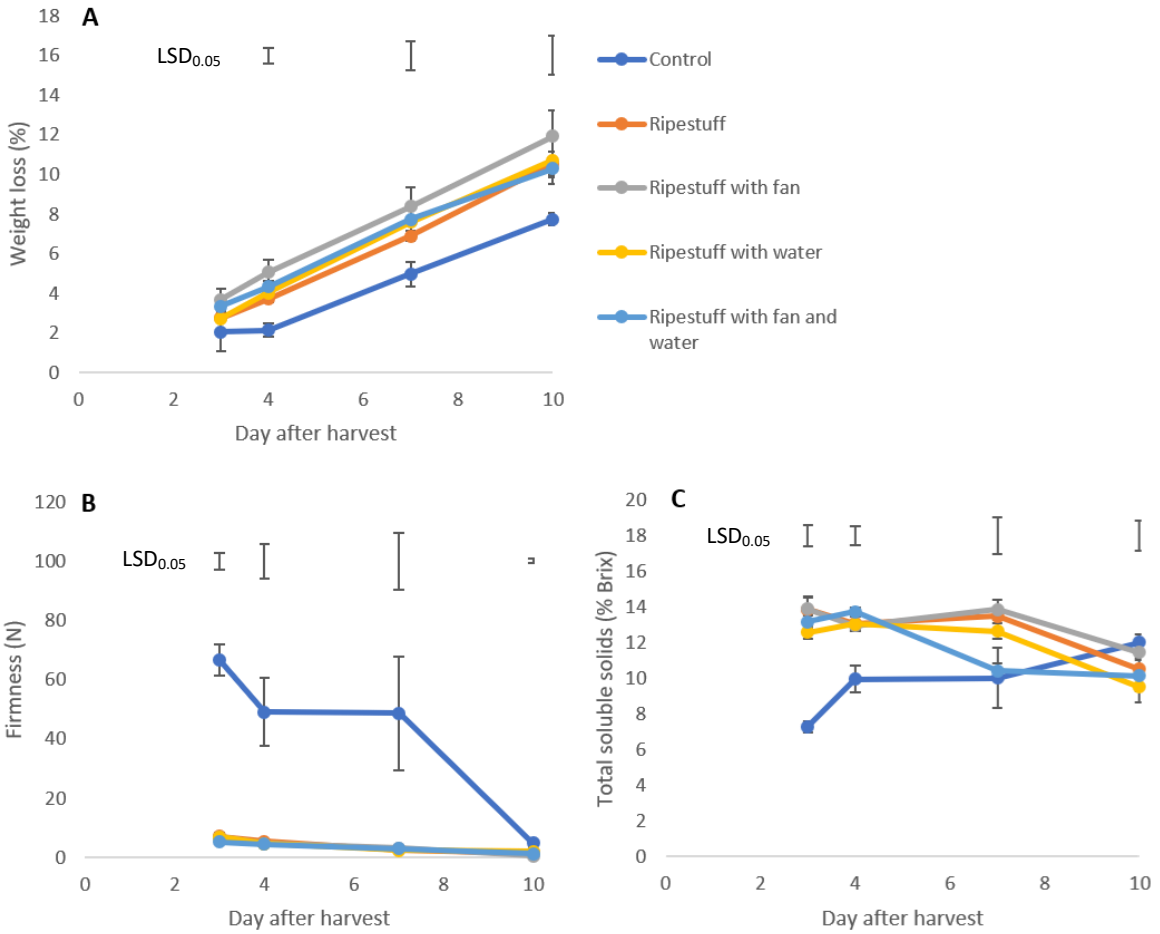

Figure 7. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango treated with Ripestuff™ in combination with fan and/or water, and stored in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Deterioration of the fruit's visual quality was faster in those treated with Ripestuff™ whether with or without fan and/or water as compared to the untreated mangoes (Figure 8). On the other hand, the occurrence of blotchy surface on the skin, stem-end rot and anthracnose, did not vary between those treated with Ripestuff™ (with fan and/or water in the surrounding) and the control.

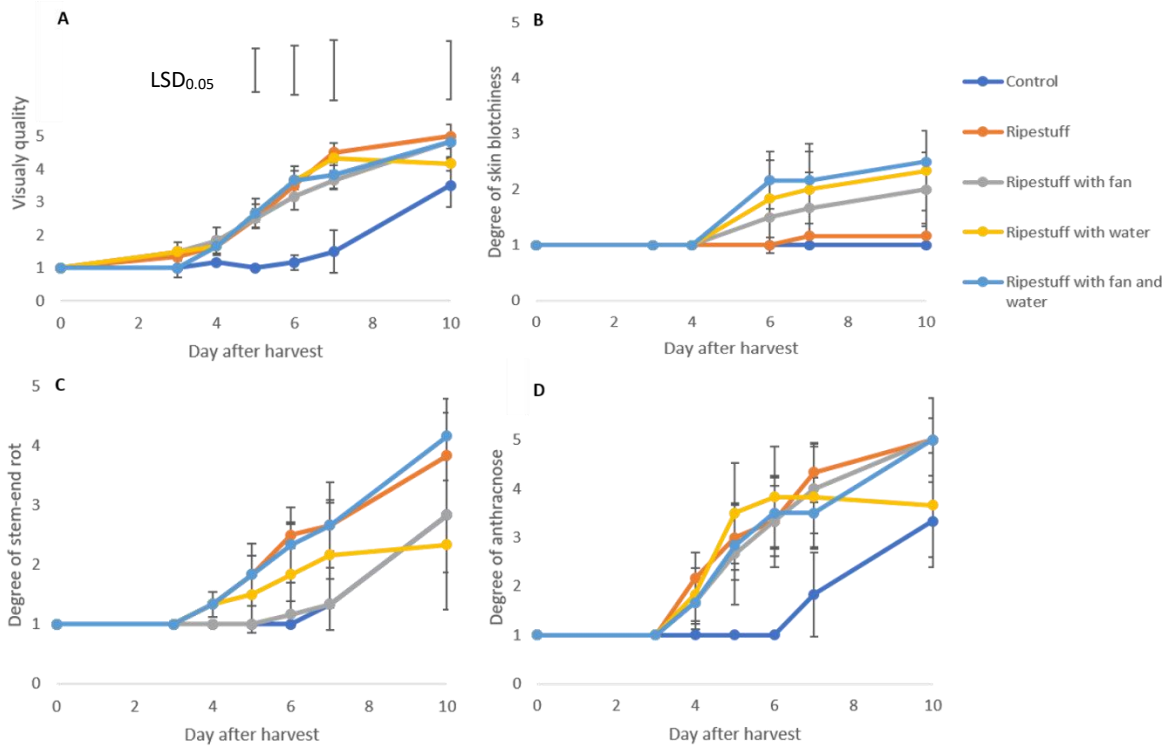

Figure 8. Visual quality of (A), and degree of skin blotchiness (B), stem-end rot (C) and anthracnose (D) in 'Carabao' mango treated with Ripestuff™ in combination with fan and/or water, and stored in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH). Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Application of Ripestuff™ resulted in mangoes with shorter time to reach saleability which was 3 to 4.3 d compared to the untreated mangoes at 9.2 d (Table 1). All Ripestuff™-treated mangoes, whether with fan and/or water in the surrounding, had a similar shelf life of 6.5 to 7.7 d while the control mangoes had 10.5 d. Mangoes that were treated with Ripestuff™ had shorter shelf life because it ripened sooner than the control. However, saleable days did not vary between them as the onset of stem-end rot and anthracnose occurred at the same time which limited its saleability.

Table 1. Saleability and shelf life of 'Carabao' mango treated with Ripestuff™ in combination with fan and/or water, then stored in ambient room conditions (27.1±2.0°C, 87.0±8.6% RH).

Treatment	Days to saleability[z]	Saleable days[NS]	Shelf life[z] (d)
Control	9.2[a]	1.3	10.5[a]
Ripestuff™	4.2[bc]	2.3	6.5[b]
Ripestuff™ with fan	3.0[c]	4.7	7.7[b]
Ripestuff™ with water	4.0[bc]	2.8	6.8[b]
Ripestuff™ with fan and water	4.3[b]	2.5	6.8[b]

[z]Means in a column with common letter/s are not significantly different using LSD at $P \leq 0.05$.
[NS]Not significant

Temperature inside treatment chambers was lower when Ripestuff™ was treated in combination with fan or water (28-29°C) compared to chambers with Ripestuff™ alone or in combination with fan and water (32-33°C) (Appendix Figure 1A). The RH reached 100% inside chambers with Ripestuff™ with fan and/or water (Appendix Figure 1B). On the other hand, the chambers without fan and/or water, sustained only 95% RH. As the RH were high, the discrepancy between those with or without fan and/or water might not have caused difference in the ethylene release rate that is ultimately responsible for fruit ripening, however high temperatures could have influenced the fruit ripening in some treatment lots. The differences in temperature could be attributed to the uneven distribution of heat in the room and the lack of airflow inside it. This could be the reason why mangoes treated with Ripestuff™ with fan in the chamber had more advance peel color after 72 h of treatment compared to the other Ripestuff™ treatments.

1.4 Conclusion

Ripestuff™ demonstrated the ability to initiate ripening in ‘Carabao’ mango even without fan and/or water inside an enclosed chamber. However, the ethylene released from Ripestuff™ seemed insufficient to turn the fruit into its readily saleable stage (i.e., fully yellow peel color) after 72 h treatment. Optimization of the release of ethylene from the Ripestuff™ powder is necessary to obtain an effective and more uniform ripening in mangoes. Further, it was recommended to eliminate the sources of error that could have caused fluctuations in the room temperature which can in turn influence fruit ripening. This could be addressed by distributing the treatments randomly in the room, increasing the airflow, and controlling the temperature by turning on the air-conditioning unit during treatment and storage of mangoes. These measures were incorporated in the next experiments.

2 Experiment 2- Effect of different amounts of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets

2.1 Introduction

This experiment tested different amounts of Ripestuff™ powder in proportion to mango fruit weight with an aim to determine the optimum amount of Ripestuff™ that results in effective ripening of 'Carabao' mango inside bamboo baskets.

2.2 Materials and Methods

There were two trials conducted in this experiment. For the first trial, freshly harvested mature green 'Carabao' mango were procured from the Southern Philippines Fresh Fruit Corporation in May 2018. In Trial 2, mangoes were harvested from a farm in Banaybanay, Davao Oriental at 104 days after flower induction, in August 2018. In each trial, 75 kg mangoes with uniform size and excellent quality were procured and sanitized with 200 µL L^{-1} NaOCl for 3 min then air-dried. Thereafter, the samples were treated with 25 g calcium carbide (i.e., 5 g kg^{-1}) or Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with newspaper linings (Figure 9). The total mass 2.5, 3.75, and 5 g of Ripestuff™ was based on the rates: 0.5, 0.75, and 1 g Ripestuff™ per kg of fruit, respectively. Each basket (V= 12 L) contained 5 kg mango fruit. Ripestuff™ powder (obtained from UQ in July 2016) was placed inside a 60 mL specimen container with lid pierced four times with Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). In Trial 1, untreated mangoes held in ambient served as the control. In Trial 2, the control fruit were packed inside baskets with empty specimen containers with four holes in the lid. The fruit inside bamboo baskets were covered with newspapers (5 sheets) and secured with polypropylene twine. The temperature and RH during treatment for 72 h were recorded using data loggers (Tinytag Ultra 2 TGU-4500, Gemini Data Loggers Ltd., England). Mango fruit quality was evaluated at 3 (after 72 h of treatment), 4 and 7 days after harvest (DAH). Data collected were weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel color index (Appendix 14.2.1) and measurements (*L*, a*, b**, and color difference (∆E)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). The experiment was arranged in CRD with three replicates each treatment. Each replicate had 10 fruit samples. Data were analyzed using ANOVA and differences in means were detected using Fisher's LSD at 5% level of significance.

Figure 9. Treatment of 'Carabao' mango using calcium carbide (25 g) and Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg mango fruit (A) wrapped in newspaper and secured with polypropylene twine (B).

2.3 Results and Discussion

2.3.1 Trial 1

The first trial of this experiment showed that Ripestuff™ was able to initiate ripening in 'Carabao' mango (Figure 10). Based on peel color parameters (Figure 11), the treatment of 5 g Ripestuff™ in 5 kg mangoes had comparable results with those treated with calcium carbide which was significantly different from the untreated mangoes. However, its ripening effect may have not been enough as reflected by the uneven ripening on the skin color after 72 h of treatment.

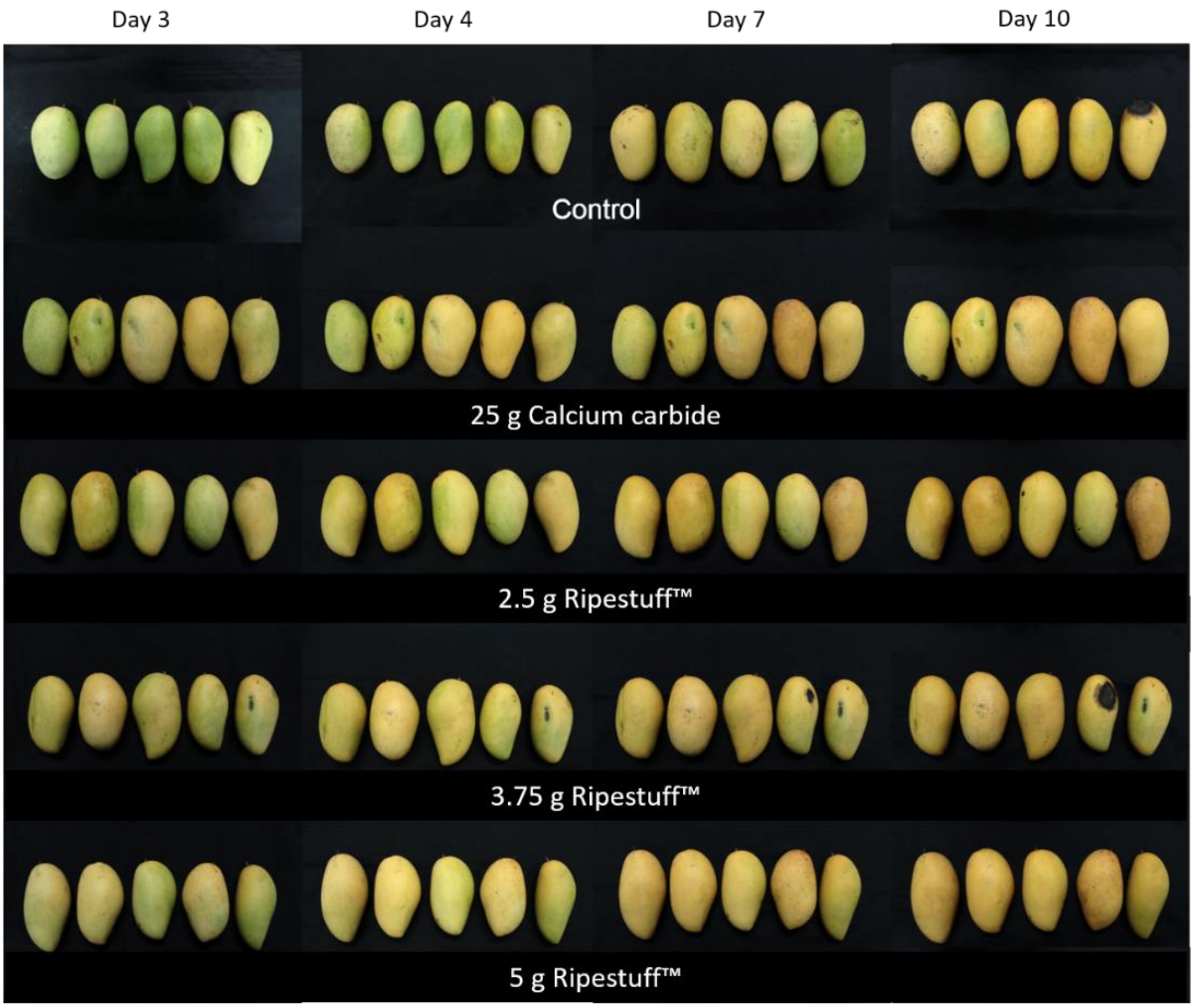

Figure 10. Appearance of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg mango fruit; then stored in ambient room conditions (27.1±2.0ºC; 87.0±8.6% RH).

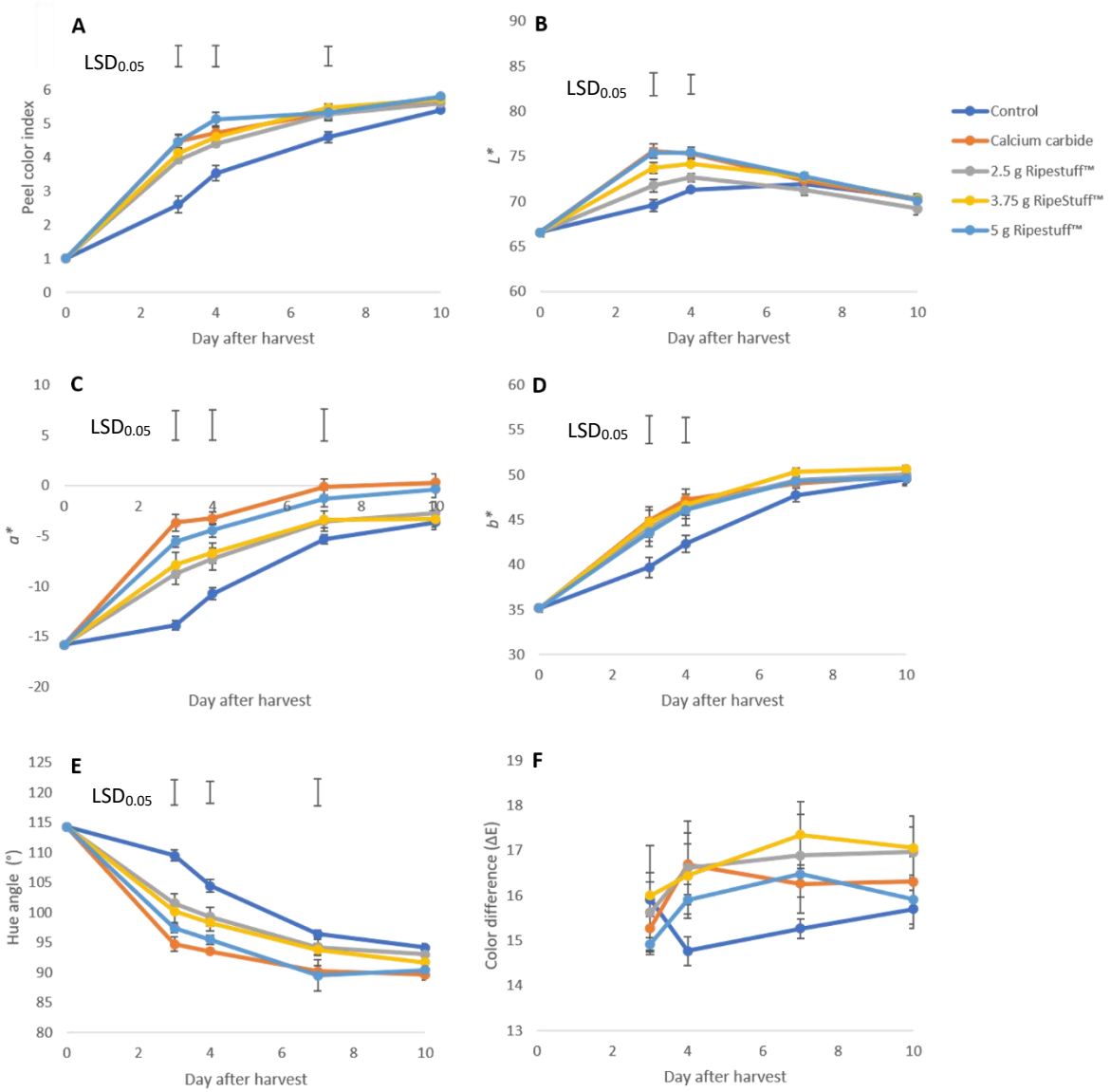

Figure 11. Peel color index (A), *L** (B), *a** (C), *b** (D), hue angle (E), and color difference (F) of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg mango fruit; then stored in ambient room conditions (27.1±2.0ºC; 87.0±8.6% RH). Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Weight loss did not vary among treatments except at 7 DAH where mangoes treated with 5 g Ripestuff™ had lower weight loss than the control (Figure 12A). After 72 h of treatment, the TSS and firmness of Ripestuff™-treated mangoes were similar to those treated with calcium carbide, regardless of the amount of Ripestuff™ powder used (Figure 12B-C).

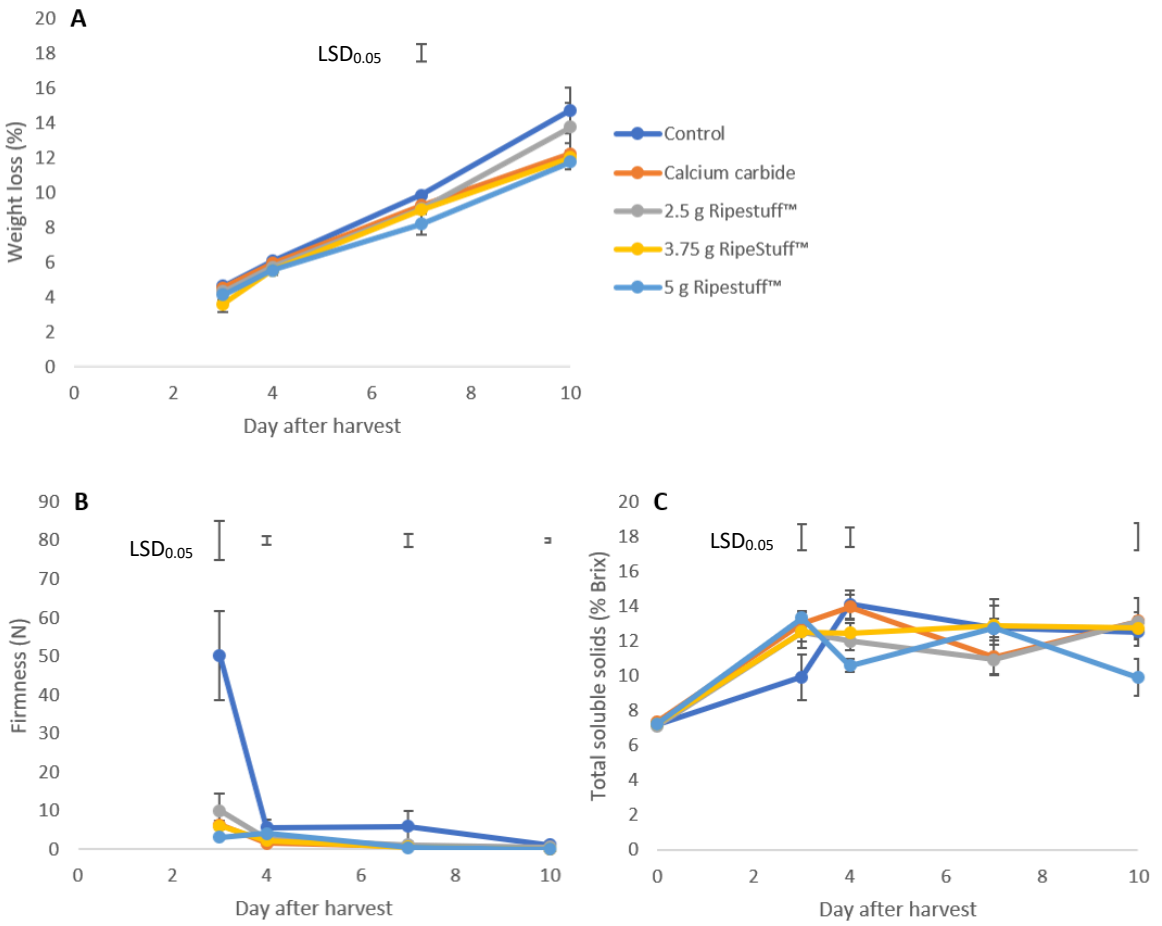

Figure 12. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg fruit; then stored in ambient room conditions (27.1±2.0ºC; 87.0±8.6% RH). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Mangoes treated with Ripestuff™, especially at a dose of 5 g per 5 kg mangoes, had better visual quality than those treated with calcium carbide and the control (Figure 13A). The onset of blotches on the skin occurred earlier in those treated with calcium carbide or 2.5 g Ripestuff™ (Figure 13B). The degree of stem-end rot and anthracnose did not vary among the treatments (Figure 13C-D).

Moreover, the treatment of Ripestuff™, regardless of its amount (2.5-5 g per 5 kg mangoes) resulted in fruit that reached saleability stage as fast as those treated with calcium carbide (Table 2). The use of Ripestuff™ resulted in saleable days similar to those treated with calcium carbide. Mangoes treated with Ripestuff™ also had longer shelf life than those treated with calcium carbide and the control.

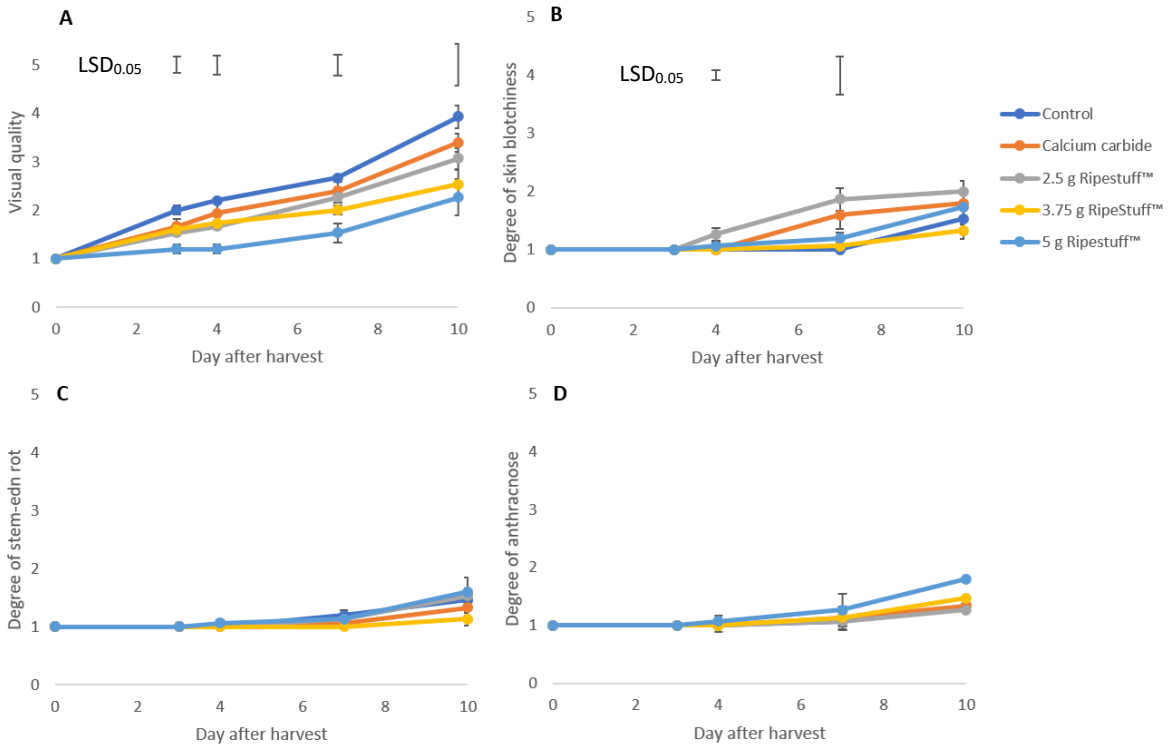

Figure 13. Visual quality (A), degree of blotchiness (B), stem-end rot (C), and anthracnose (D) in 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg fruit; then stored in ambient room conditions (27.1±2.0ºC; 87.0±8.6% RH). Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Table 2. Saleability and shelf life of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg fruit then stored in ambient room conditions (27.1±2.0ºC; 87.0±8.6% RH).

Treatment	Concentration (g Ripestuff™/ calcium carbide kg^{-1} mango)	Days to saleability[z]	Saleable days[z]	Shelf life[z] (d)
Control		7.7[a]	2.7[c]	10.3[d]
25 g Calcium carbide	5.0	4.7[b]	6.1[ab]	10.7[cd]
2.5 g Ripestuff™	0.5	5.6[b]	5.5[b]	11.1[bc]
3.75 g Ripestuff™	0.75	4.7[b]	6.9[ab]	11.6[ab]
5 g Ripestuff™	1.0	4.3[b]	7.6[a]	11.9[a]

[z]Means in a column with common letter/s are not significantly different using LSD at $P \leq 0.05$.

The temperature inside baskets with Ripestuff™ treatments was 3ºC lower than the basket with calcium carbide in it (Appendix Figure 2.1A). The RH inside bamboo baskets was at 92-94% while (Appendix Figure 2.1B).

2.3.2 Trial 2

In contrast to the first trial, Trial 2 of this experiment showed that the treatment of Ripestuff™ was not able to initiate ripening in 'Carabao' mango unlike calcium carbide (Figure 14). This could be attributed to the difference in maturity and quality of the fruit used in both experiments. Based on the fruit's peel color, only fruit treated with calcium carbide ripened successfully after 72 h of treatment (Figure 15). Further, mango fruit treated with calcium carbide had higher weight loss, were less firm and sweeter than the fruit treated with Ripestuff™ at various amounts (2.5, 3.75, or 5 g) (Figure 16). The effect of Ripestuff™ treatments on mango did not vary with the control. A day after opening the baskets, the firmness and TSS content of the fruit from all treatments became similar with the control. The deterioration of visual quality and development of skin blotchiness, stem-end rot, and anthracnose were advance in mangoes treated with calcium carbide (Figure 17). The onset of diseases in mangoes occurred earlier due to the general quality of the fruit at harvest which was not on par with the ones used in the first trial.

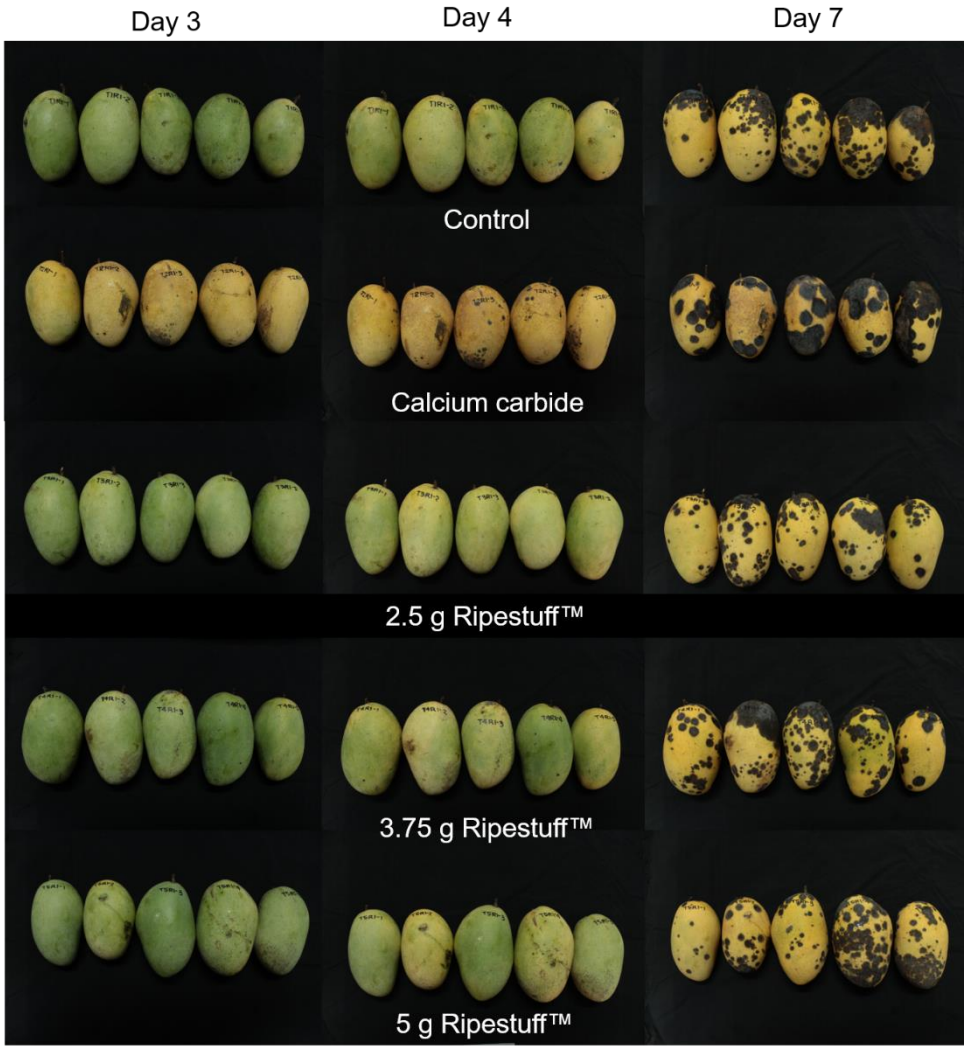

Figure 14. Appearance of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets then stored in ambient room conditions (28.1±1.2ºC; 92.8±7.9% RH). Each bamboo basket contained 5 kg mangoes.

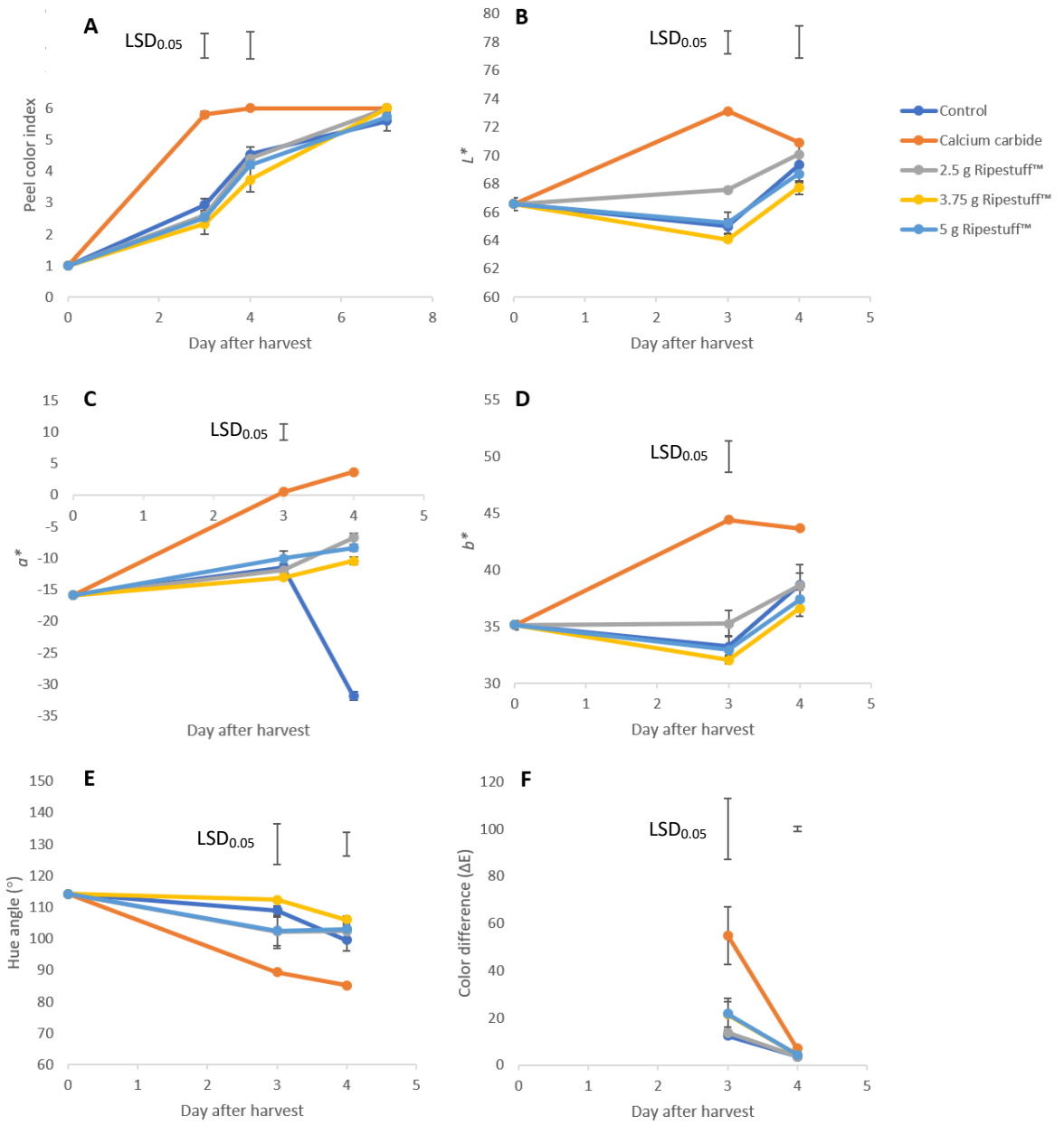

Figure 15. Peel color index (A), *L** (B), *a** (C), *b** (D), hue angle (E), and color difference (F) of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg fruit; then stored in ambient room conditions (28.1±1.2ºC; 92.8±7.9% RH). Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

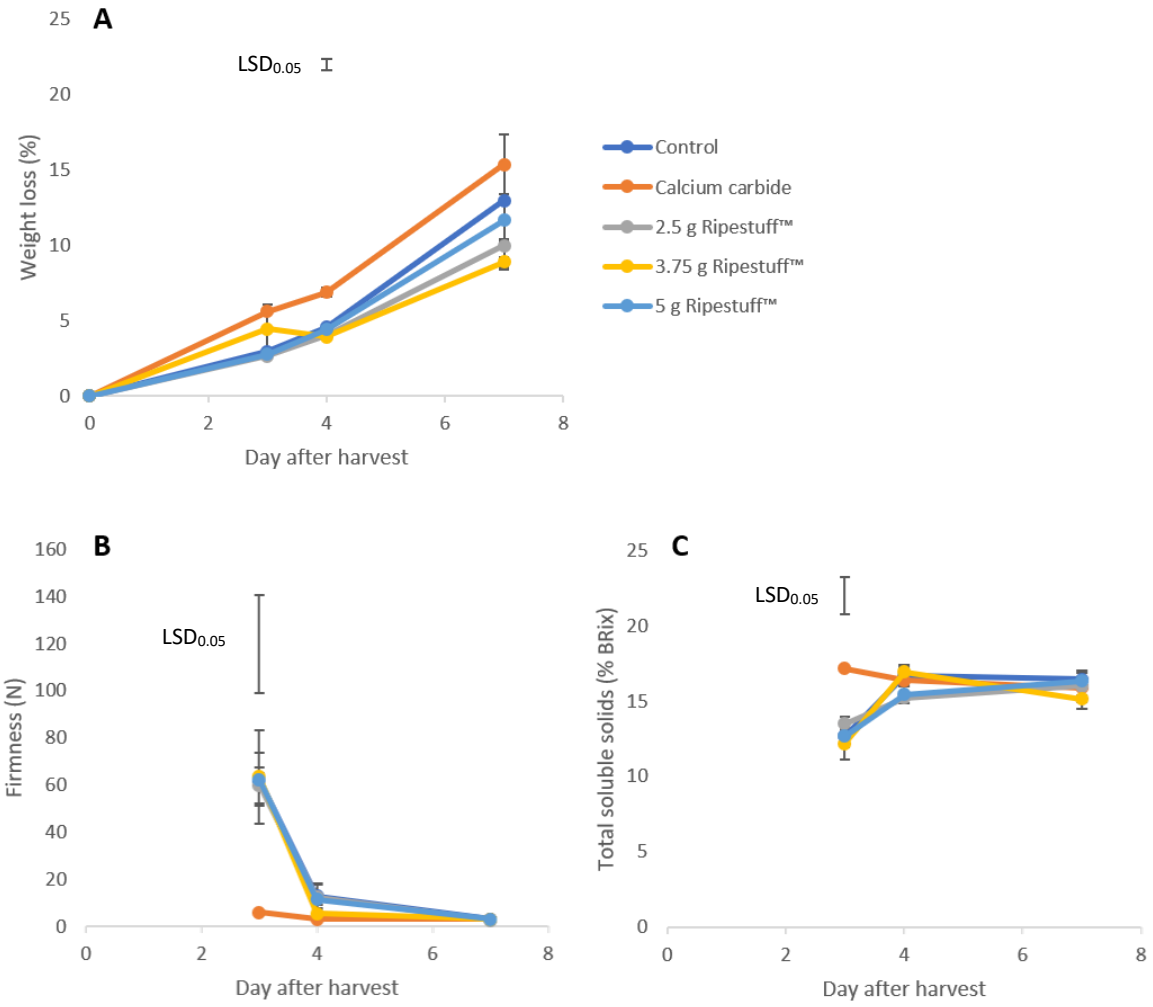

Figure 16. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango treated with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg fruit; then stored in ambient room conditions (28.1±1.2ºC; 92.8±7.9% RH). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

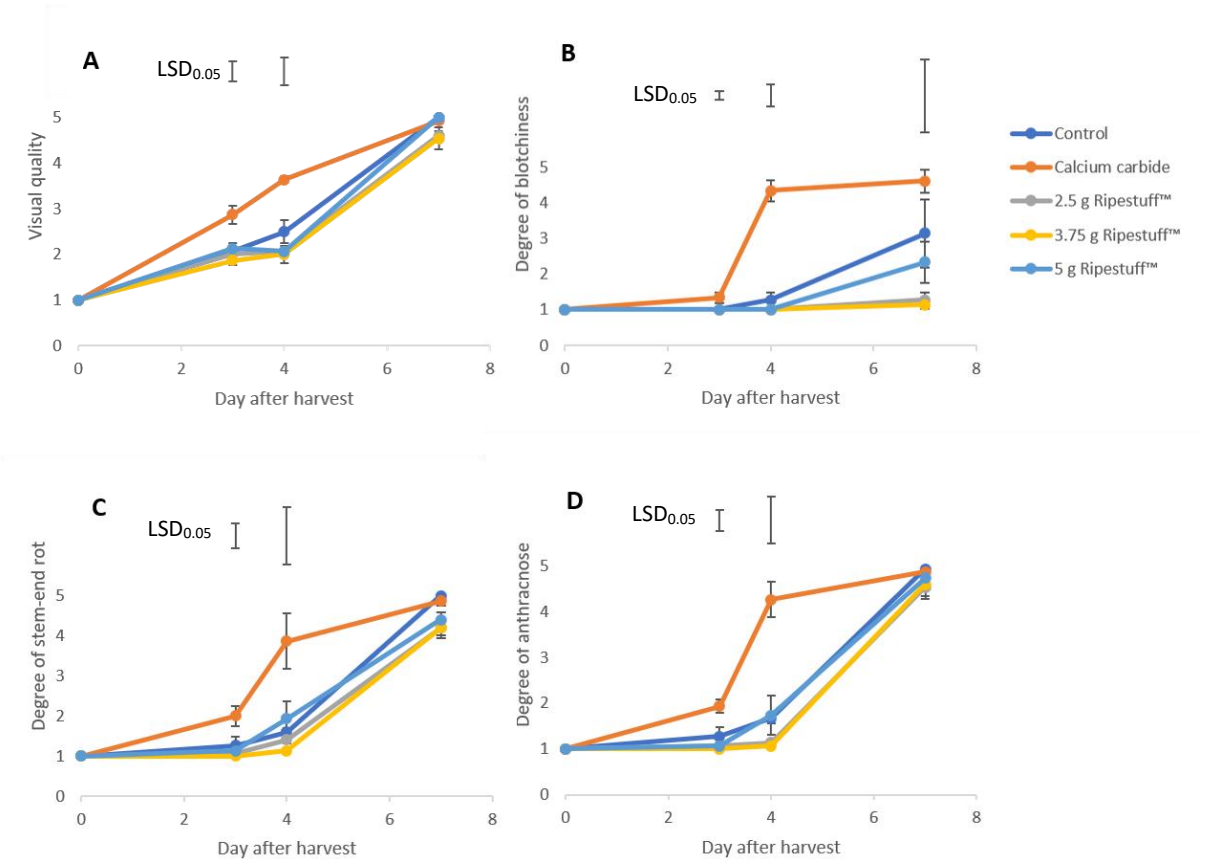

Figure 17. Visual quality (A), degree of blotchiness (B), stem-end rot (C), and anthracnose (D) in 'Carabao' mango treated with calcium carbide or different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets with 5 kg fruit; then stored in ambient room conditions (28.1±1.2ºC; 92.8±7.9% RH). Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

The saleable stage was reached sooner by mangoes treated with calcium carbide at 3 d compared to Ripestuff™-treated mangoes at 5.9-6.5 d (Table 3). However, the saleable days did not vary among treatments. As mangoes treated with calcium carbide ripened soonest, it also had the shortest shelf life.

Table 3. Saleability and shelf life of 'Carabao' mango treated with calcium carbide or different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets[z] then stored in ambient room conditions (28.1±1.2ºC; 92.8±7.9% RH).

Treatment	Concentration (g Ripestuff™/ calcium carbide kg^{-1} mango)	Days to saleability[x]	Saleable days[NS]	Shelf life[x] (d)
Control		4.1[b]	2.2	4.3[bc]
25 g Calcium carbide	5.0	3.0[b]	0.2	3.2[c]
2.5 g Ripestuff™	0.5	5.9[a]	0.7	6.6[a]
3.75 g Ripestuff™	0.75	6.3[a]	0.7	6.6[a]
5 g Ripestuff™	1.0	6.5[a]	2.8	4.8[b]

[z]Each basket contained 5 kg fruit.
[x]Means in a column with common letter/s are not significantly different using LSD at $P \leq 0.05$.
[NS]Not significant

2.4 Conclusion

In Trial 1, Ripestuff™ treatment with the three amounts tested (2.5, 3.75, or 5 g), could initiate ripening in 'Carabao' mango fruit inside bamboo baskets which was comparable to the effect of calcium carbide. However, in Trial 2, only those treated with calcium carbide ripened effectively after 72 h of treatment in baskets whereas Ripestuff™-treated mangoes did not. The disparity of the results could be attributed to the difference in maturity in mangoes and overall quality at harvest. Despite the fruit's positive response to Ripestuff™ in Trial 1, Ripestuff™ may still not be at its optimum potential as it did not turn the mangoes in its readily saleable stage after 72 h of treatment. Further optimization on the use of Ripestuff™ was addressed in the next experiments.

3 Experiment 3- Effect of different amounts of Ripestuff™ and the addition of water on the ripening of 'Carabao' mango inside bamboo baskets

3.1 Introduction

In view of faster release of ethylene from Ripestuff™ with increased relative humidity (RH), this experiment tested the effect of the addition of water in the surrounding environment to enhance the release of ethylene from Ripestuff™ powder. Effective release of ethylene would in turn result in rapid and uniform ripening in mango fruit.

3.2 Materials and Methods

Mature green 'Carabao' mango harvested from Carmen, Davao del Norte were procured from the Southern Philippines Fresh Fruit Corporation in November 2018. A total of 100 kg mangoes with uniform size and excellent quality were procured and sanitized with 200 µL L^{-1} NaOCl for 3 min then air-dried. Five kg mangoes were placed inside each bamboo baskets (V= 12 L). The samples were then treated with 25 g calcium carbide (5 g kg^{-1}) or Ripestuff™ (1.25, 2.5, 3.75, and 5 g based on the rates 0.25, 0.50, 0.75, and 1 g Ripestuff™ kg^{-1} of fruit, respectively) inside bamboo baskets with 5 sheets of newspaper linings (Figure 18). Ripestuff™ powder (obtained from UQ in July 2016) was placed inside a specimen container with lid pierced four times with Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). The Ripestuff™ container was placed inside a 250 mL beaker containing 50 mL distilled water and allowed to 'sit'. Untreated mangoes held inside the bamboo baskets without the presence of water served as control. The mangoes inside the basket were covered with newspapers and secured with polypropylene twine. The temperature and RH were recorded during treatment for 72 h using data loggers (Tinytag Ultra 2 TGU-4500, Gemini Data Loggers Ltd., England). Mango fruit quality was evaluated at 3 (after 72 h of ripening treatment), 4, 7 and 10 days after harvest (DAH). Data collected were weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel color index (Appendix 14.2.1) and measurements (*L*, a*, b**, and color difference (∆E)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). The experiment was arranged in CRD with three replicates each treatment. Each replicate had 10 fruit samples. Data were analyzed using ANOVA and differences in means were detected using Fisher's LSD at 5% level of significance.

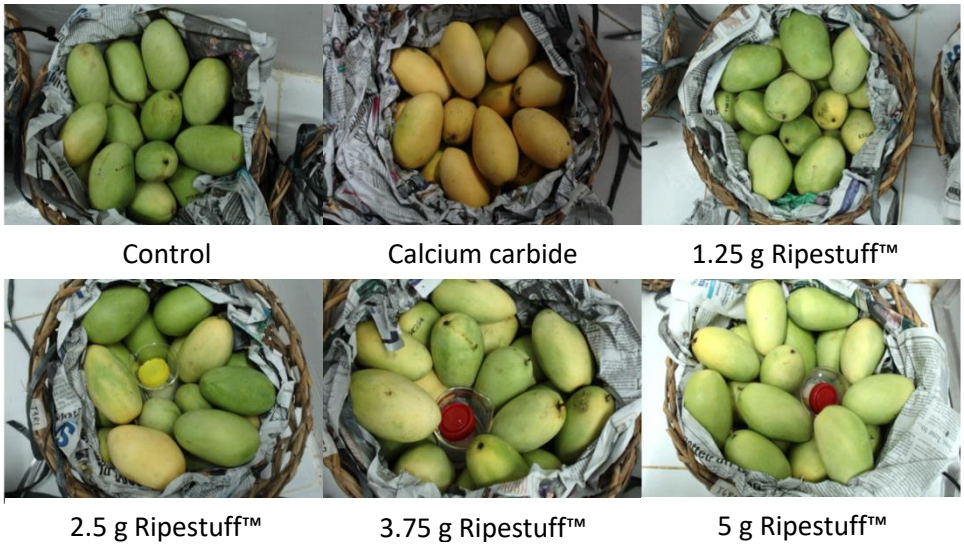

Figure 18. 'Carabao' mango inside bamboo baskets with newspaper linings and treatment with different amounts of Ripestuff™ (1.25, 2.5, 3.75, or 1 g) with addition of water in the surrounding. Each basket contained 5 kg mango fruit. Photo taken at opening of baskets after 72 h of treatment.

3.3 Results and Discussion

This experiment showed that the addition of water in the surrounding environment of Ripestuff™ container did not effectively ripen the fruit after treatment for 72 h inside bamboo baskets (Figure 19). In terms of peel color development (Figure 20), weight loss, firmness, and total soluble solids (Figure 21), the effect of Ripestuff™ did not vary with the control. Calcium carbide, on the other hand, ripened 'Carabao' mangoes with its peel color already in the saleable stage after 72 h treatment. In relation to ripening, the deterioration of visual quality also occurred faster in those treated with calcium carbide (Figure 22A). The degree of blotchiness, stem-end rot, and anthracnose did not vary among treatments (Figure 22B-D).

Mangoes treated with calcium carbide took only 2.8 d to reach saleability, followed by those treated with 5 g Ripestuff™ at 5.2 d (Table 4). The rest of the treatments were not significantly different from the control which became saleable only at 6.3 d. Shelf life and saleable days did not vary among treatments.

Figure 19. Appearance of ‘Carabao’ mango as influenced by different amounts of Ripestuff™ (1.25, 2.5, 3.75, and 5 g) with added water in the surrounding, and fruit were stored in ambient room conditions (26.5±0.7ºC; 80.1±4.3% RH). Each basket contained 5 kg mangoes.

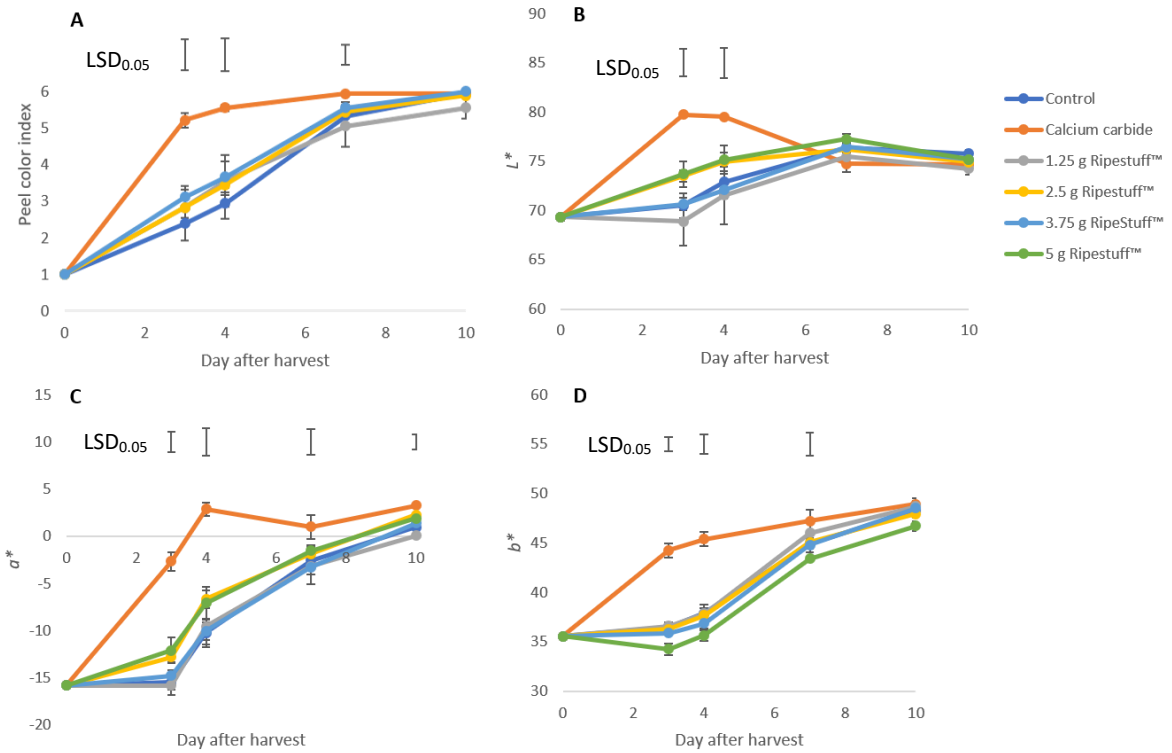

Figure 20. Peel color index (A), *L** (B), *a** (C), and *b** (D) of 'Carabao' mango as influenced by different amounts of Ripestuff™ (1.25, 2.5, 3.75, and 5 g) with added water in the surrounding, and fruit were stored in ambient room conditions (26.5±0.7ºC; 80.1±4.3% RH). Each basket contained 5 kg mangoes. Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

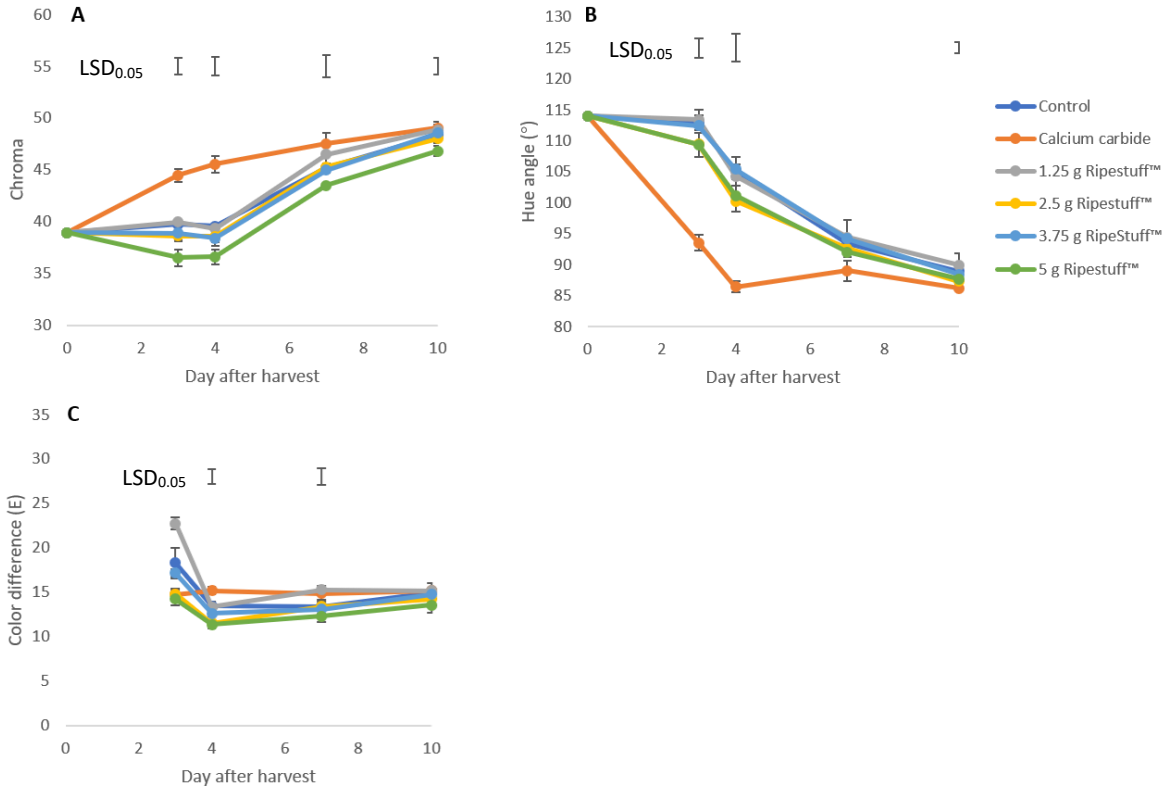

Figure 21. Chroma (A), hue angle (B), and color difference (C) of 'Carabao' mango peel as influenced by different amounts of Ripestuff™ (1.25, 2.5, 3.75, and 5 g) with added water in the surrounding, and fruit were stored in ambient room conditions (26.5±0.7ºC; 80.1±4.3% RH). Each basket contained 5 kg mangoes. Data points with LSD bar are significantly different at *P*≤0.05. Error bars= SEM.

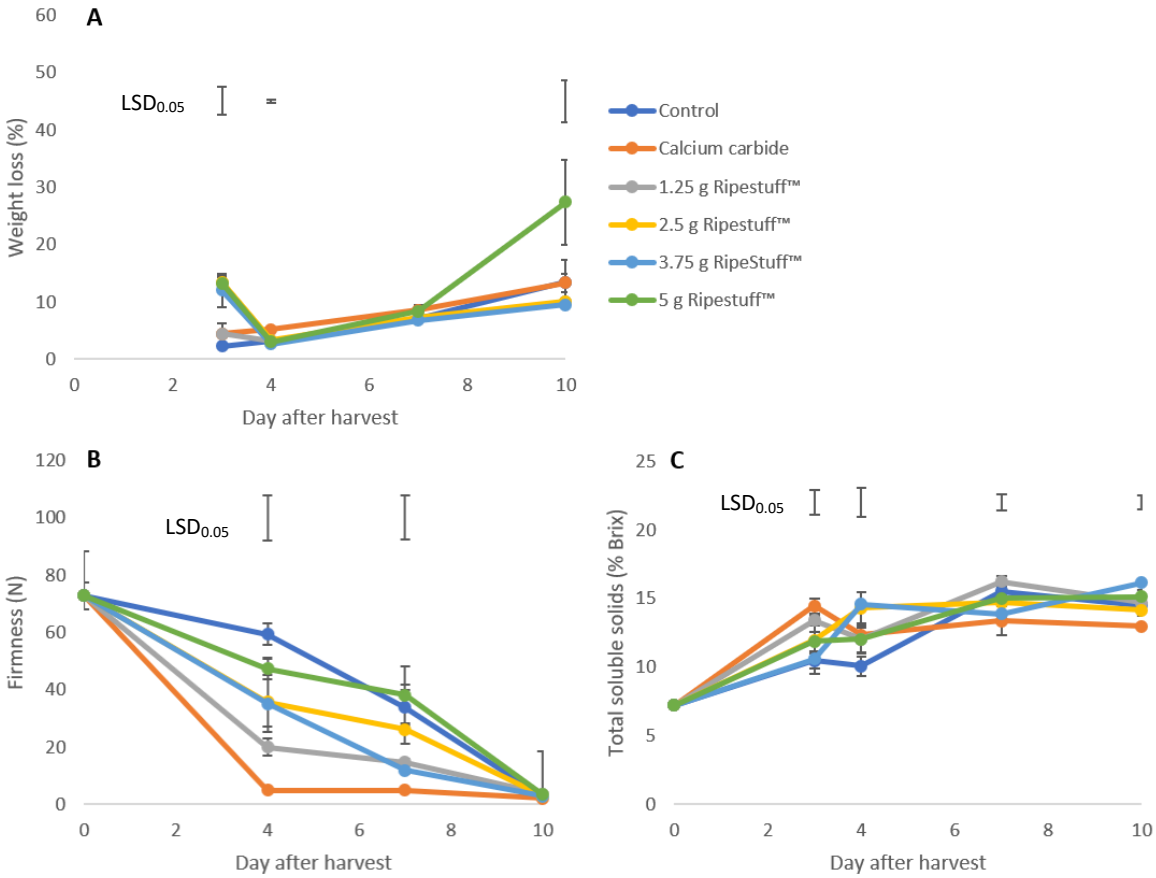

Figure 22. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango as influenced by different amounts of Ripestuff™ (1.25, 2.5, 3.75, and 5 g) with added water in the surrounding, and fruit were stored in ambient room conditions (26.5±0.7ºC; 80.1±4.3% RH). Each basket contained 5 kg mangoes. Data points with LSD bar are significantly different at *P*≤0.05. Error bars= SEM.

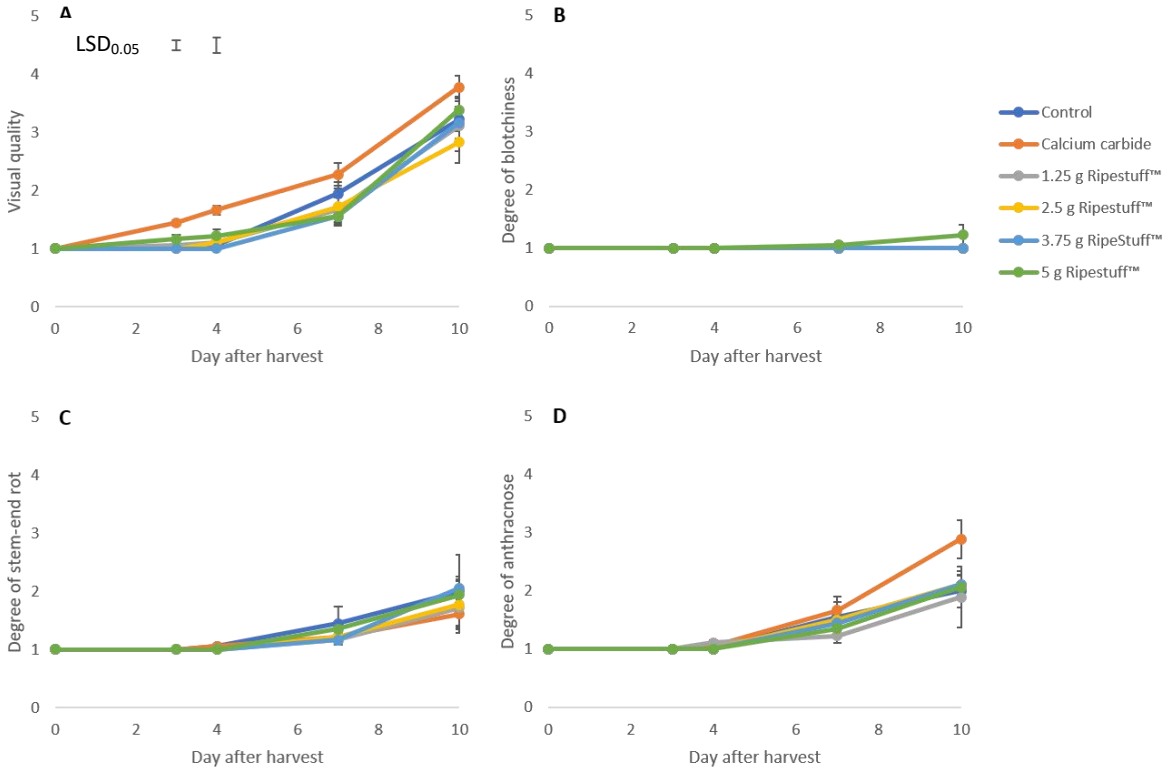

Figure 23. Visual quality (A), degree of skin blotchiness (B), stem-end rot (C), and anthracnose (D) in 'Carabao' mango as influenced by different amounts of Ripestuff™ (1.25, 2.5, 3.75, and 5 g) with added water in the surrounding, and fruit were stored in ambient room conditions (26.5±0.7ºC; 80.1±4.3% RH). Each basket contained 5 kg mangoes. Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Table 4. Saleability and shelf life of 'Carabao' mango as influenced by different amounts of Ripestuff™ and addition of water in the surrounding; fruit were stored in ambient room conditions (26.5±0.7ºC; 80.1±4.3% RH).

Treatment	Concentration (g Ripestuff™/ calcium carbide kg^{-1} mangoes)	Days to saleability^{z}	Saleable days^{NS}	Shelf life^{NS} (d)
Control		6.3^{a}	5.2	11.5
Calcium carbide	5.0	2.8^{c}	7.1	9.9
1.25 g Ripestuff™	0.25	6.1^{ab}	5.1	11.2
2.5 g Ripestuff™	0.5	5.7^{ab}	5.8	11.5
3.75 g Ripestuff™	0.75	5.6^{ab}	5.1	10.7
5 g Ripestuff™	1.0	5.2^{b}	5.3	10.5

^{z}Means in a column with common letter/s are not significantly different using LSD at $P \leq 0.05$.
^{NS}Not significant

In terms of temperature, baskets treated with calcium carbide had higher temperature reaching 29°C inside the basket while the rest of the treatments remained between 25-26°C (Appendix Figure 3A). With the addition of water in the surrounding of Ripestuff™ treatments, the RH remained at 90-100% levels while the control (without water) was only at 80-85% (Appendix Figure 3B). Baskets with calcium carbide also had high RH at around 95% probably due to the high moisture that transpired from the fruit brought about by the high temperature from the reaction of calcium carbide with moisture.

3.4 Conclusion

Unlike the previous experiments, this experiment showed that Ripestuff™ was not able to ripen ‘Carabao’ mango even with the addition of water in the surrounding to increase the RH in the environment and ultimately release ethylene from the Ripestuff™ powder. This result could be attributed to the differences in maturity of the fruit used in the experiment. This was addressed in the next experiment by identifying the maturity (viz., floater or sinker) and establishing harvest characteristics such as dry matter, initial peel color, firmness, and TSS.

4 Experiment 4- Effect of Ripestuff™ with added water in the vessel on the ripening of 'Carabao' mango inside enclosed chamber

4.1 Introduction

Results of the previous experiments showed that Ripestuff™ was able to initiate ripening in 'Carabao' mango but it did not match the ripening effect of calcium carbide. On the other hand, mangoes treated with Ripestuff™ ripened ahead of the untreated fruit. The slight delay in ripening of Ripestuff™-treated mangoes indicated that ethylene was not fully released from the cyclodextrin inclusion complex powder. Based on the release kinetics experiment conducted in the University of Queensland (UQ) (Figure 24), ethylene was released faster when water was added in the vessel with four holes on the lid (Perkins and Joyce, 2019a). In their experiment, the use of four holes on the lid resulted in 100% ethylene release from the Ripestuff™ powder within 12 h. It is surmised that if only one hole will be used, the ethylene would have a relatively controlled release. This experiment aimed to optimize the application method of Ripestuff™ based on the results of the release kinetics experiment conducted in UQ. Furthermore, this experiment determined the effect of Ripestuff™ (with added water in the vessel) on the ripening of 'Carabao' mango.

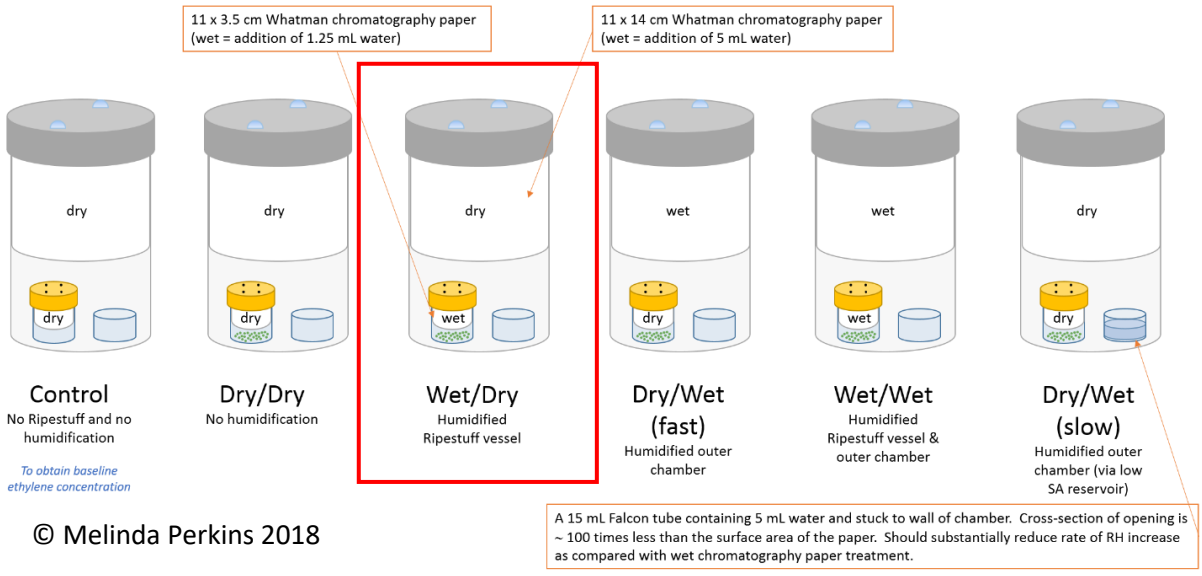

Figure 24. Ripestuff™ release kinetics experimental set-up conducted in UQ. The treatment highlighted in red facilitated the most efficient release of ethylene from Ripestuff™. Photo credits: Melinda Perkins (2018).

4.2 Materials and Methods

Due to limited availability of chambers, this experiment was conducted in two batches representing two replicates. A total of 60 kg 'Carabao' mangoes (19% dry matter) harvested at 108-110 days after flower induction were sourced from Digos City, Davao del Sur (58% sinkers) and Mati City, Davao Oriental (61% sinkers) between January to February 2019. The samples were transported to the Postharvest Biology Laboratory through an air-conditioned

vehicle. In the laboratory, the temperature was set at 25°C using an air-conditioning unit to attain a uniform temperature and facilitate airflow inside the room.

Mango fruit quality at harvest was characterized. Dry matter (%) was determined by drying 5 g of mango fruit flesh in a hot air-oven at 130°C for 2 h or until constant weight was attained. The mango fruit were subjected to flotation using 1% NaCl solution to determine the maturity (i.e., floater= immature; sinker= mature). The fruit were then sanitized with 200 µL L^{-1} NaOCl for 3 min and air-dried. The fruit were weighed and the volume of 10 sample fruit were determined using water displacement method. The total volume of the fruit was determined by multiplying the average volume of the fruit by the total number of samples in the chamber. Sinker and floater mangoes were randomly distributed among treatments.

The treatments used in this experiment were: calcium carbide (25.37 g), Ripestuff™ (0.26 g) without water in the vessel, and Ripestuff™ with water (5 mL) in the vessel. Untreated mangoes with empty specimen container inside the chamber served as control. The volume of 5 kg mango fruit was used to calculate the amount of Ripestuff™ powder to release 30 µL L^{-1} ethylene in the chamber headspace. Ripestuff™ powder Batch '7 Feb B' (0.42 mol·mol^{-1} ethylene) was sourced from UQ Gatton and used in this experiment. A mass of 0.26 g Ripestuff™ powder was used per treatment to achieve a full release of 30 µL L^{-1} ethylene in a static chamber having 95.57 L volume. The Ripestuff™ powder was sieved using a strainer to eliminate the lumps before weighing through an analytical balance. The powder was then placed in a 60 mL specimen container and covered with a screw-cap lid pierced once using Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). Five mL distilled water was added to the container when the chambers were about to be closed. The amount of calcium carbide used was calculated based on the acetylene concentration from calcium carbide (80% technical grade) that has the same biological effect as ethylene (i.e., 270 µL L^{-1} acetylene per 0.1 µL L^{-1} ethylene) (Saltveit, 1999). The total acetylene released from calcium carbide into the chamber headspace was 81,000 µL L^{-1}. Calcium carbide followed a traditional manner of treatment which was by wrapping in paper.

The treatments were applied on mangoes inside pre-sanitized chambers for 72 h (Figure 25). Each chamber had a fan to facilitate airflow which mimics the function of holes in the bamboo baskets. There were two replications in each treatment distributed in a completely randomized design. To achieve a more consistent condition during treatment and storage, the linear temperature gradient in the laboratory was eliminated by setting the air-conditioning unit at 25°C.

Figure 25. Treatment of calcium carbide or Ripestuff™, with or without water in the vessel, in 'Carabao' mango inside enclosed chambers. Untreated mangoes inside chamber served as control. Each chamber contained 5 kg mangoes.

Ethylene concentration in the chamber headspace was measured every h for the first 8 h of treatment, then every 24 h for 72 h using a portable ethylene gas analyser (Ethan, Bioconservacion, Barcelona, Spain). Two opposite lateral sides of the chamber were attached with sampling ports (i.e., one on each side) composed of 30 cm x 4 mm polyvinyl chloride (PVC) flexible tube (Ezy Flex Tube, Holman Industries, Western Australia) fitted with 4 mm barbed in-line tap (Pope, Toro Australia Pty Ltd, South Australia). The external tubes (inlet and outlet) of the gas analyser were attached to each port upon gas sampling to return the gas back into the chamber. In this manner, the concentration of ethylene in the chamber remained unchanged. The gas analyser has an internal pump which provides a continuous flow to and from the detector. The instrument uses an electrochemical detector that is sensitive to ethylene.

After 72 h of treatment, the fruit samples were transferred in trays and stored in ambient room conditions (24.3±0.6°C, 92.0±7.8% RH). Mango fruit quality was evaluated at 3 (after 72 h of ripening treatment), 4, 7 and 10 days after harvest (DAH). Data collected were respiration rate in terms of CO_2 using a portable gas analyser (CheckPoint O_2/CO_2, Dansensor, Denmark), ethylene production rate using Ethan ethylene analyser, weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel color index (Appendix 14.2.1), peel and flesh color measurements (*L*, a*, b**, and color difference (∆E)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). The experiment was arranged in CRD with two replicates each treatment. The data for the third replicate was extrapolated from the first two replicates. Each replicate had 30 fruit samples. Data were analyzed using ANOVA and differences in means were detected using Fisher's LSD at 5% level of significance.

4.3 Results and Discussion

Ripestuff™ with added water in the vessel released ethylene faster than the one without water (Figure 26). After 1 h of treatment, ethylene concentration in the chamber headspace already reached 6 µL L^{-1} comprising 18% release of total ethylene from Ripestuff™ powder. At 5 h after treatment, it reached the highest concentration recorded at 10 µL L^{-1} ethylene which was 30% of maximum ethylene released in the chamber headspace from the Ripestuff™ powder. After that, ethylene continuously dropped down to 7 µL L^{-1} at the 6th h until no ethylene from Ripestuff™ was left in the chamber after 72 h of treatment. Although ethylene was not accumulated in the chamber, data on residual ethylene shows that ethylene from Ripestuff™ with added water was almost fully released as evidenced by the very low concentration left in the vessel headspace (Table 5).

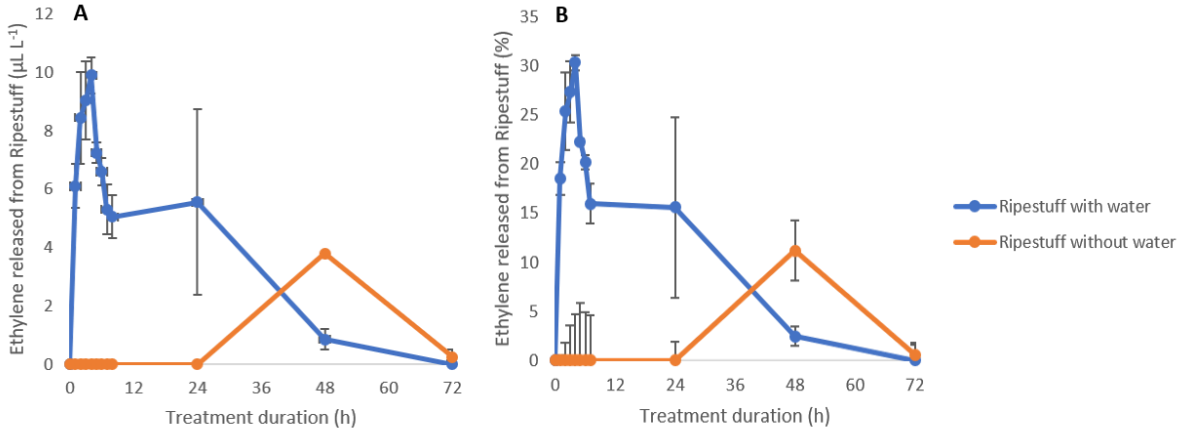

Figure 26. Ethylene released from Ripestuff™ with or without added water in the vessel (A) and its corresponding percentage (B). Full Ripestuff™ ethylene release at 30 µL L^{-1}. Error bars= SEM.

Table 5. Residual ethylene from Ripestuff™ left in the vessel after 72 h of treatment in enclosed chambers.

Treatment	Residual ethylene after 72 h (µL L^{-1}) ± SEM
Ripestuff™ without water	46.4 ± 6.9
Ripestuff™ with water	2.6 ± 2.5

Ethylene was supposed to be accumulated in the chamber as it was assumed to be tightly sealed. However, upon leakage test using water, leaks were detected on the sides of the chamber even after closing the locks (Figure 27). The leaks on the sides of the chamber could explain why ethylene gas was not accumulated in the chamber and 100% release of ethylene from Ripestuff™ was not recorded.

Figure 27. Leakage detection in chambers using water leak test.

In terms of ripening, mangoes treated with Ripestuff™ with added water had the highest respiration rate among others indicating physiological changes rapidly occurring in the fruit (Figure 28A). It also had an increased ethylene production after treatment, an indicative of ripening process taking place (Figure 28B).

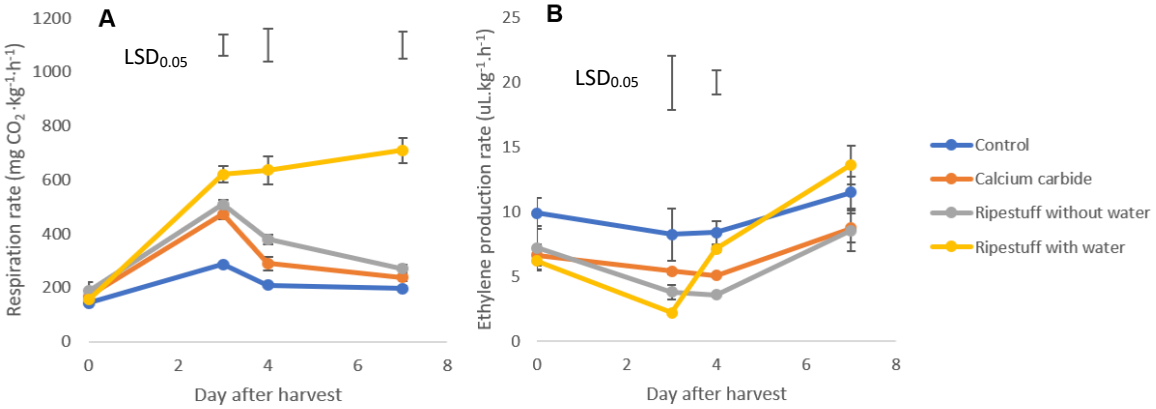

Figure 28. Respiration (A) and ethylene production (B) rates of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water, during storage in air-conditioned room (24.3±0.6°C, 92.0±7.8% RH). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Based on the peel color and overall appearance of the mangoes, Ripestuff™ with added water was able to initiate ripening faster than the control but not as fast as calcium carbide (Figures 29-30). Ripestuff™ without water was not effective in ripening as the mangoes were the same as the untreated ones based on peel color development. However, when it comes to flesh color (Figure 31) and firmness (Figure 32B), it seemed that mangoes treated with Ripestuff™ with added water ripened similar to the effect of calcium carbide. Fruit skin and flesh are supposed to respond similarly however, there were cases in artificially ripened 'Carabao' mango wherein asynchronous ripening happened for a time and then firmness or sweetness become uniform thereafter (Lacap et al., 2019).

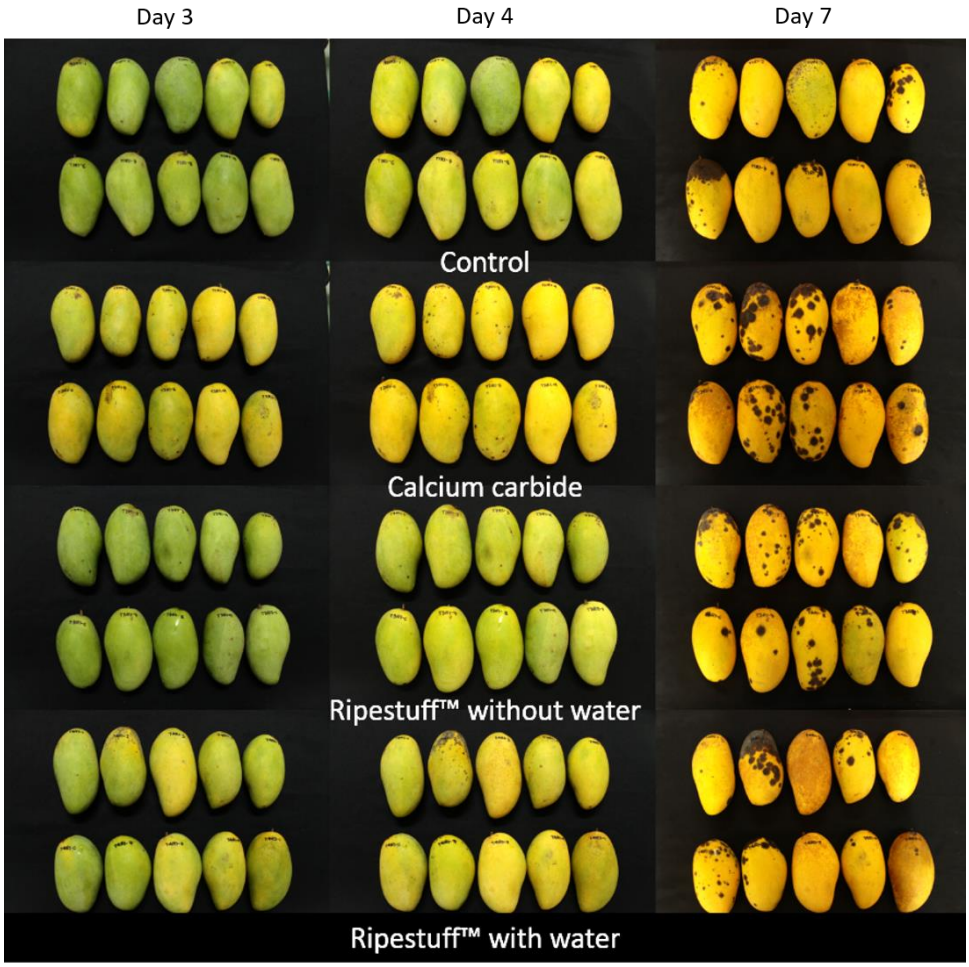

Figure 29. Appearance of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water, during storage in air-conditioned room (24.3±0.6°C, 92.0±7.8% RH).

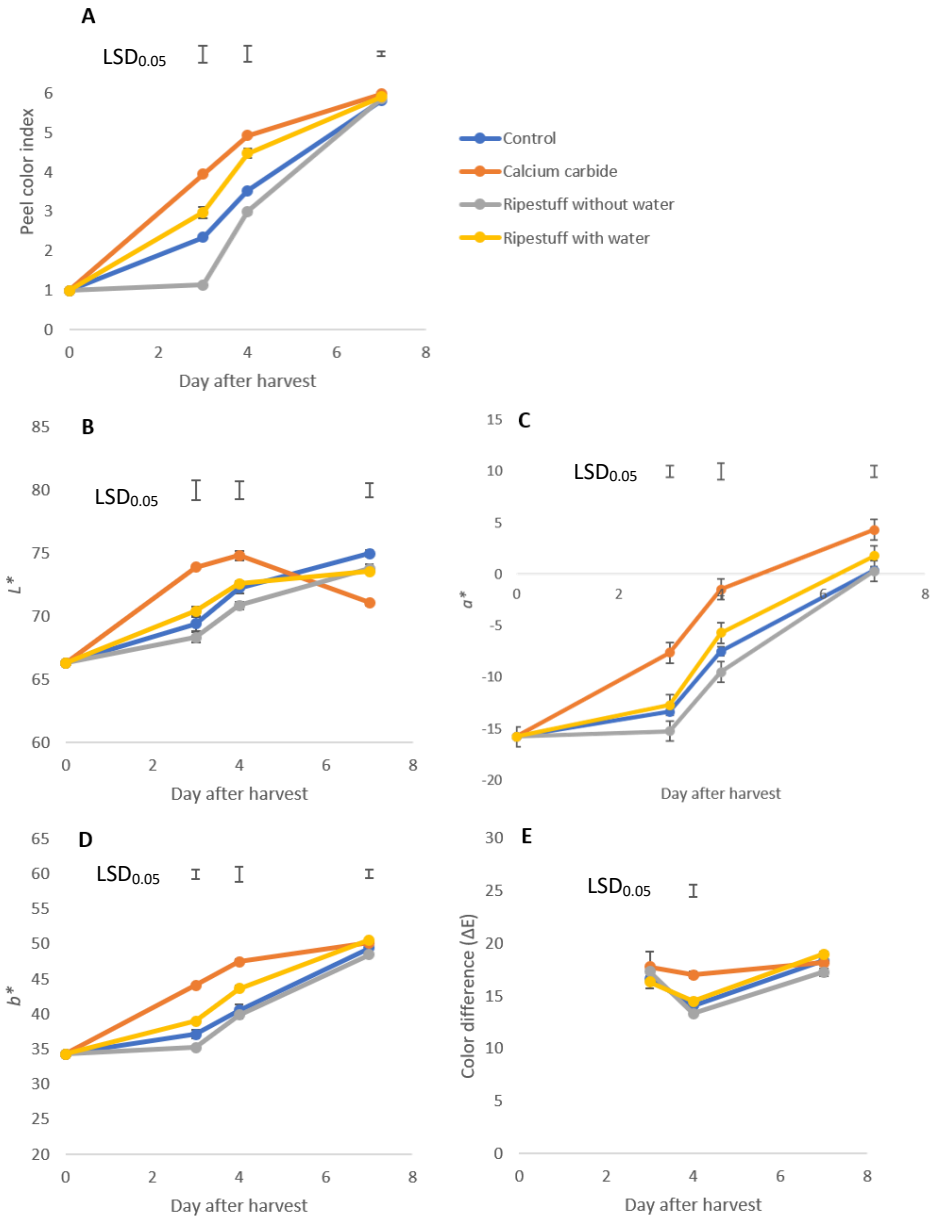

Figure 30. Peel color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water, during storage in air-conditioned room (26.5±0.7ºC; 80.1±4.3% RH). Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

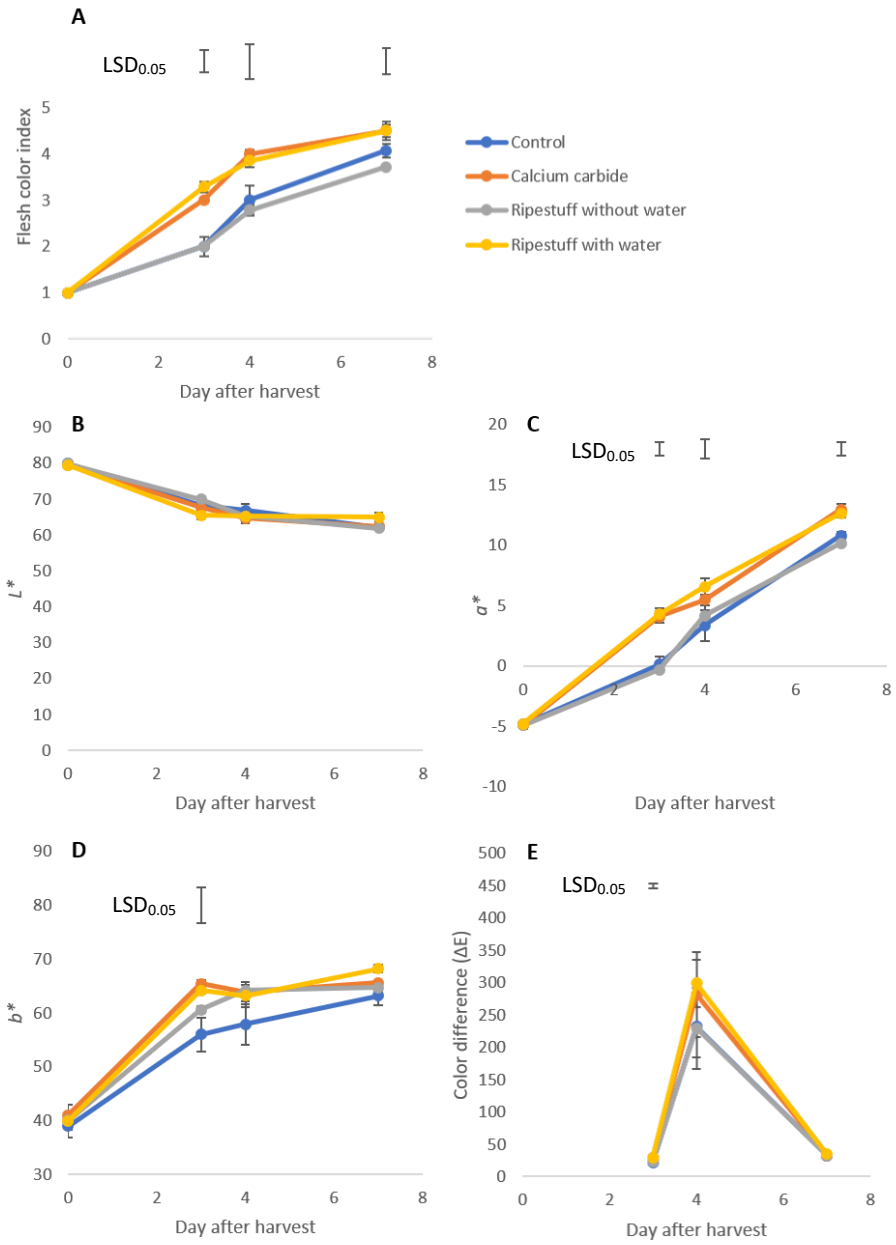

Figure 31. Flesh color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water, during storage in air-conditioned room (26.5±0.7ºC; 80.1±4.3% RH). Flesh color index: 1= white-yellow; 2= light yellow; 3= bright yellow; 4= yellow-orange; 5= orange. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Mangoes treated with calcium carbide had higher weight loss than the Ripestuff™-treated mangoes and the control (Figure 31A). This could be a result of higher transpiration of moisture from the fruit due to heat produced from the reaction of calcium carbide and moisture to produce acetylene (Appendix Figure 4). Total soluble solids of the fruit did not differ among treatments (Figure 31C).

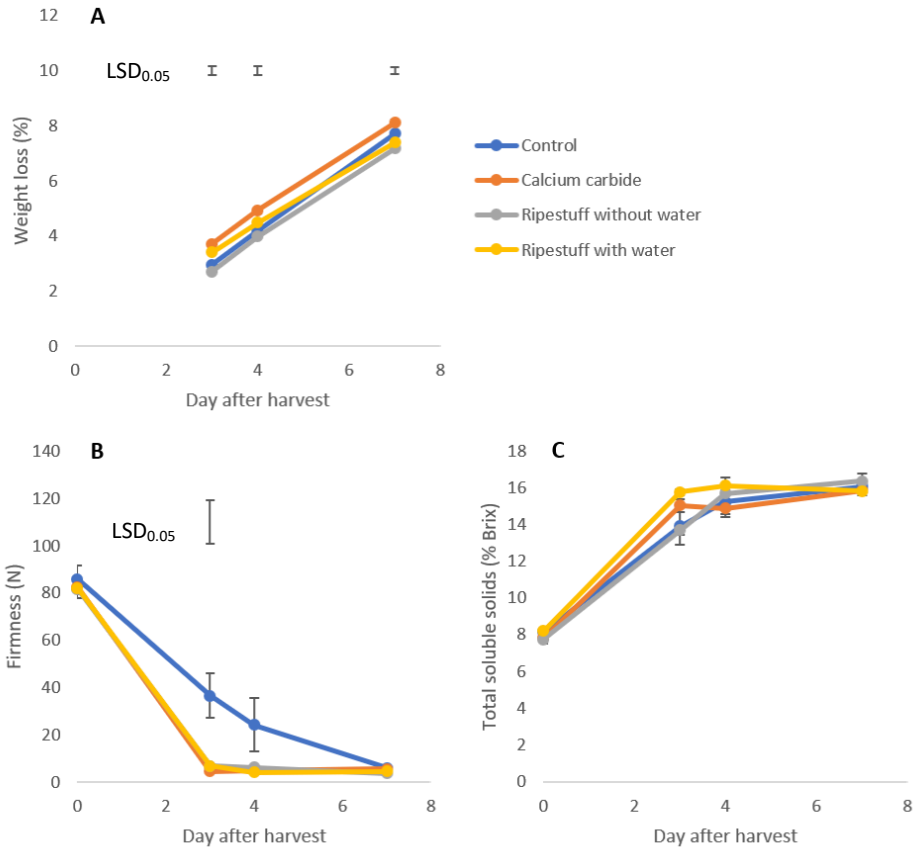

Figure 32. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water, during storage in air-conditioned room (26.5±0.7ºC; 80.1±4.3% RH). Data points with LSD bar are significantly different at *P*≤0.05. Error bars= SEM.

Deterioration of visual quality occurred faster in mangoes treated with calcium carbide—a symptom of senescence progression (Figure 33A). Latent diseases (i.e., diseases that occur only when the fruit is ripe) such as stem-end rot and anthracnose also ocurred earlier in mangoes treated with calcium carbide (Figure 33B-C). These diseases occurred next in mangoes treated with Ripestuff™ with added water, and lastly in mangoes treated with Ripestuff™ without water, and the untreated mangoes.

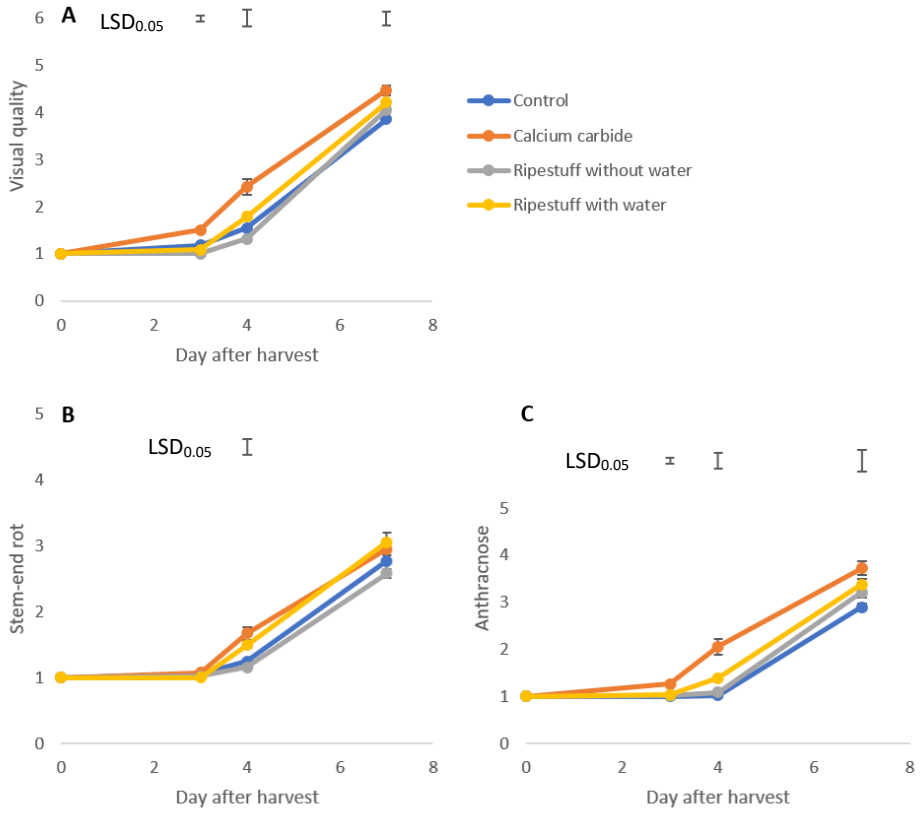

Figure 33. Visual quality (A), stem-end rot (B), and anthracnose (C) of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water, during storage in air-conditioned room (26.5±0.7ºC; 80.1±4.3% RH). Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

In terms of saleability, mangoes treated with calcium carbide reached the saleable stage faster at 3.9 d followed by those treated with Ripestuff™ with added water at 4.6 d, and lastly by those treated with Ripestuff™ without water which was the same as the control at 5 d (Table 6). As a result, mangoes treated with calcium carbide had longer saleable days. The mangoes treated with Ripestuff™ with added water was comparable with those treated with calcium carbide however, it did not differ from the ones treated with dry Ripestuff™ or the control. Shelf life did not differ among treatments.

Table 6. Saleability and shelf life of 'Carabao' mango as influenced by calcium carbide or Ripestuff™ with or without water.

Treatment	Days to saleability[z]	Saleable days[z]	Shelf life[NS] (d)
Control	5.4[a]	1.8[b]	7.2
Calcium carbide	3.9[c]	2.5[a]	6.5
Ripestuff™ without water	5.6[a]	1.4[b]	7.1
Ripestuff™ with water	4.6[b]	2.1[ab]	6.7

[z]Means in a column with common letter/s are not significantly different using LSD at $P \leq 0.05$.
[NS]Not significant

4.4 Conclusion

This experiment showed that the addition of water inside the Ripestuff™ vessel proved to be effective in releasing ethylene from Ripestuff™ powder which was in conjunction to the results of the release kinetics experiment conducted in UQ. Although 100% ethylene release from Ripestuff™ was not contained due to leakage in the chamber, residual ethylene in the vessel hinted an almost full release when Ripestuff™ was added with water.

Ripestuff™ with added water was able to initiate ripening in 'Carabao' mango faster than those treated with Ripestuff™ without water, and the control. However, the released ethylene from Ripestuff™ seemed not enough to ripen the mangoes as fast as calcium carbide. The next experiments probed on the factors that could affect the release of ethylene from Ripestuff™ (e.g., Ripestuff™ mass, number of holes on the lid, and airflow) and the factors that could influence the response of mangoes to ethylene from Ripestuff™ (e.g., maturity).

5 Experiment 5- Release kinetics of ethylene from Ripestuff™ powder as influenced by the number of holes in the lid and air circulation

5.1 Introduction

With the aim of optimizing the application of Ripestuff™ to achieve an effective ripening in 'Carabao' mango that is on par with the effect of calcium carbide, this experiment aimed to probe on the factors that could affect the release of ethylene from Ripestuff™. This experiment specifically determined the effect of the number of holes in the lid of Ripestuff™ vessel, and presence of airflow through the fan in jars (i.e., representative of air circulation through the holes of bamboo baskets).

5.2 Materials and Methods

This experiment was conducted in March 2019. The ethylene release from Ripestuff™ powder was monitored in a set up with two factors— 1) with or without fan in the jar and 2) number of holes (0, 1, 2, 3, or 4) in the lid of Ripestuff™ vessel (Figure 34). The holes in the lid were pierced using Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). A mass of 0.006±0.002 g Ripestuff™ powder Batch '7 Feb B' (0.42 $mol \cdot mol^{-1}$ ethylene; UQ Gatton, Queensland, Australia) was weighed in a 60 mL specimen container using an analytical balance. Two mL distilled water was injected in the vessel upon closing of the jars (V= 1.7 L). A vessel containing Ripestuff™ powder with added water, and covered with a lid without holes served as control. Each vessel was placed in a sealed glass jar with or without fan. The lids of the glass jars were attached with sampling ports composed of 30 cm x 4 mm flexible PVC tube (Ezy Flex Tube, Holman Industries, Western Australia) fitted with barbed in-line tap (Pope, Toro Australia Pty Ltd, South Australia) to monitor ethylene release using the a portable ethylene analyser (Ethan, Bioconservacion, Barcelona, Spain). Ethylene in the jar headspace was monitored every h for the first 5 h and at 24 h. Full release of ethylene from Ripestuff™ into the jar headspace was predicted to be at 44 $\mu L\ L^{-1}$.

Figure 34. Glass jars with or without fan containing Ripestuff™ in a vessel with different number of holes (0, 1, 2, 3, or 4) in the lid.

The experiment was arranged in CRD with three replicates each treatment. Data were analyzed using two-way ANOVA with factors being the number of holes in the lid and air circulation (i.e., with or without fan). The differences in means were detected using Fisher's LSD at 5% level of significance.

5.3 Results and Discussion

Results of the experiment showed that ethylene from Ripestuff™ powder contained in a vessel with four holes in the lid was released faster into the jar headspace than those with less holes in it (Figure 35A). On the other hand, ethylene release from Ripestuff™ was not affected by air circulation (i.e., with or without fan) (Figure 35B). At 35-40% release after 2 h, the ethylene concentration started to plateau until the 4th h then it started to decrease thereafter. After 72 h, almost no ethylene was left in the jar headspace. This wasn't expected as ethylene should be accumulated in the jar until it reached full release. The possibility of leakage in the jar was high due to the frequency of gas sampling through the ports into the portable gas analyser which was done every h. Ethylene might have escaped from the jar when the taps were opened. Nonetheless, when the residual ethylene in the vessel was measured after 24 h, results showed that ethylene was fully released from Ripestuff™ into the jar headspace (Table 7). Moreover, there was no interaction between the number of holes in the lid of Ripestuff™ vessel and air circulation that could affect the release of ethylene.

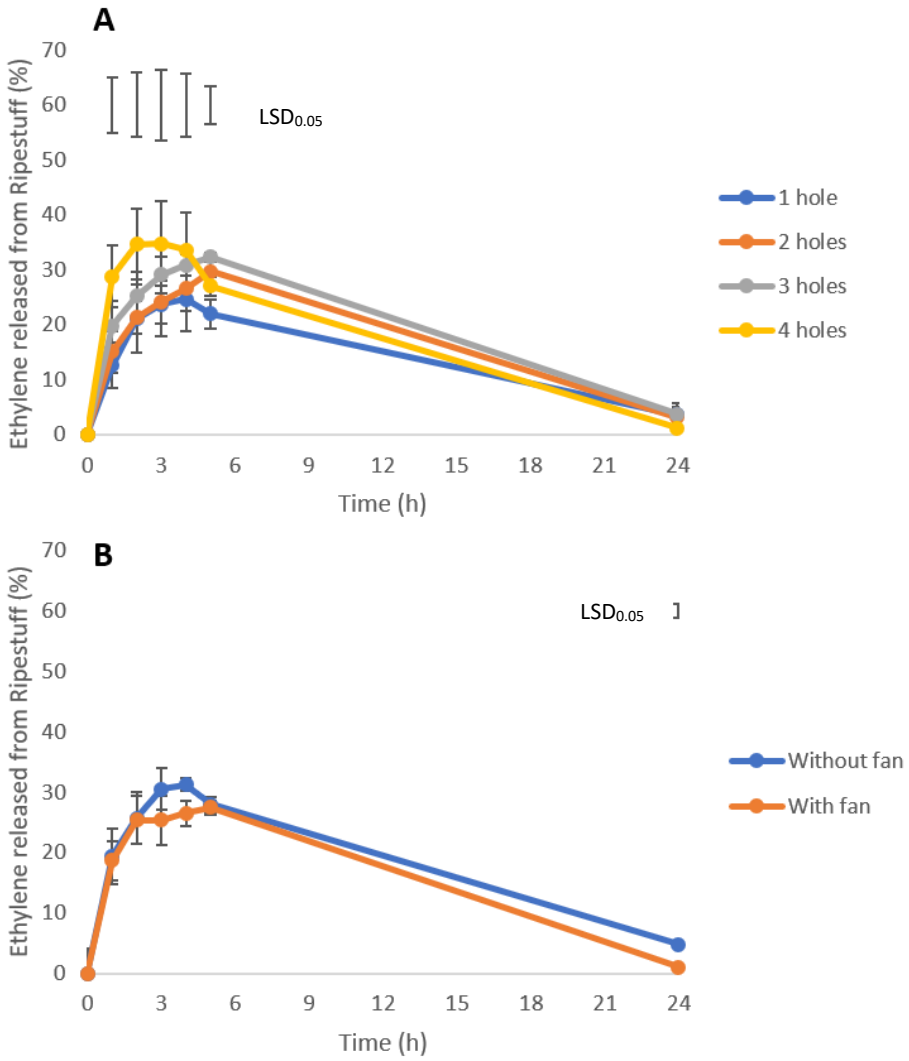

Figure 35. Ethylene released from Ripestuff™ contained in vessels with different number of holes on the lid (A) and placed in jars with or without fan (B). Data points with LSD bars are significantly different at *P*≤0.05. Error bars= SEM.

Table 7. Ethylene released from Ripestuff™ powder based on the residual ethylene in the vessel measured after 24 h.

Treatment	Ethylene released[z] (%) after 24 h
No hole	0[b]
1 hole	99.9[a]
2 holes	99.9[a]
3 holes	99.9[a]
4 holes	99.9[a]
Airflow	
Without fan	80.0[A]
With fan	79.9[A]
Interaction	ns

[z]Means with a common letter are not significantly different using LSD at *P*≤0.05.
[NS]Not significant

5.4 Conclusion

This experiment showed that ethylene from Ripestuff™ powder was released faster if the vessel had more holes in the lid. A lid with lesser holes will result in slower release of ethylene towards its environment. It also eliminated airflow as a factor that could influence the release of ethylene from Ripestuff™.

6 Experiment 6- Effect of Ripestuff™ and harvest maturity on the ripening of 'Carabao' mango

6.1 Introduction

Maturity of 'Carabao' mango at harvest is an important determinant of postharvest quality and one of the factors that could potentially affect the fruit's response to Ripestuff™. Immature fruit do not ripen evenly (Agillon, 2003). A technique used to determine the harvest maturity in mango is through flotation in 1% NaCl solution. Mature mangoes sink while immature fruit float. Ideally, the proportion of sinkers in the solution must be 75% to consider the fruit in its proper harvest maturity (Lizada, 1991). This study tested the effect of Ripestuff™ on the ripening of 'Carabao' mango with different harvest maturities (sinker or floater).

6.2 Materials and Methods

This experiment was conducted in March 2019. Mango fruit from a farm in the Island Garden City of Samal, Davao del Norte were harvested at 115 days after flower induction. Dry matter (%) was determined by drying 5 g of mango fruit flesh in a hot air-oven at 130°C for 2 h or until constant weight was attained. The fruit were subjected to flotation using 1% NaCl solution, sanitized with 200 µL L^{-1} NaOCl then air-dried. The sinker fruit were separated from the floaters and both were treated with Ripestuff™ (Batch '7 Feb B', 0.42 mol·mol^{-1} ethylene) in separate chambers (V= 95.57 L) for 72 h (Figure 36). Each chamber contained 5 kg mango. A mass of 0.26 g Ripestuff™ was weighed in a specimen container using analytical balance and covered with screw cap lid pierced twice with Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). Five mL distilled water was injected into the Ripestuff™ vessel when the chamber was about to be closed. The treatments were applied for 72 h in mangoes inside enclosed chambers. After treatment, the samples were transferred to an air-conditioned room (25.9±1.1°C, 69.0±9.9% RH).

Mango fruit quality was evaluated at 3 (after 72 h of ripening treatment), 4, and 7 days after harvest (DAH). Data collected were fruit respiration rate in terms of CO_2 using a portable gas analyser (CheckPoint O_2/CO_2, Dansensor, Denmark), ethylene production rate using a portable ethylene analyser (Ethan, Bioconservacion, Barcelona, Spain), weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel color index (Appendix 14.2.1), peel and flesh color measurements (*L*, a*, b**, and color difference (ΔE)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). The experiment was arranged in CRD with two replicates each treatment. The data for the third replicate was extrapolated from the first two replicates. Each replicate had 30 fruit samples. Data were analyzed using ANOVA and differences in means were detected using Fisher's LSD at 5% level of significance.

Figure 36. ‘Carabao’ mango with different maturities (sinker or floater) treated with Ripestuff™ inside sealed chamber.

6.3 Results and Discussion

At harvest, sinker fruit had higher dry matter and TSS (25.8% dry matter; 9% Brix) compared to the floaters (22.7% dry matter; 8.3% Brix). In terms of respiration, sinker fruit treated with Ripestuff™ had higher rate than the other treatments. This could be an indicator of enhanced ripening process by Ripestuff™ in sinker fruit but not in floaters (Figure 37).

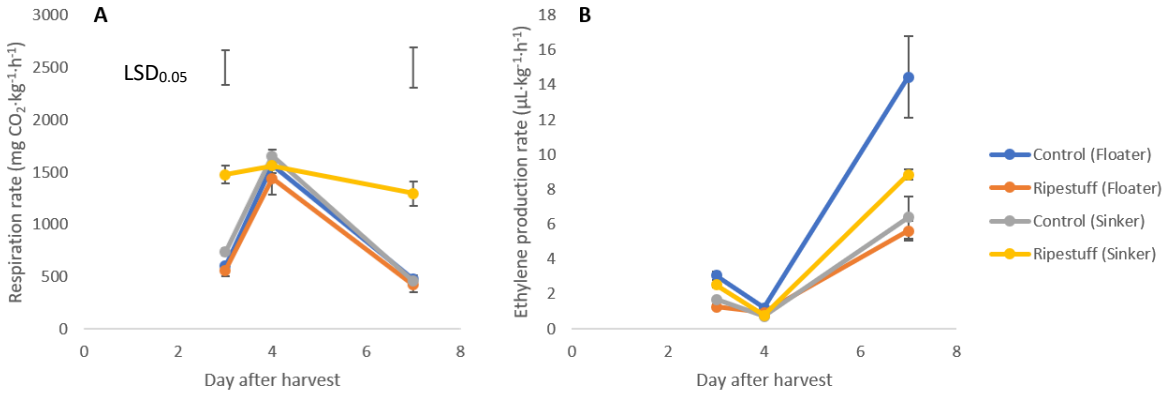

Figure 37. Respiration (A) and ethylene production (B) rates of ‘Carabao’ mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

In terms of ripening, mangoes treated with Ripestuff™, whether sinker or floater, ripened faster and reached the saleability stage sooner (4 to 5 d) than the untreated mangoes (6 to 7 d) (Table 8) as assessed by peel and flesh color (Figures 38-40), and firmness (Figure 41). Sinker mangoes treated with Ripestuff™ were sweeter than the floaters which was in concurrence with high dry matter at harvest. The control sinkers had slightly lower degree of stem-end rot and anthracnose than the floaters (Figure 42). Weight loss did not vary among treatments. Based on the results, Ripestuff™ could ripen 'Carabao' mango whether the fruit is mature or immature, but sinkers were sweeter when it ripened.

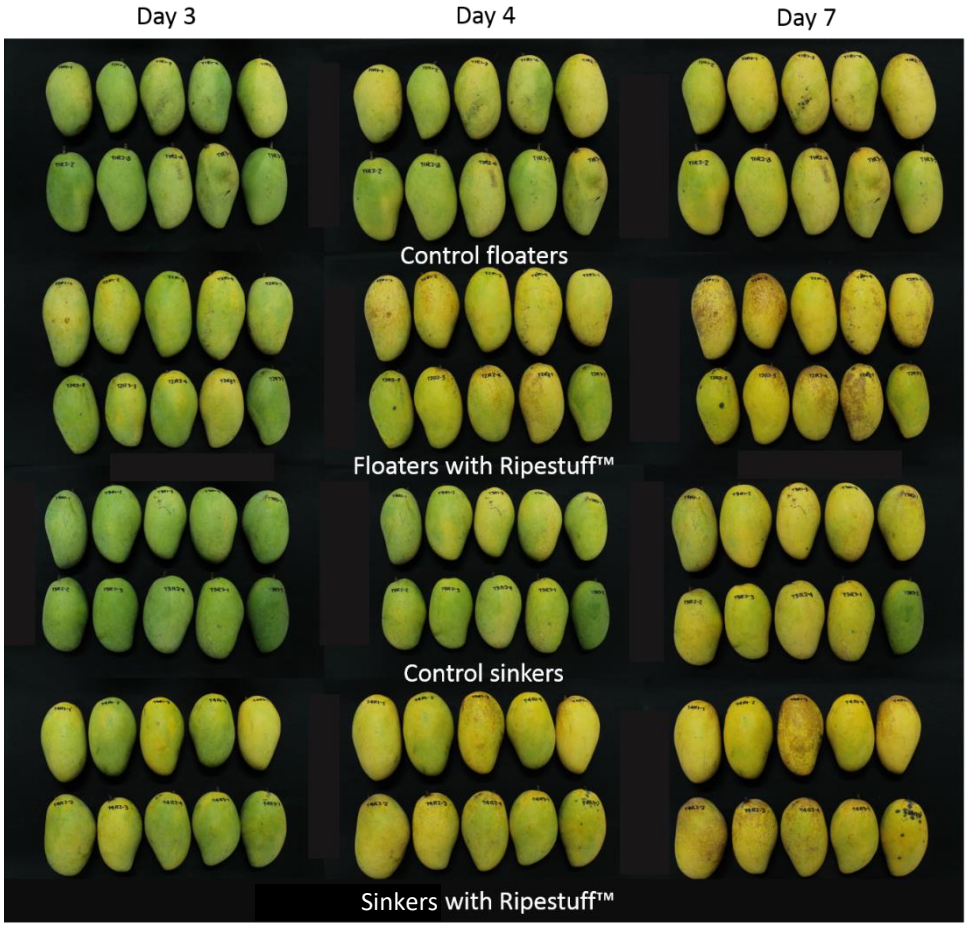

Figure 38. Appearance of 'Carabao' mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker).

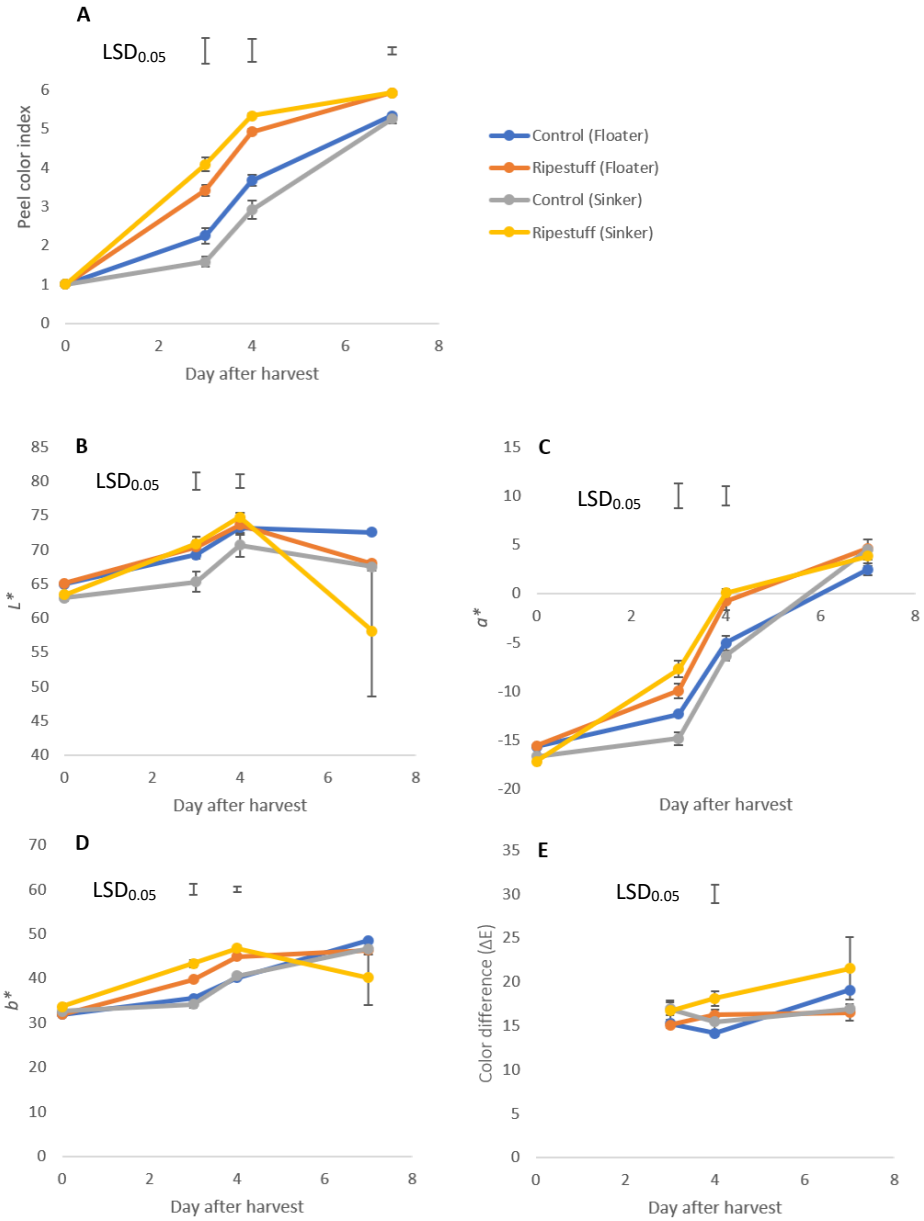

Figure 39. Peel color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) in 'Carabao' mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker). Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

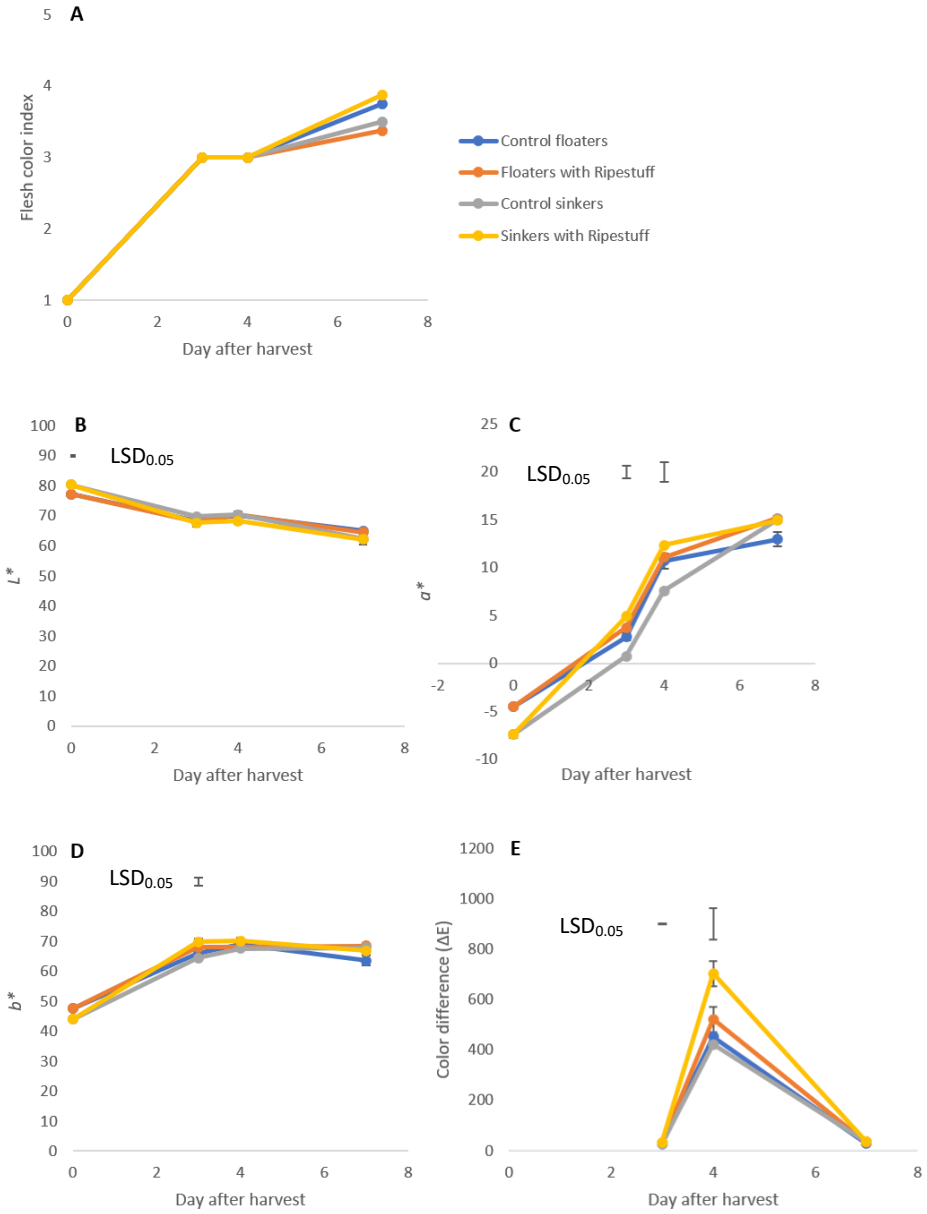

Figure 40. Flesh color *L** (A), *a** (B), *b** (C), and color difference (D) in 'Carabao' mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker). Flesh color index: 1= white-yellow; 2= light yellow; 3= bright yellow; 4= yellow-orange; 5= orange. Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

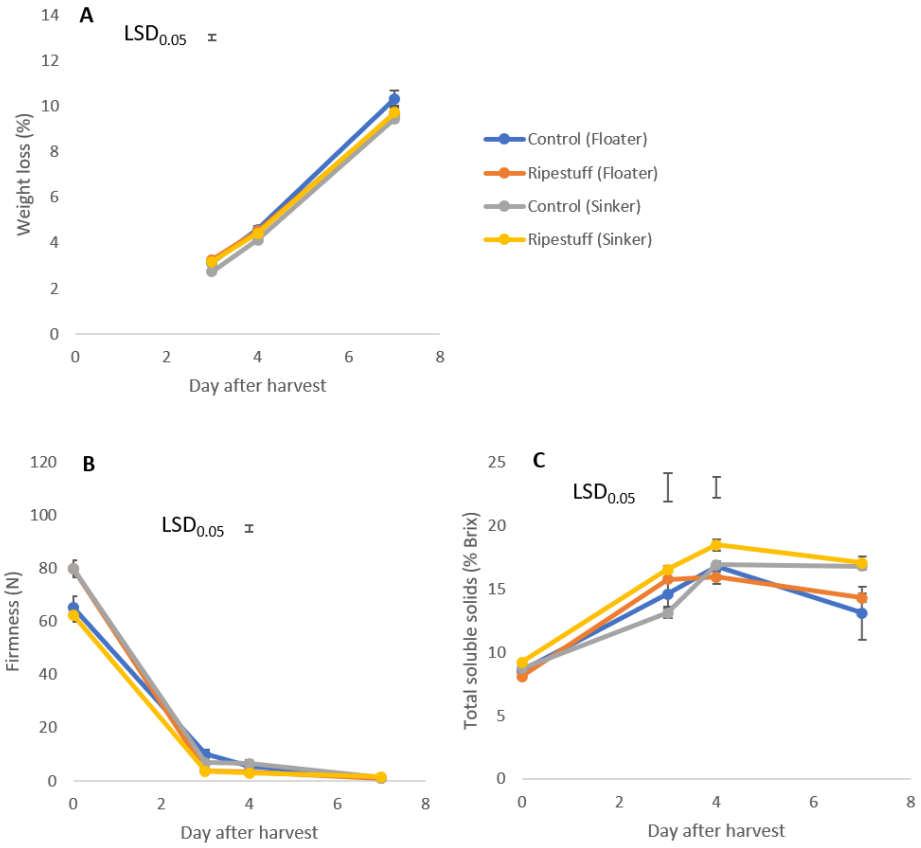

Figure 41. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

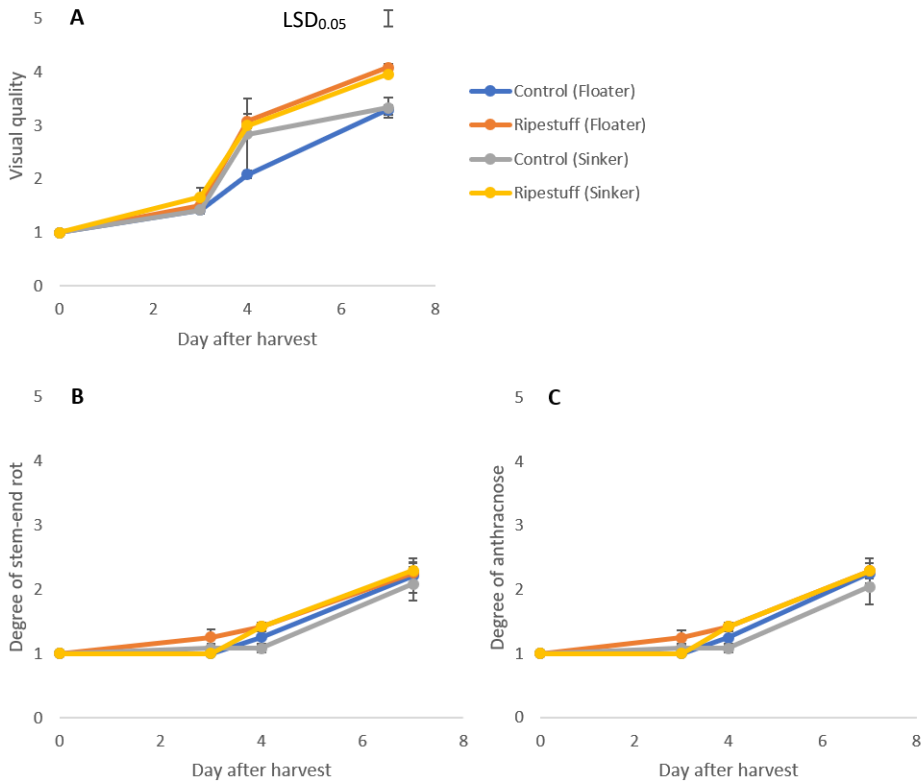

Figure 42. Visual quality (A), degree of stem-end rot (B) and anthracnose (C) of 'Carabao' mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker). Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Treated mangoes whether sinker or floater had shorter shelf life due to the appearance of blotchiness on the skin resulting in poor quality. Blotchiness was high in mangoes treated with Ripestuff™ (with water and 2 holes on the container lid). Blotchiness probably resulted from the upsurge of ethylene diffusing faster through the two holes of the Ripestuff™ container, or the high CO_2 concentration in the headspace of the treated chambers leading to CO_2 injury (Figure 43).

Table 8. Saleability and shelf life of 'Carabao' mango as influenced by Ripestuff™ treatment and harvest maturity (floater or sinker).

Maturity	Treatment	Days to saleability[z]	Saleable days[NS]	Shelf life[z] (d)
Floater	Control	6.5[a]	1.3	7.3[a]
	Ripestuff™	5.3[b]	0.5	4.6[b]
Sinker	Control	6.5[a]	1.4	7.5[a]
	Ripestuff™	4.8[b]	1.2	5.3[b]

[z]Means in a column with common letter are not significantly different using Fisher's LSD at $P \leq 0.05$.
[NS]Not significant

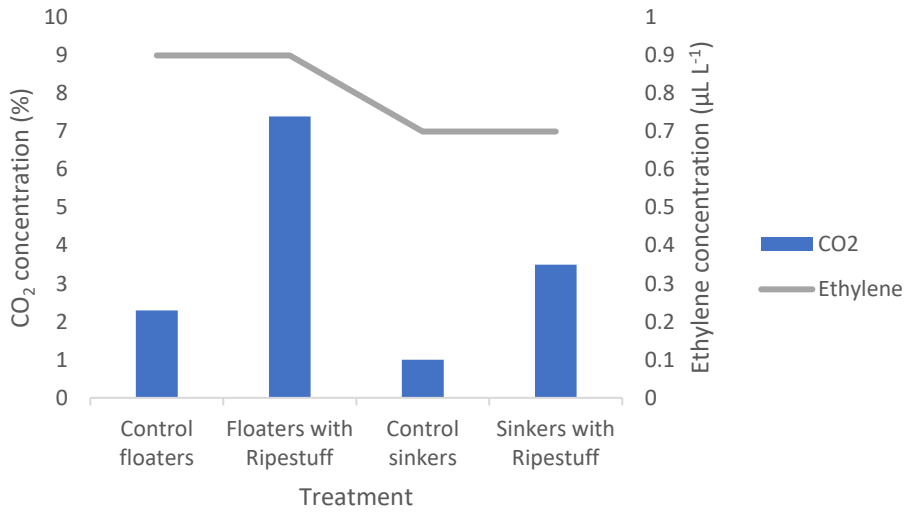

Figure 43. Concentrations of CO_2 (primary axis) and ethylene (secondary axis) inside the chamber after 72 h of treatment with Ripestuff™ in 'Carabao' mango with different maturities (sinker or floater). Ripestuff™ maximum ethylene release: 30 µL L^{-1}.

6.4 Conclusion

This study showed that sinker or floater 'Carabao' mangoes had similar ripening responses to Ripestuff™ in terms of color development and firmness. However, sinker mangoes tended to become sweeter when it ripened due to high dry matter content at harvest.

7 Experiment 7- Effect of different doses of Ripestuff™ on the ripening of ‘Carabao’ mango treated in bamboo baskets

7.1 Introduction

The local market in the Philippines uses bamboo baskets as container for the treatment of calcium carbide in ‘Carabao’ mango (Figure 44). This experiment aimed to apply the principles of Ripestuff™ treatment from a static chamber (Experiments 4 and 6) to baskets with an assumption that there is no loss of ethylene from the basket due to the barrier created by the newspaper linings. From the previous experiments, the treatment of Ripestuff™ was only able to initiate ripening in mango but not fully transform the fruit into its readily saleable stage. It was surmised that if 30 µL L^{-1} headspace ethylene from Ripestuff™ was not enough to ripen mangoes evenly, then higher dosage was probably required. This could mean to increase the mass of Ripestuff™ in the vessel, however, results of an experiment in UQ showed that introducing a greater quantity of Ripestuff™ to the system leads to incomplete filling of the α-cyclodextrin cavity thereby not releasing the ethylene (Perkins and Joyce, 2019c). Addition of water would also create a barrier for ethylene to diffuse easily towards the headspace. To address this problem, one unit of vessel was treated as one dose and increasing the dosage would mean to multiply the Ripestuff™ containers. This experiment aimed to determine the best dose of Ripestuff™ that equates to the effect of calcium carbide in the ripening ‘Carabao’ mango treated inside bamboo baskets.

Figure 44. Bamboo basket (V= 12 L) used as container for ‘Carabao’ mangoes in the local market in the Philippines. Above basket has a capacity of 5 kg. Ripeners use similar bamboo baskets with a capacity of 25 kg.

7.2 Materials and Methods

This experiment was conducted in April 2019. Freshly harvested ‘Carabao’ mango (26.3% dry matter, 72.5% sinkers) at 115 days after flower induction was harvested from Digos, Davao del Sur and brought in the Postharvest Biology Laboratory in UP Mindanao, Davao City through an air-conditioned vehicle. Dry matter (%) was determined by drying 5 g

of mango fruit flesh in a hot air-oven at 130°C for 2 h or until constant weight was attained. The fruit were subjected to flotation using 1% NaCl solution to determine the maturity of the fruit. Fruit were then sanitized with 200 µL L^{-1} NaOCl for 3 min and air-dried. Each bamboo basket (V= 12 L) was allotted with 5 kg mango fruit. The mangoes were placed inside bamboo baskets lined with five sheets of newspaper. Meanwhile, Ripestuff™ powder Batch '7 Feb B' (0.42 mol·mol^{-1} ethylene) was sieved using a strainer and 20 mg powder was weighed into a 60 mL vessel using an analytical balance. The Ripestuff™ vessel was covered with a screw-cap lid pierced once with Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). Distilled water (5 mL) was injected into the vessel when the baskets were about to be covered with newspaper sheets. The treatments were described in Table 9 and Figure 45A. The vessels containing Ripestuff™ with water were placed at the middle-centre portion of the basket together with the mangoes. Calcium carbide with a total mass of 186 mg (aimed to release 81,000 µL L^{-1} headspace acetylene as in Experiment 4) was wrapped in paper and placed together with mangoes in the basket. Tubes composed of 30 cm x 4 mm flexible PVC (Ezy Flex Tube, Holman Industries, Western Australia) fitted with in-line barbed tap (Pope, Toro Australia Pty Ltd, South Australia) were inserted in the basket to serve as sampling ports for ethylene, acetylene and CO_2 measurements (Figure 45B). The mangoes were covered with newspaper and secured with polypropylene twine. The mangoes were treated for 72 h. Ethylene and acetylene concentrations in the basket headspace were monitored every 24 h for 72 h. Ethylene was measured using a portable ethylene analyser (Ethan, Bioconservacion, Barcelona, Spain). Acetylene concentration was measured using Kitagawa detector tubes (50-1000 µL L^{-1}, Kitagawa Precision Gas Detector Tubes, Komyo Rikagaku Kogyo, Japan) and aspirating pump (Kitagawa AP-20 Aspirating Pump, Komyo Rikagaku Kogyo, Japan). CO_2 concentration in the basket after 72 h treatment was determined using a gas analyser (CheckPoint O_2/CO_2, Dansensor, Denmark). After 72 h of treatment, the mangoes were transferred in trays and stored in 25.9±1.1°C, 69.0±9.9% RH.

Table 9. Description of treatments used in determining the best dose of Ripestuff™ in ripening 'Carabao' mango inside bamboo baskets. Each basket contained 5 kg fruit.

Treatment	Ripestuff™ mass (mg)	Volume of fruit (mL)	Volume of water (mL)[z]	Total ethylene content (µmol)	Basket headspace volume (mL)	Headspace ethylene conc. at 100% release (µmol.L^{-1})	Headspace ethylene conc. at 100% release[x] (µL.L^{-1})
Control	0	5000	0	0	6885	0	**0**
Calcium carbide	0	5000	0	0	6885	0	**0**
Ripestuff™ 1 dose	20	5000	5	8.53	6820	1.251	**30**
Ripestuff™ 2 doses	40	5000	10	17.06	6755	2.525	**60**
Ripestuff™ 3 doses	60	5000	15	25.59	6690	3.825	**90**
Ripestuff™ 4 doses	80	5000	20	34.12	6625	5.149	**120**

[z]One dose of Ripestuff™ contains 5 mL distilled water

[x]Values were calculated based on a hermetically sealed environment and assuming that there was no loss of ethylene from the baskets over time

Calculation credits: Melinda Perkins

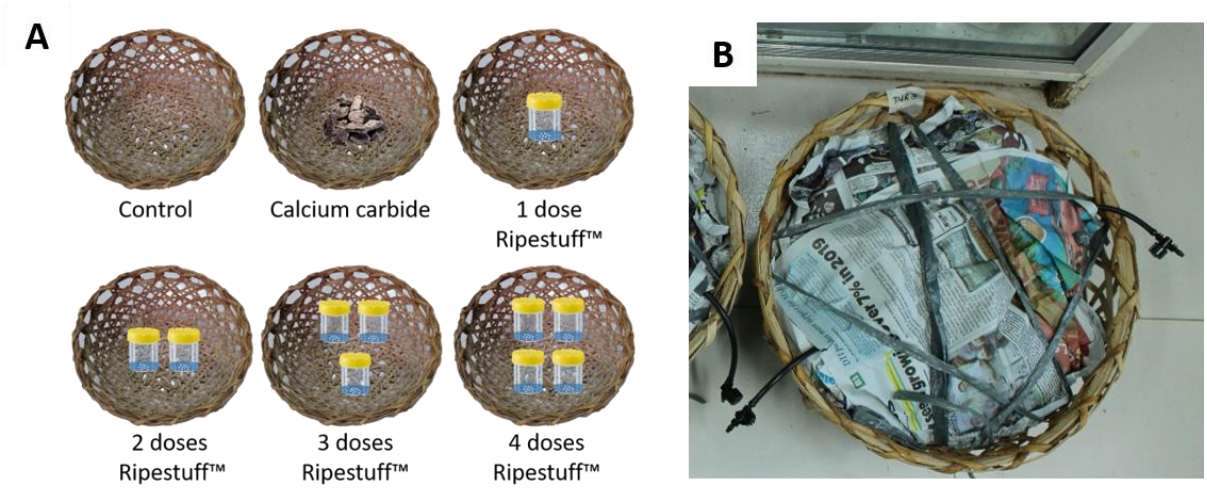

Figure 45. Description of treatments used in this experiment (A) and a basket containing mangoes covered with newspaper sheets inserted with gas sampling ports altogether bound by polypropylene twine (B).

Mango fruit quality was evaluated at 3 (after 72 h of treatment), 4, 7 and 10 days after harvest. The effect of the treatments were determined using the following parameters: weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel (Appendix 14.2.1) and flesh (Appendix 14.2.2) color indices and measurements (*L*, a*, b**, and color difference (ΔE)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). Each replicate had 10 fruit samples. The experiment was arranged in CRD and data were analysed using ANOVA. Significant differences in means were detected using LSD at 5% level of significance.

7.3 Results and Discussion

Results showed that the mass of Ripestuff™ (20 mg) per dose releasing 30 μL L^{-1} in the basket headspace was not enough in ripening 'Carabao' mango treated in bamboo baskets even if the dosage was increased four times (Figure 46A). Also, the use of calcium carbide at lower amount (186 mg) which releases 81,000 μL L^{-1} headspace acetylene and equivalent to the biological efficacy of 30 μL L^{-1} ethylene was not enough to initiate mango ripening (Figure 46B). The concentrations of ethylene and acetylene detected were even lower due to the leakage in the baskets. However, the use of 3-4 doses of Ripestuff™ released higher concentrations of Ripestuff™ ethylene compared to lower doses and the control but only a small portion was detected due to leakage (target: 90-120 μL L^{-1} headspace ethylene). Mangoes treated with 3-4 doses of Ripestuff™ also registered a rise in ethylene production after 72 h treatment implying a climacteric phase of ripening (Figure 47B). However, respiration rate was not affected by the treatments (Figure 47A).

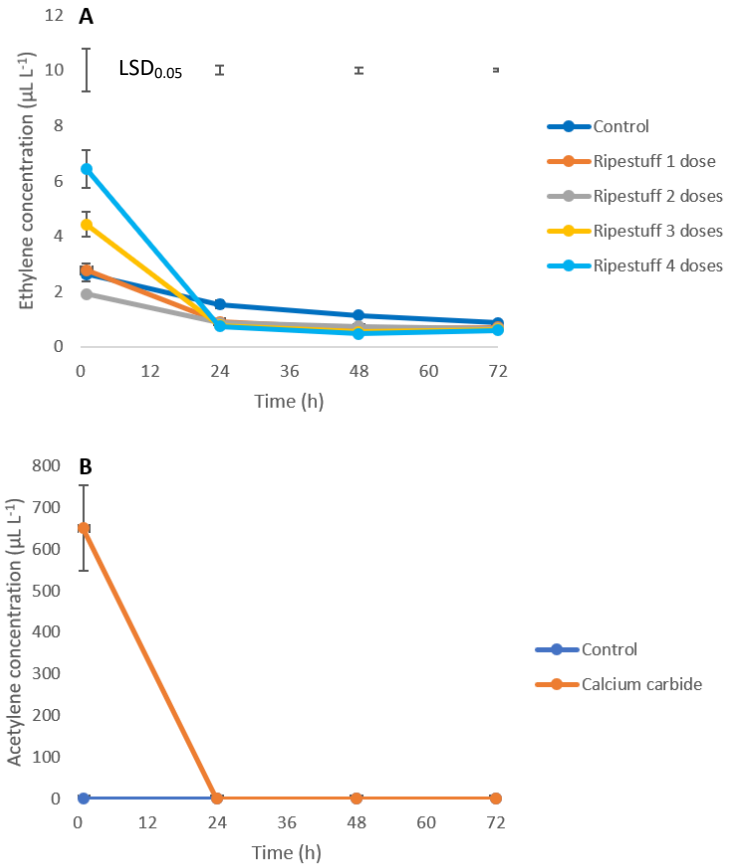

Figure 46. Ethylene (A) and acetylene (B) concentrations inside baskets containing 'Carabao' mangoes treated with calcium carbide or different doses of Ripestuff™ for 72 h. First measurement was done after 1 h of treatment. Data points with LSD bar are significantly different at *P*≤0.05. Error bars= SEM.

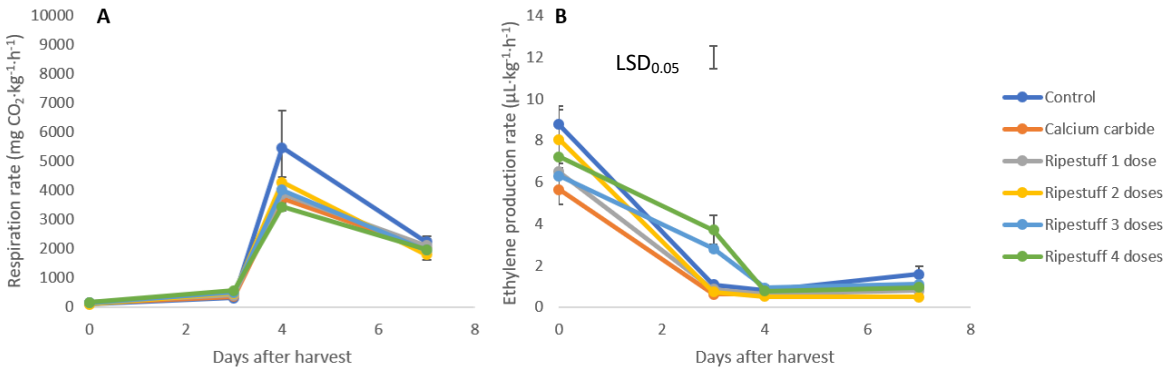

Figure 47. Respiration (A) and ethylene production (B) rates of 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h and stored in air-conditioned room (25.9±1.1°C, 69.0±9.9% RH). Data points with LSD bar are significantly different at *P*≤0.05. Error bars= SEM.

Based on the peel color, the treatment with calcium carbide or different doses of Ripestuff™ was not able to ripen mangoes because of the low concentration applied (Figures 48-49). Although the treatment of 30 µL L^{-1} Ripestuff™ ethylene in a static chamber from previous experiments initiated ripening in mangoes, the application of the same concentration into a basket configuration was not effective as it required less amount of Ripestuff™ thus less ethylene released, and leakage in the basket made headspace ethylene even lesser.

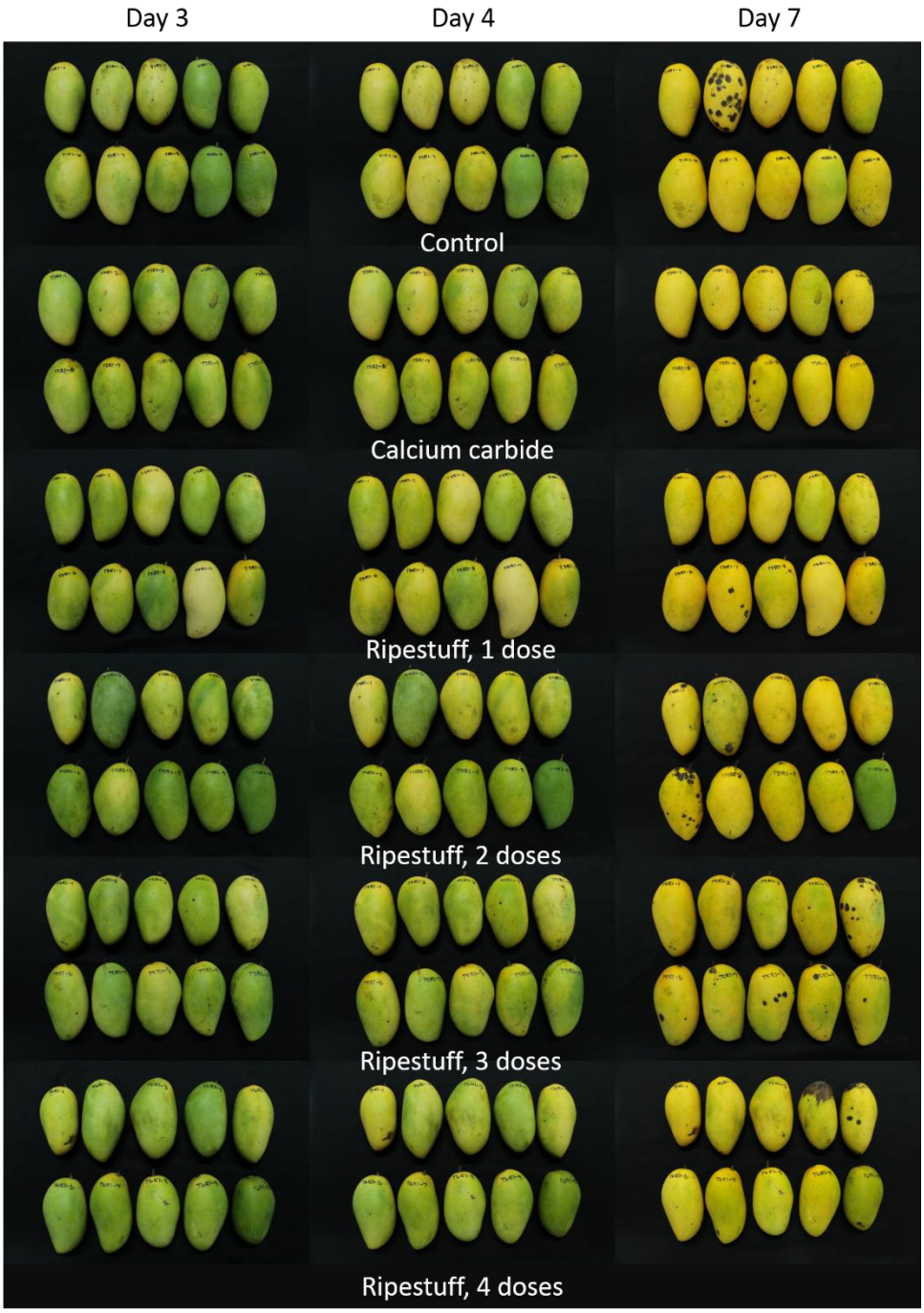

Figure 48. Appearance of 'Carabao' mangoes treated with calcium carbide or different doses of Ripestuff™ for 72 h and stored in air-conditioned room (25.9±1.1°C, 69.0±9.9% RH).

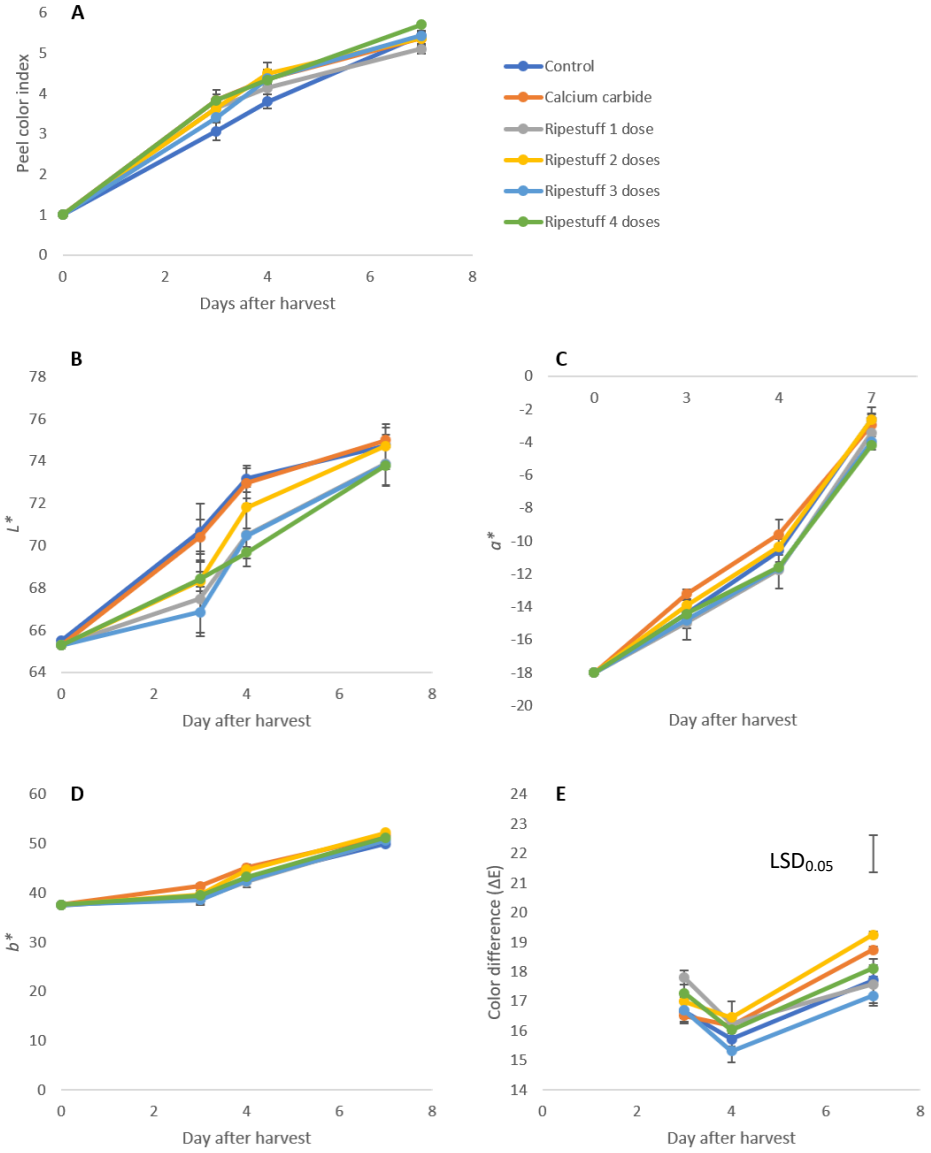

Figure 49. Peel color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) in 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h and stored in air-conditioned room (25.9±1.1°C, 69.0±9.9% RH). Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

On the other hand, the results on flesh color, weight loss, firmness, and total soluble solids showed that ripening process might have occurred in mangoes treated with calcium carbide or different doses of Ripestuff™ but it was asynchronous with the peel color (Figures 50-51). Cases of asynchronous ripening in 'Carabao' mango has been observed in previous studies (Lacap et al., 2019). Untreated mangoes were significantly different from the treated fruit in terms of flesh color, firmness and total soluble solids, suggesting unripe qualities for control mangoes. The visual quality, degree of stem-end rot and anthracnose (Figure 52), saleability, and shelf life (Table 10) did not differ among treatments.

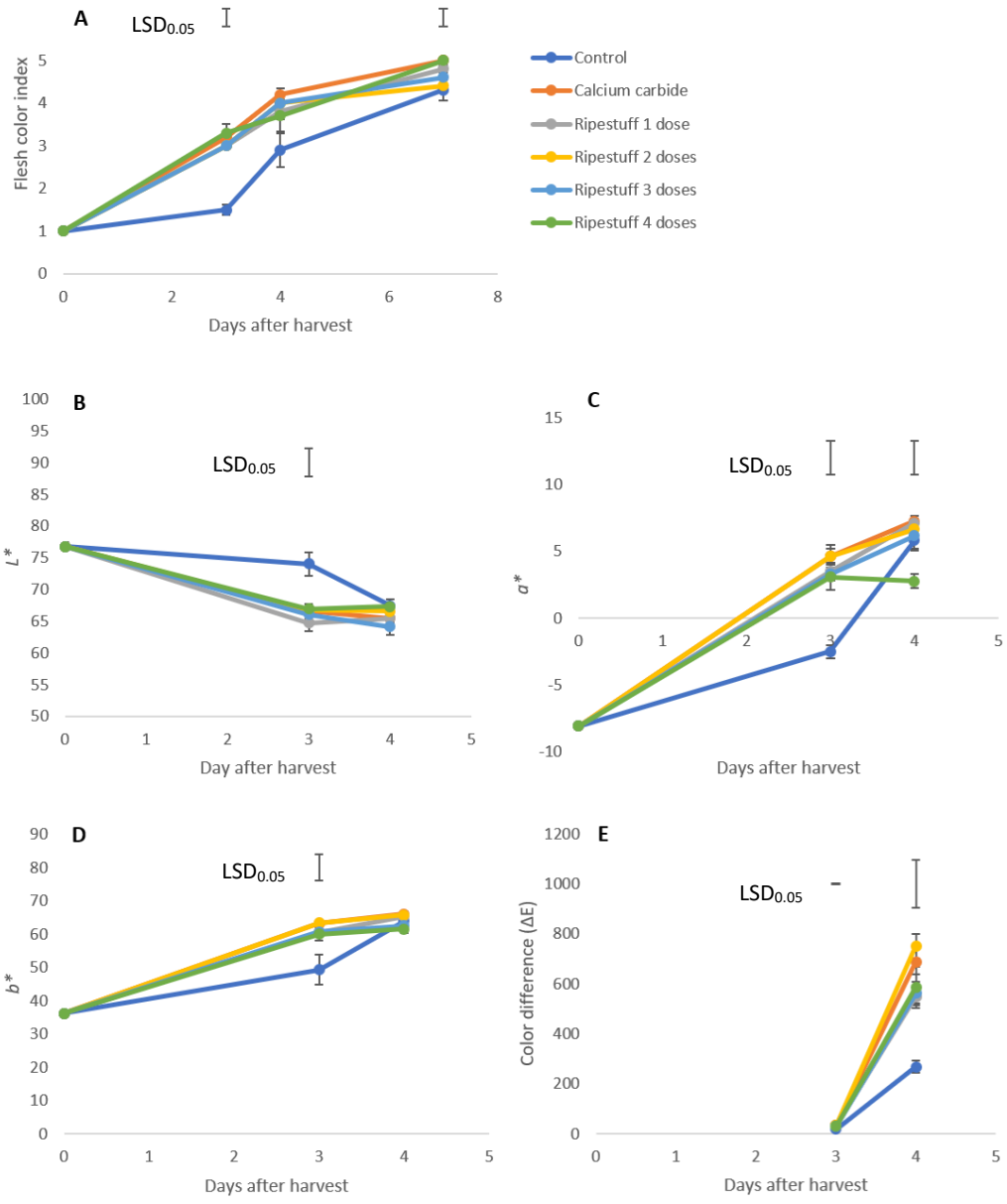

Figure 50. Flesh color *L** (A), *a** (B), *b** (C), and color difference (D) in 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h and stored in air-conditioned room (25.9±1.1°C, 69.0±9.9% RH). Flesh color index: 1= white-yellow; 2= light yellow; 3= bright yellow; 4= yellow-orange; 5= orange. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

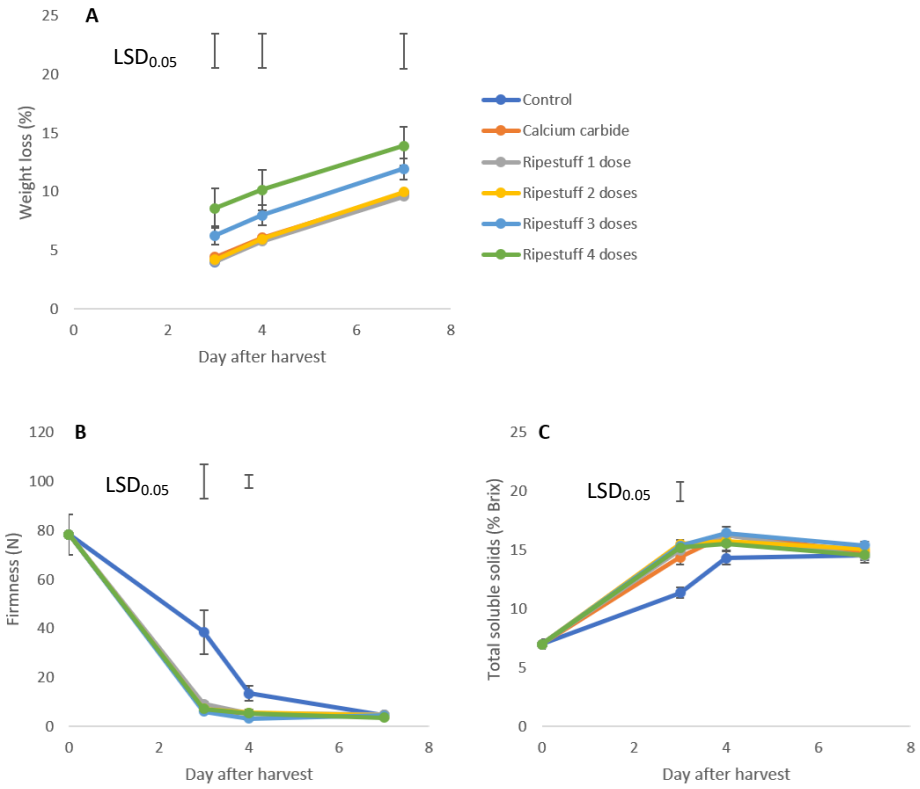

Figure 51. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h and stored in air-conditioned room (25.9±1.1°C, 69.0±9.9% RH). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

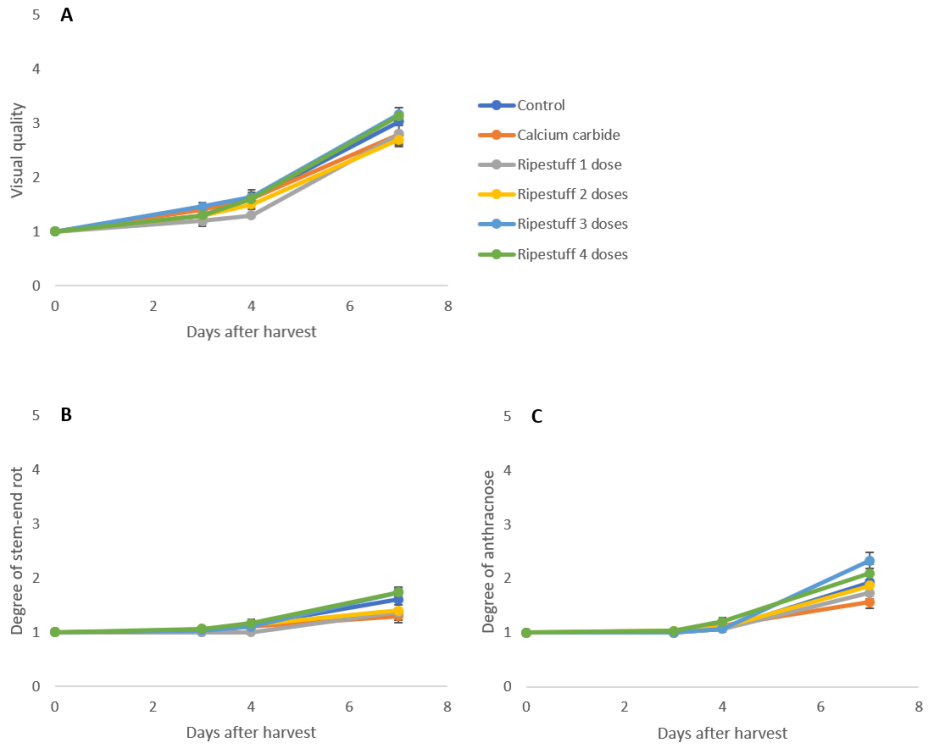

Figure 52. Visual quality (A), degree of stem-end rot (B) and anthracnose (C) in 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h and stored in air-conditioned room (25.9±1.1°C, 69.0±9.9% RH). Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Error bars= SEM.

Table 10. Saleability and shelf life of 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h inside bamboo baskets.

Treatment	Shelf life^{NS} (d)	Days to saleability^{NS}	Saleable days^{NS}
Control	8.5	5.9	2.6
Calcium carbide	9.6	5.4	4.3
1 dose of Ripestuff™	9.1	5.8	3.4
2 doses of Ripestuff™	8.9	5.3	3.6
3 doses of Ripestuff™	8.7	5.2	3.5
4 doses of Ripestuff™	8.2	4.7	3.5

^{NS}Not significant

7.4 Conclusion

This experiment showed that the application of Ripestuff™ based on calculated maximum ethylene release of 30 μL L^{-1} in a static chamber will not yield similar results if applied in a basket configuration. The low Ripestuff™ mass (20 mg) per dose also released very low concentration of ethylene (2-4 μL L^{-1}) in the basket headspace that was not enough to trigger ripening in 'Carabao' mangoes. Further, the 'leaky' conformation of the basket further influenced the low concentration of ethylene left in the basket. It is suggested to increase the mass of Ripestuff™ in the next experiments.

8 Experiment 8- Effect of increased amount of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets

8.1 Introduction

The result of the previous experiment (Experiment 7) showed that the low amount of Ripestuff™ (20-80 mg) that aimed to release 30-120 µL L^{-1} ethylene inside a bamboo basket containing 5 kg fruit was not able to ripen 'Carabao' mango after 72 h of treatment. The low amount of Ripestuff™ might not have been enough to cause an effective ripening. This experiment aimed to achieve a more uniform ripening response from mango by using higher amounts of Ripestuff™. From 20-80 mg Ripestuff™, the amounts were increased to 250 and 500 mg. The 250 mg amount was benchmarked from the mass of Ripestuff™ used in Experiment 4 using an enclosed chamber.

8.2 Materials and Methods

This experiment was conducted in May 2019. 'Carabao' mango fruit (26.7% dry matter, 72.5% sinkers) at 115 days after flower induction were harvested from Kiblawan, Davao del Sur. A total of 60 kg mangoes was brought to the Postharvest Biology Laboratory in UP Mindanao using an air-conditioned vehicle. The fruit were subjected to flotation using 1% NaCl solution to determine the harvest maturity then sanitized with 200 µL L^{-1} NaOCl and air-dried. Meanwhile, Ripestuff™ treatment was prepared by sieving the powder using a strainer to remove the lumps. In a 60 mL specimen container with four holes in the lid, 250 or 500 mg Ripestuff™ powder (Batch '7 Feb B', 0.42 mol·mol^{-1} ethylene) was weighed using an analytical balance. The holes in the lid were pierced using Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). Distilled water (5 mL) was injected into the hole of the vessel using a syringe. This was done when the baskets together with the treatments were about to be wrapped. A specimen container with four holes in the lid added with 5 mL distilled water but without Ripestuff™ powder served as control.

In a bamboo basket (V= 12 L) lined with newspaper sheets, 5 kg mango fruit were arranged and treated with CaC_2 (25 g) or Ripestuff™ (250 or 500 mg) for 72 h inside bamboo baskets (Figure 53). Two tubes composed of 30 cm x 4 mm flexible PVC (Ezy Flex Tube, Holman Industries, Western Australia) fitted with in-line barbed tap (Pope, Toro Australia Pty Ltd, South Australia) were inserted in the basket to serve as inlet and outlet ports for ethylene, acetylene and CO_2 measurements. The mangoes were treated for 72 h. Ethylene and acetylene concentrations in the basket headspace were monitored every 3 h for 12 h and every 24 h thereafter for 72 h. Ethylene was measured using a gas analyser (Ethan, Portable Ethylene Gas Analyzer, Bioconservacion, Barcelona, Spain). Acetylene concentration was measured using Kitagawa detector tubes (50-1000 µL L^{-1}, Kitagawa Precision Gas Detector Tubes, Komyo Rikagaku Kogyo, Japan) and aspirating pump (Kitagawa AP-20 Aspirating Pump, Komyo Rikagaku Kogyo, Japan). CO_2 concentration in the basket after 72 h treatment was determined using a portable gas analyser (CheckPoint O_2/CO_2, Dansensor, Denmark). After 72 h of treatment, the mangoes were transferred in trays and stored in an air-conditioned room (26.2±1.0°C, 81.3±6.5% RH).

Mango fruit quality was evaluated at 3 (after 72 h of treatment), 4, and 7 days after harvest. The effect of the treatments were determined using the following parameters: weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure

Tester, Wagner Instruments, USA), peel (Appendix 14.2.1) and flesh (Appendix 14.2.2) color indices and measurements (*L*, a*, b**, and color difference (ΔE)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). The experiment was arranged in a CRD with three replicates each treatment. Each replicate had 10 fruit samples. Data were analyzed using ANOVA and differences in means were compared using Fisher's LSD at 5% level of significance.

Figure 53. Bamboo baskets containing 5 kg 'Carabao' mango wrapped in newspaper and tightly sealed with polypropylene twine. The treatments (i.e., control, CaC_2, 250 or 500 mg Ripestuff™) were arranged in a Completely Randomized Design.

8.3 Results and Discussion

The use of 500 mg Ripestuff™ powder released higher ethylene than 250 mg Ripestuff™ by a difference of 10 µL L^{-1} (Figure 54A). After an hour of treatment, about 26 µL L^{-1} ethylene concentration was detected in the basket with 500 mg Ripestuff™ treatment while 16 µL L^{-1} for the basket with 250 mg Ripestuff™. These concentrations were enough to trigger ripening in mango as it only requires 0.1 µL L^{-1} to stimulate ripening (Burg and Burg, 1962). However, this concentration was not sustained during 72 h treatment. After the peak at the

first hour, ethylene concentration dropped to 7 µL L^{-1} until no Ripestuff™ ethylene was detected starting at the 12th hour of treatment. This could be attributed to leakage brought about by the basket's configuration. The residual ethylene detected in the vessel with 250 mg Ripestuff™ showed that almost all ethylene was released into the basket headspace while there was still a small amount of ethylene (~4 µL L^{-1}) left in the vessel with 500 mg Ripestuff™ (Figure 54B). On the other hand, calcium carbide released 1700 µL L^{-1} acetylene in the basket within the first 12 h of treatment and declined thereafter (Figure 55). After 72 h of treatment, the CO_2 concentration in the baskets did not differ among treatments (Figure 56).

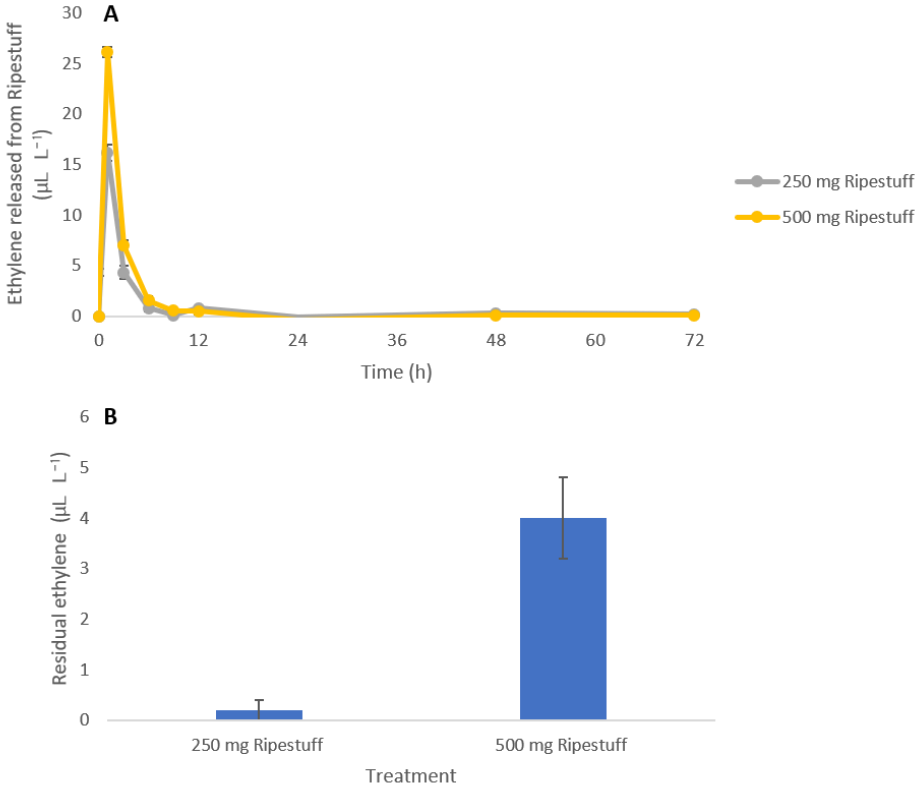

Figure 54. Ethylene concentration released from Ripestuff™(A) and residual ethylene left in the vessel after 72 h treatment (B). Error bars= SEM.

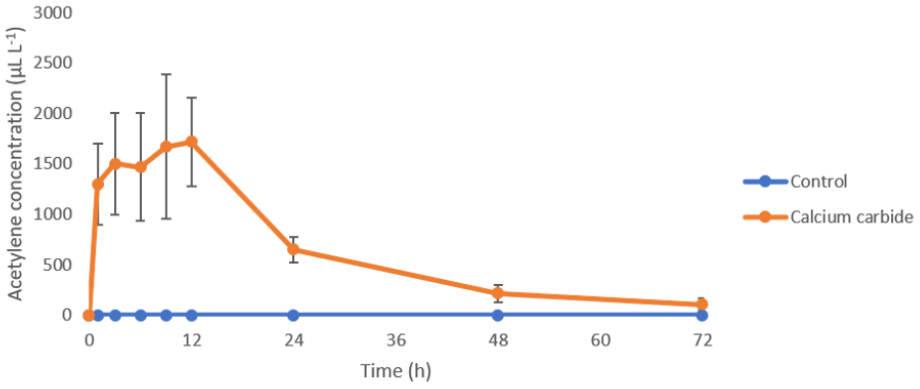

Figure 55. Acetylene concentration released from calcium carbide during 72 h treatment on 'Carabao' mango inside bamboo baskets. Error bars= SEM.

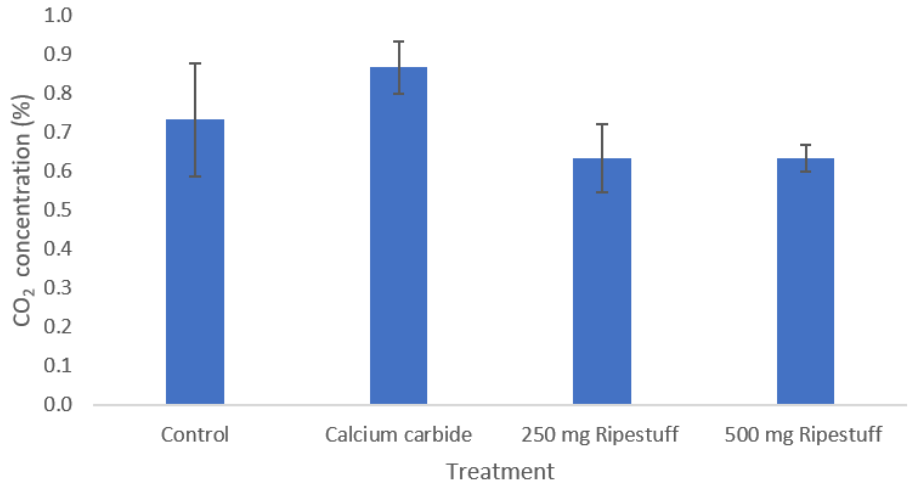

Figure 56. CO_2 concentration inside bamboo baskets after 72 h treatment of calcium carbide or different concentrations of Ripestuff™ (250 or 500 mg). Error bars= SEM.

Results showed that mango treated with 500 mg Ripestuff™ ripened faster than those treated with only 250 mg powder (Figure 57). Also, the ripening effect of Ripestuff™ was comparable to the effect of calcium carbide in terms of peel (Figure 58) and flesh color (Figures 59-60), firmness (Figure 61B), and total soluble solids (Figure 61C). Further, mangoes treated with Ripestuff™ had lower weight loss (Figure 61A) and better visual quality (Figure 62A) compared to mangoes treated with calcium carbide which developed a higher degree of blotchiness and anthracnose (Figure 62B&D) on the skin. Stem-end rot did not differ among treatments (Figure 62C). Mangoes treated with calcium carbide or 500 mg Ripestuff™ took only 3.6 days to reach saleable stage compared to the control at 5.7 d. Ripestuff™ resulted in mangoes with longer shelf life (9.5 d) compared to calcium carbide-treated fruit (8 d) after storage in 26.3±1°C, 81.4±6.4% RH (Table 11).

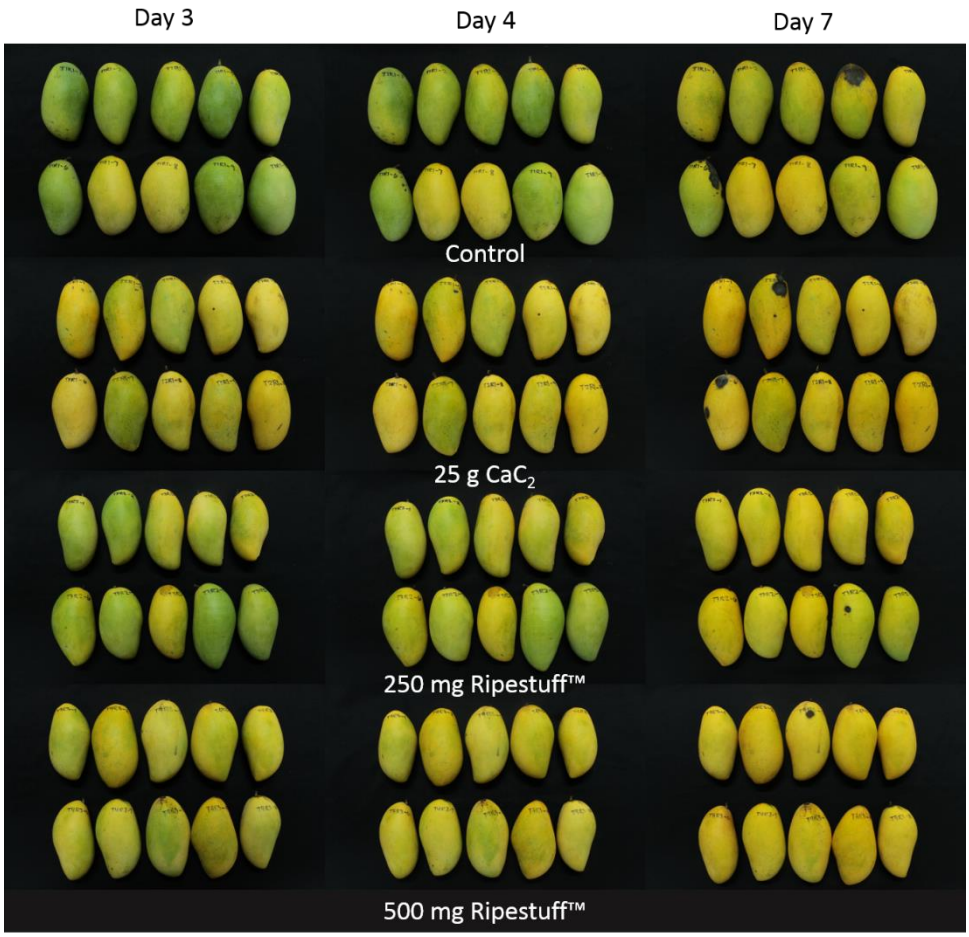

Figure 57. Peel color changes in 'Carabao' mango during storage in air-conditioned room (26.2±1.0°C, 81.3±6.5% RH) as influenced by treatment of calcium carbide or Ripestuff™ (250 or 500 mg) for 72 h. Each basket contained 5 kg fruit.

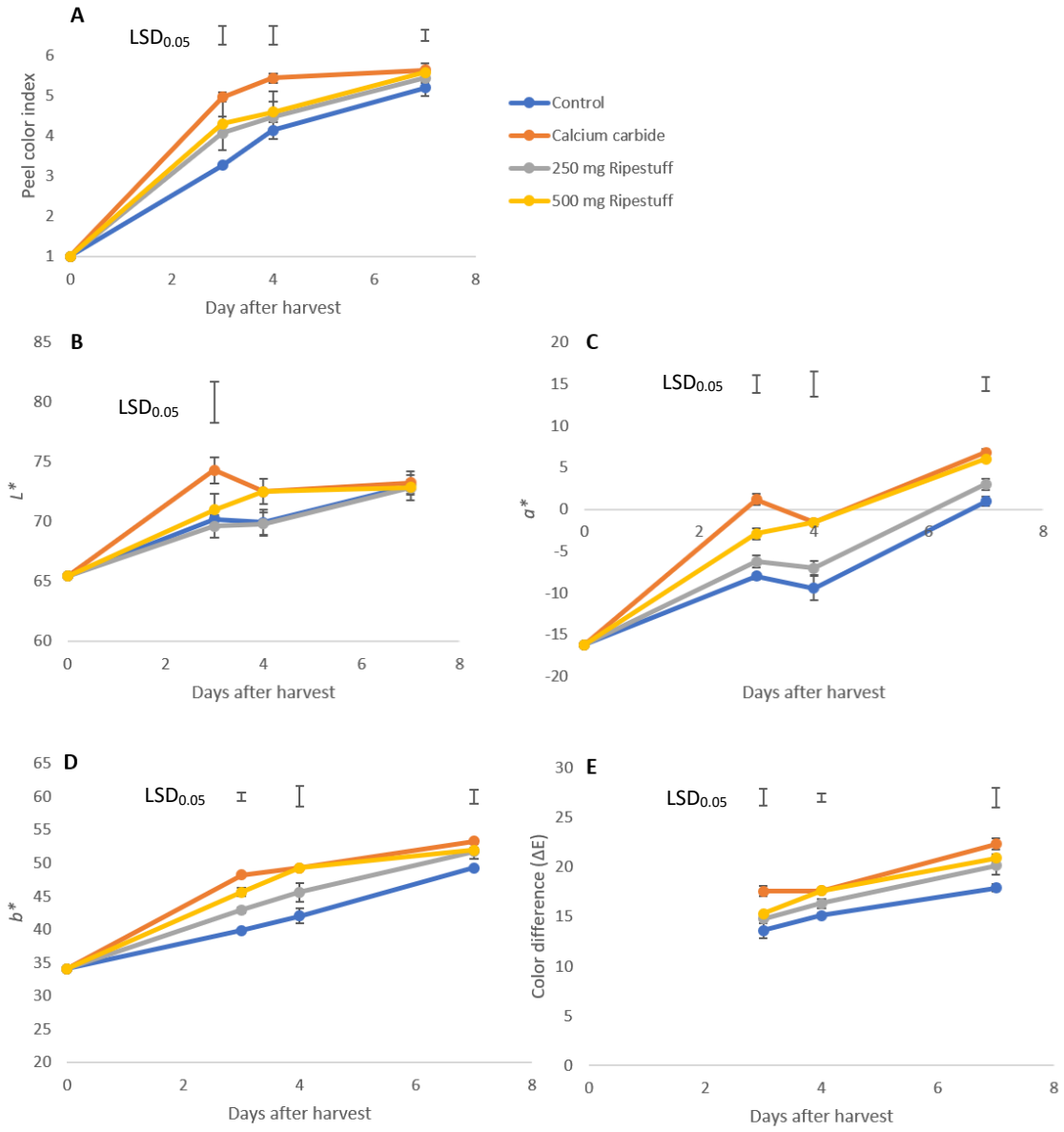

Figure 58. Peel color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) in 'Carabao' mango treated with calcium carbide or Ripestuff™ (250 or 500 mg) for 72 h, and stored in an air-conditioned room (26.2±1.0°C, 81.3±6.5% RH). Each basket contained 5 kg fruit. Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

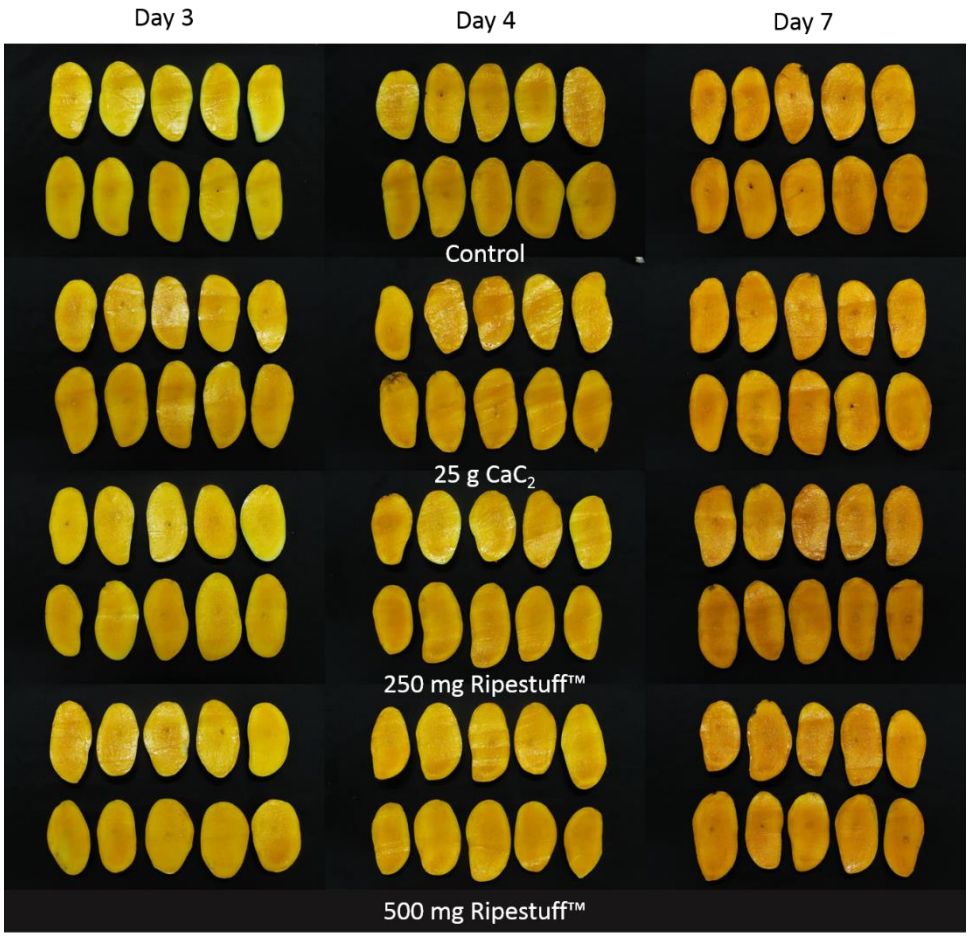

Figure 59. Flesh color changes in 'Carabao' mango during storage in airconditioned room (26.2±1.0°C, 81.3±6.5% RH) as influenced by treatment of CaC_2 or Ripestuff™ (250 or 500 mg) for 72 h. Each basket contained 5 kg fruit.

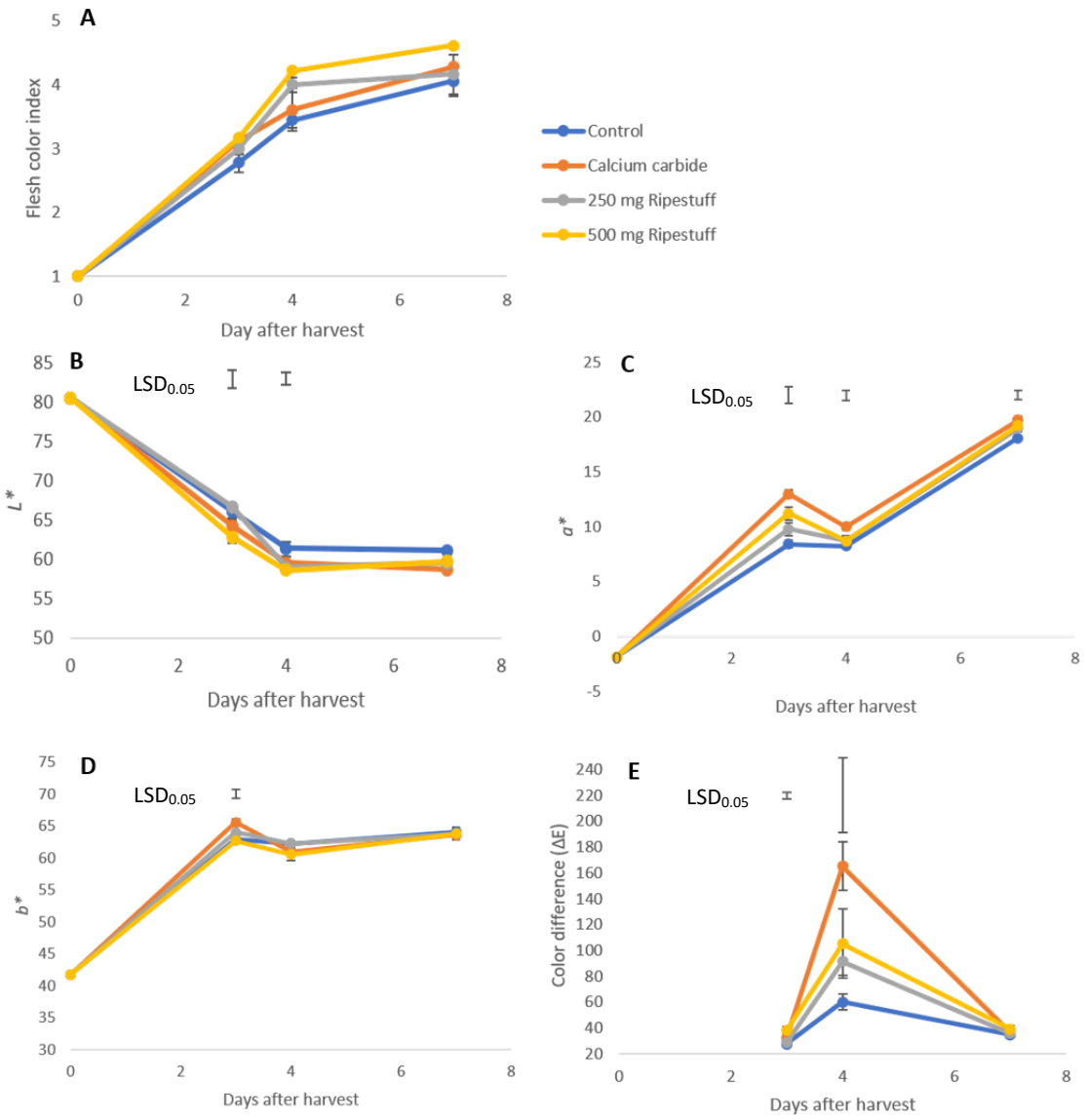

Figure 60. Flesh color *L** (A), *a** (B), *b** (C), and color difference (D) in 'Carabao' mango treated with calcium carbide or Ripestuff™ (250 or 500 mg) for 72 h, and stored in an air-conditioned room (26.2±1.0°C, 81.3±6.5% RH). Each basket contained 5 kg fruit. Flesh color index: 1= white-yellow; 2= light yellow; 3= bright yellow; 4= yellow-orange; 5= orange. Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

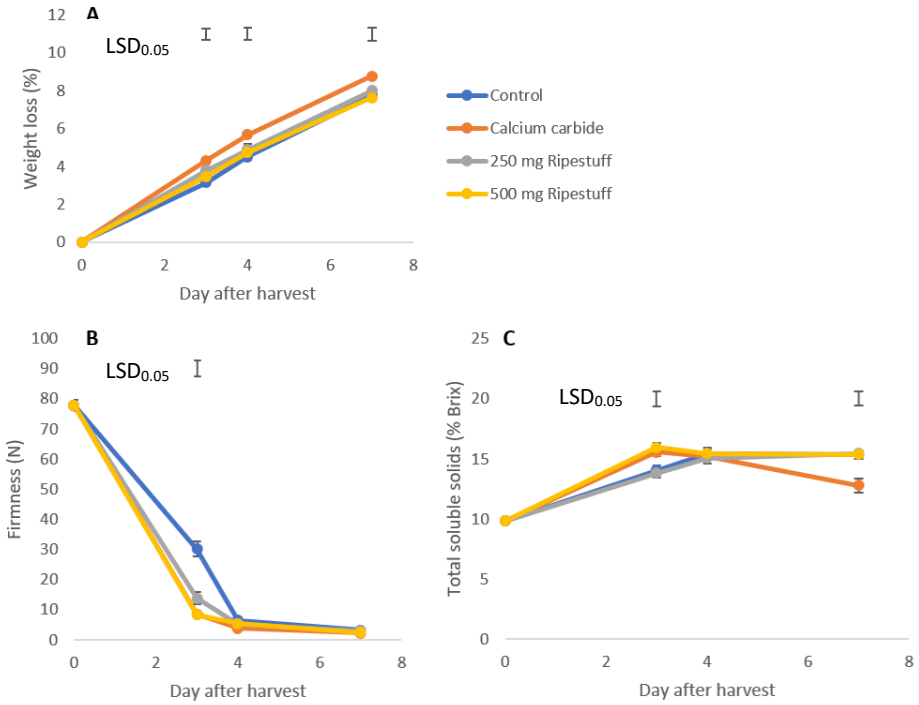

Figure 61. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango treated with calcium carbide or Ripestuff™ (250 or 500 mg) for 72 h, and stored in an air-conditioned room (26.2±1.0°C, 81.3±6.5% RH). Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

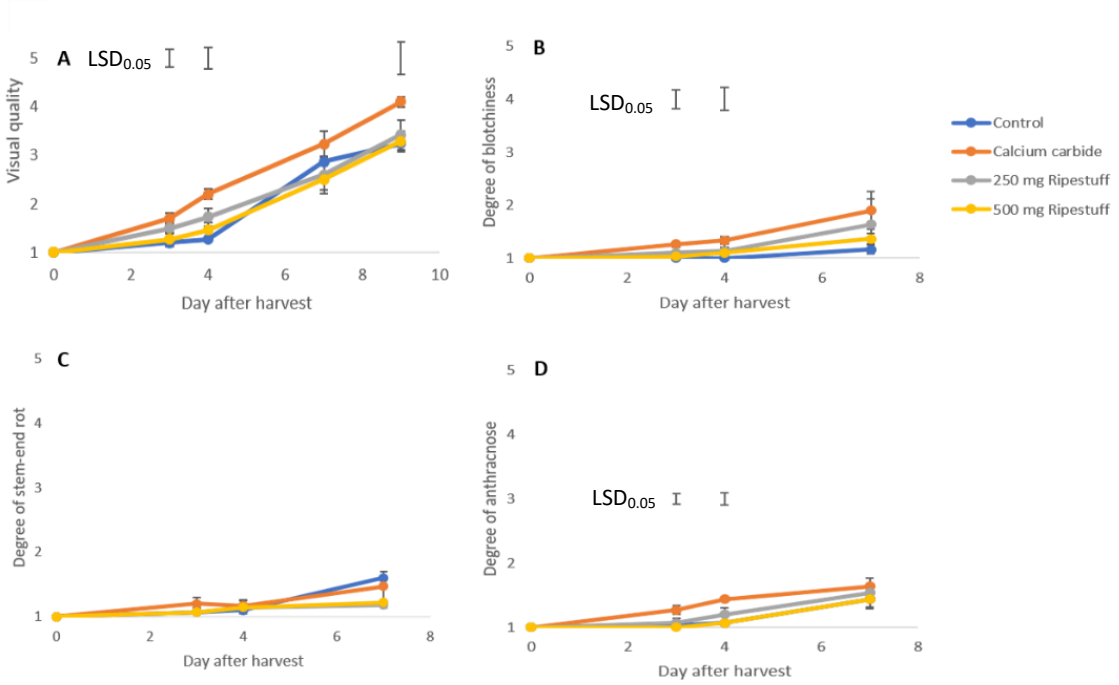

Figure 62. Visual quality (A), degree of skin blotchiness (B), stem-end rot (C) and anthracnose (D) in 'Carabao' mango treated with calcium carbide or Ripestuff™ (250 or 500 mg) for 72 h, and stored in an air-conditioned room (26.2±1.0°C, 81.3±6.5% RH). Each basket contained 5 kg fruit. Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bar are significantly different at $P \leq 0.05$. Error bars= SEM.

Table 11. Saleability and shelf life of 'Carabao' mango treated with calcium carbide or Ripestuff™ (250 or 500 mg) inside bamboo baskets[z] for 72 h and stored in an air-conditioned room (26.2±1.0°C, 81.3±6.5% RH).

Treatment	Days to saleability[x]	Saleable days[NS]	Shelf life[x] (d)
Control	5.6[a]	4.7	10.4[a]
Calcium carbide	3.6[c]	4.4	8.0[c]
250 mg Ripestuff™	4.8[b]	4.4	9.3[bc]
500 mg Ripestuff™	4.4[c]	5.1	9.5[ab]

[z]Each basket contained 5 kg Ripestuff™.
[x]Means with common letter are not significantly different using LSD at $P \leq 0.05$.
[NS]Not significant

8.4 Conclusion

Based on this experiment, the use of 250 mg Ripestuff™ was not enough to ripen 'Carabao' mango in baskets although it was able to trigger ripening faster than the untreated ones. Doubling this amount at 500 mg Ripestuff™ powder resulted in better ripening effect that was on par with the effect of calcium carbide. This treatment could be an alternative method of ripening against the harmful calcium carbide. However, ethylene must be sustained inside the bamboo basket in order to achieve a more uniform ripening in 'Carabao' mango.

9 Experiment 9- Ethylene leakage from bamboo basket

9.1 Introduction

Monitoring the concentrations of ethylene from Ripestuff™ and acetylene from calcium carbide in the previous experiment (Experiment 8) showed that after a peak at the first hour of treatment, the concentration of the gases declined thereafter. This experiment would like to determine how 'leaky' the baskets were by measuring the ethylene that comes out from the basket towards an enclosed chamber.

9.2 Materials and Methods

This experiment was conducted in June 2019. A total of 30 kg freshly harvested mature green 'Carabao' mango (20.9% dry matter, 25.5% sinkers) was harvested from Island Garden City of Samal, Davao del Norte at 115 days after flower induction. The mangoes were transported to the Postharvest Biology Laboratory in UP Mindanao, Davao City. The fruit were prepared and treated as in Experiment 8 but only using 500 mg Ripestuff™ with 5 mL water contained in a 60 mL specimen container with four holes in the lid pierced using Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). A specimen container with the same amount of water and holes in the lid but without Ripestuff™ served as control.

Each basket (V= 12 L) containing 5 kg mangoes with its corresponding treatment were placed inside an enclosed chamber (V= 90.57 L) with fan (Figure 63A). Each chamber was attached with gas sampling ports on two opposite lateral sides for monitoring the concentration of ethylene that leaked out from the basket towards the chamber headspace. Ethylene concentration inside the chamber was monitored at 0, 1, 3, 6, 9, 12, 24, 48, and 72 h of treatment using the Ethan portable ethylene analyser (Ethan, Portable Ethylene Gas Analyzer, Bioconservacion, Barcelona, Spain) (Figure 63B). In the middle of monitoring ethylene leakage from the basket, it was found that the chamber was also leaking as confirmed by the water leakage test. The leakage in the chamber was fixed by wrapping all the adjacent sides inside and outside the chamber with clear adhesive tape.

CO_2 concentration in the basket after 72 h treatment was determined using a portable gas analyser (CheckPoint O_2/CO_2, Dansensor, Denmark) (Figure 63C). Temperature and RH inside the basket and chamber were monitored using a data logger (Tinytag Ultra 2 TGU-4500, Gemini Data Loggers Ltd., England). The experiment was arranged in CRD with three replicates each treatment. Data was analyzed using t-Test at $P \leq 0.05$.

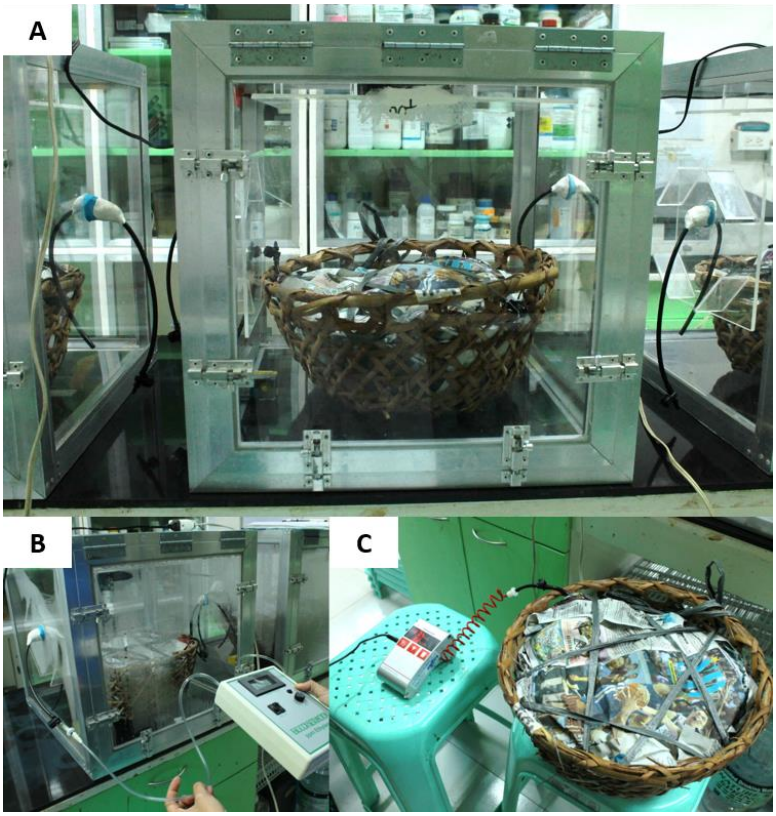

Figure 63. Bamboo basket containing mangoes treated with Ripestuff™ placed in an enclosed chamber (A) to monitor the ethylene leakage using the Ethan portable ethylene analyser (B). CO_2 concentration after 72 h of treatment was determined prior to opening of the basket.

9.3 Results and Discussion

Results showed that sealing the chambers with clear adhesive tape was effective in preventing the leakage of gases. With the leaking chamber, the peak of leaked Ripestuff™ ethylene hardly reached 10 µL L^{-1} which continuously decreased after 6 h of monitoring (Figure 65A). After sealing, ethylene from the basket accumulated in the chamber which registered up to 50 µL L^{-1} after 72 h of treatment (Figure 65B). This confirms that the basket configuration, although laid with newspaper linings, was very leaky and thus unable to fully contain the ethylene that was released from Ripestuff™. Unsustained ethylene release could result in an uneven ripening in mangoes. In contrast to the untreated mangoes, fruit treated with 500 mg Ripestuff™ ripened although there were traces of green left in the peel (Figure 66). Proportion of mangoes with corresponding peel color showed that 45% of the fruit treated with 500 mg Ripestuff™ had more yellow than green peel color and 65% showed bright yellow flesh color after 72 h (Figure 67). Ripestuff™-treated mangoes were also less firm and sweeter than the control fruit though not statistically significant (Figure 68). Although the treated mangoes showed a difference from the control, this may still require further optimization to come up with a more uniform ripening in mangoes.

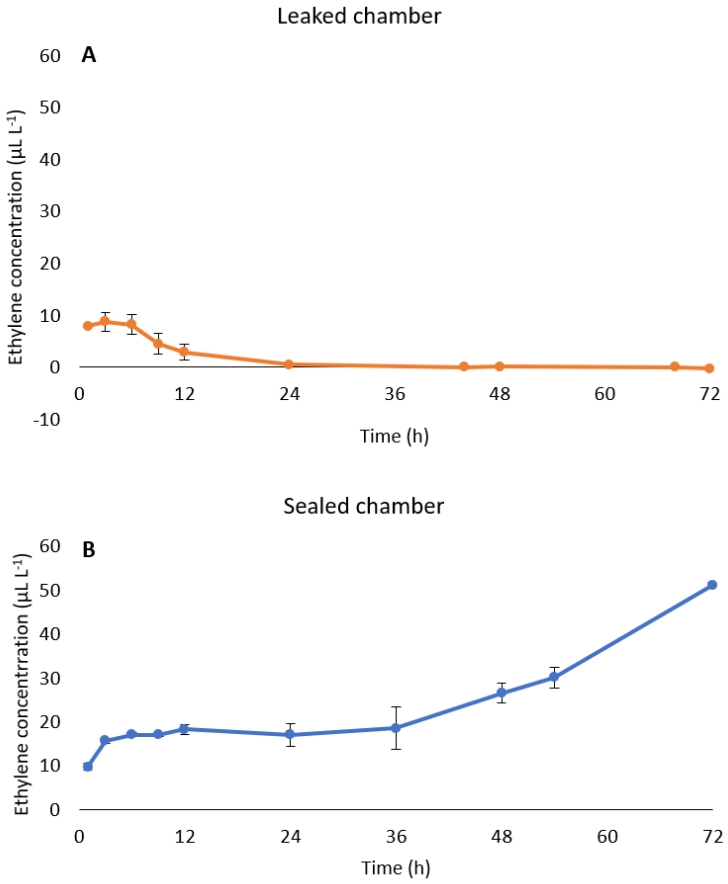

Figure 64. Concentration of ethylene in the chamber that leaked out from the basket before (A) and after (B) sealing the chamber. Error bars= SEM.

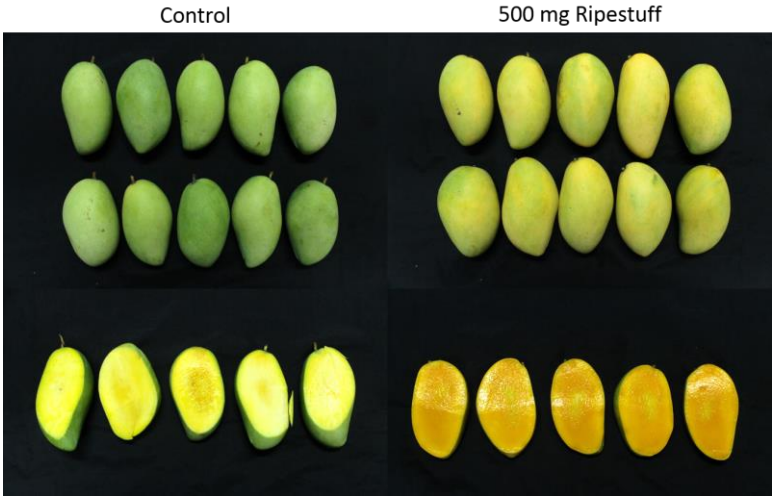

Figure 65. Peel and flesh color of 'Carabao' mango with or without treatment of 500 mg Ripestuff™ for 72 h inside bamboo basket. Each basket contained 5 kg fruit.

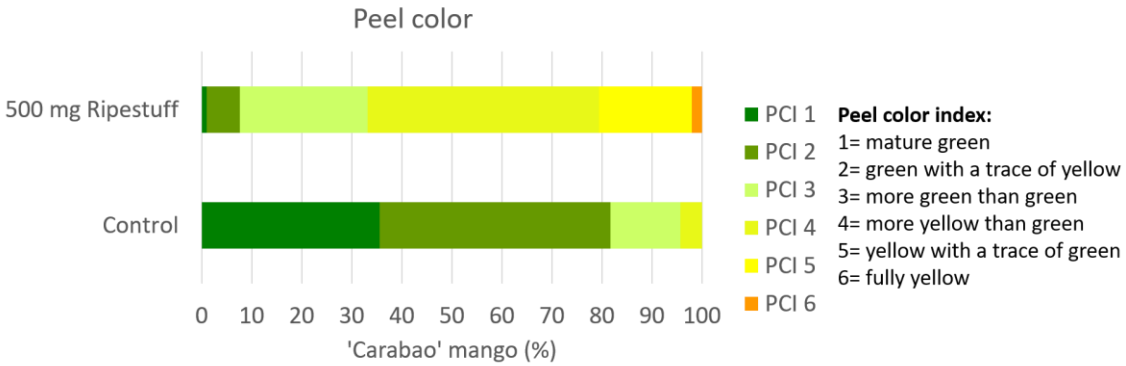

Figure 66. Percentage of 'Carabao' mango with particular peel color index (PCI) after treatment with 500 mg Ripestuff™ for 72 h inside bamboo basket. Each basket contained 5 kg fruit.

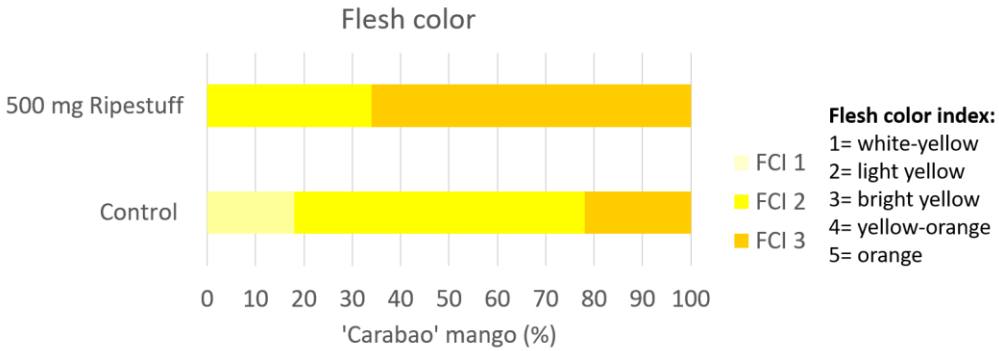

Figure 67. Percentage of 'Carabao' mango with particular flesh color index (FCI) after treatment with 500 mg Ripestuff™ for 72 h inside bamboo basket. Each basket contained 5 kg fruit.

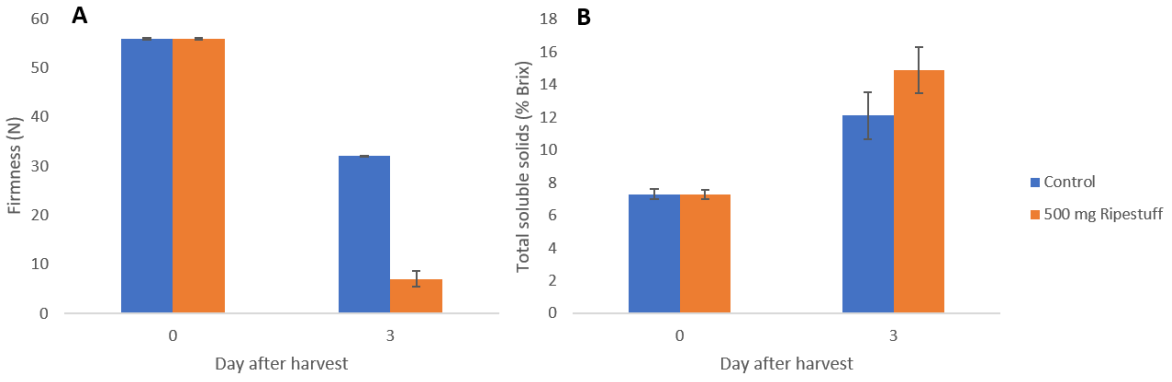

Figure 68. Firmness (A) and total solubles solids content (B) of 'Carabao' mango after treatment with 500 mg Ripestuff™ for 72 h inside bamboo baskets. Each basket contained 5 kg fruit.

9.4 Conclusion

This experiment confirmed that the basket configuration for the treatment of Ripestuff™ in mangoes was very leaky. This is the reason why almost no ethylene was left in the basket after a peak at the first 12 h of treatment. There is a need to sustain the ethylene concentration inside the basket to come up with more even ripening in mangoes. This was addressed in the next experiment.

10 Experiment 10- Effect of sustained ethylene release from Ripestuff™ on the ripening of 'Carabao' mango

10.1 Introduction

Knowing in Experiment 9 that the basket configuration could poorly sustain ethylene inside the basket due to its leaky behaviour, collaborators from UQ conducted an experiment to sustain the ethylene released from Ripestuff™ in the basket (Perkins and Joyce, 2019b). Their experiment found that sustained ethylene release from Ripestuff™ over a 72 h treatment period is theoretically possible by using a combination of wet (addition of water in the vessel) and dry Ripestuff™ (500 or 1000 mg) contained in vessels with different number of holes (1, 4, 16, 32, or 64 holes) in the lid. Each vessel containing Ripestuff™ resulted in different ethylene release rates, and combining it altogether would result in a more sustained ethylene concentration in the basket. Based on their results, this experiment utilized the 'low' sustained ethylene release from Ripestuff™ which aimed to release ethylene at a rate of 175 μL L^{-1} h^{-1}, instead of the 'high' sustained release which aimed to release at 300 μL L^{-1} h^{-1}. The 'low' sustained release was used in this experiment as it may offer uniform ripening without causing skin blotchiness in mango brought about by too high ethylene concentration.

10.2 Materials and Methods

This experiment was conducted in June 2019. 'Carabao' mango at mature green stage was harvested at 115 days after flower induction in Island Garden City of Samal, Davao del Norte. The mangoes were brought to the Postharvest Biology Laboratory in UP Mindanao, Davao City to conduct the experiment. The mangoes were subjected to flotation using 1% NaCl solution to determine its harvest maturity and were sorted as sinkers and floaters. Separately, sinker and floater mangoes were sanitized with 200 μL L^{-1} NaOCl and air-dried.

Ripestuff™ treatment was prepared by sieving the powder using a strainer to remove the lumps. In a 60 mL specimen container, Ripestuff™ powder (Batch '7 Feb B', 0.42 mol·mol^{-1} ethylene) was weighed following the amount prescribed for each treatment as described in Table 12. Distilled water (5 mL) was injected into the Ripestuff™ vessel using a syringe when the baskets together with the treatments were about to be closed. Baskets with mangoes and empty specimen containers with number of holes similar to the 'low' sustained ethylene release served as control.

In a bamboo basket (V= 12 L) lined with newspaper sheets, 5 kg mango fruit were arranged and treated with 25 g calcium carbide, 500 mg wet Ripestuff™ (1 x 04.W.500), 1000 mg dry Ripestuff™ (1 x 64.D.1000), or 'low' sustained ethylene from Ripestuff™. The holes in the lid were pierced using a Terumo® 16 mm x 25 gauge needle (Terumo Philippines Corporation, Laguna, Philippines). Two tubes composed of 30 cm x 4 mm flexible PVC (Ezy Flex Tube, Holman Industries, Western Australia) fitted with in-line barbed tap (Pope, Toro Australia Pty Ltd, South Australia) were inserted into each basket to serve as sampling ports for ethylene and acetylene monitoring. The mangoes were treated for 72 h. Ethylene and acetylene concentrations in the basket headspace were monitored every 3 h for 12 h and every 24 h thereafter for 72 h. Ethylene was measured using a portable ethylene gas analyser (Ethan, Bioconservacion, Barcelona, Spain). Acetylene concentration was measured using Kitagawa detector tubes (50-1000 μL L^{-1}, Kitagawa Precision Gas Detector Tubes, Komyo Rikagaku Kogyo, Japan) and aspirating pump (Kitagawa AP-20 Aspirating Pump, Komyo Rikagaku Kogyo, Japan). After 72 h of treatment, the mangoes were transferred in trays and stored in an air-conditioned room (26.4±0.5°C, 85.6±5.3% RH).

Mango fruit quality was evaluated at 3 (after 72 h of treatment), 4, and 7 days after harvest. The effect of the treatments were determined using the following parameters: weight loss (%), total soluble solids (TSS, % Brix) using a handheld refractometer (HI 96801, Hanna Instruments, Romania), firmness (N) using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA), peel (Appendix 14.2.1) and flesh (Appendix 14.2.2) color indices and measurements (*L*, a*, b**, and color difference (ΔE)) using Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada), visual quality (Appendix 14.2.3), degree of skin blotchiness (Appendix 14.2.4), stem-end rot (Appendix 14.2.5) and anthracnose (Appendix 14.2.6), days to saleability, saleable days, and shelf life. Days to saleability indicates the time for mangoes to reach a saleable stage of ripeness (i.e., peel color index of ≥ 5, visual quality rating of ≤ 3, and no diseases). Saleable days refer to when the fruit were judged marketable (i.e., the time when fruit was deemed ripe until the end of shelf life). Shelf life is defined as the length of time from the day of harvest until it goes beyond the limit of saleability (i.e., visual quality rating of >3, and presence of disease). Each replicate had 10 fruit samples. The experiment was arranged in CRD and data were analysed using two-way ANOVA with treatment and maturity set as factors. Significant differences in means were detected using Fisher's LSD at 5% level of significance.

Table 12. Description of treatments used in Experiment 10 for treatment of 'Carabao' mango inside bamboo basket[z].

Treatment	Quantity x No. of holes on the lid. Wet (W) or dry (D). Mass of Ripestuff™ (mg)
Control	1 x 04.W.0 2 x 04.D.0 1 x 16.D.0 1 x 64.D.0
Calcium carbide	25 g calcium carbide wrapped in paper
500 mg Ripestuff™ (wet)	1 x 04.W.500
1000 mg Ripestuff™ (dry)	1 x 64.D.1000
'Low' sustained ethylene release	1 x 04.W.500 1 x 04.D.500 1 x 04.D.1000 1 x 16.D.500 1 x 64.D.500

[z]Each basket contained 5 kg mango fruit.

10.3 Results and Discussion

The treatment which was designed to release a 'low' sustained ethylene of ~175 μL L^{-1} h^{-1} was not able to sustain the target concentration due to a very leaky characteristic of the basket configuration, as confirmed in the previous experiment (Experiment 9). However, this treatment was able to reach and maintain a relatively higher ethylene concentration than the treatment of 500 mg wet Ripestuff™ with 4 holes in the lid or 1000 mg dry Ripestuff™ with 64 holes in the lid (Figure 69A). Similar to the results in Experiment 8, the treatment of 500 mg wet Ripestuff™ spiked a release of ethylene (14 μL L^{-1}) in the basket after 1 h of treatment which decreased afterwards until no ethylene was detected after 24 h. On the other hand, the treatment of 1000 mg dry Ripestuff™ resulted in a lower but more sustained ethylene (4-5 μL L^{-1}) until the 12th h compared to the treatment of 500 mg wet Ripestuff™. The same goes to acetylene concentration in the basket treated with calcium carbide which also decreased over time due to leakage in the basket (Figure 69B).

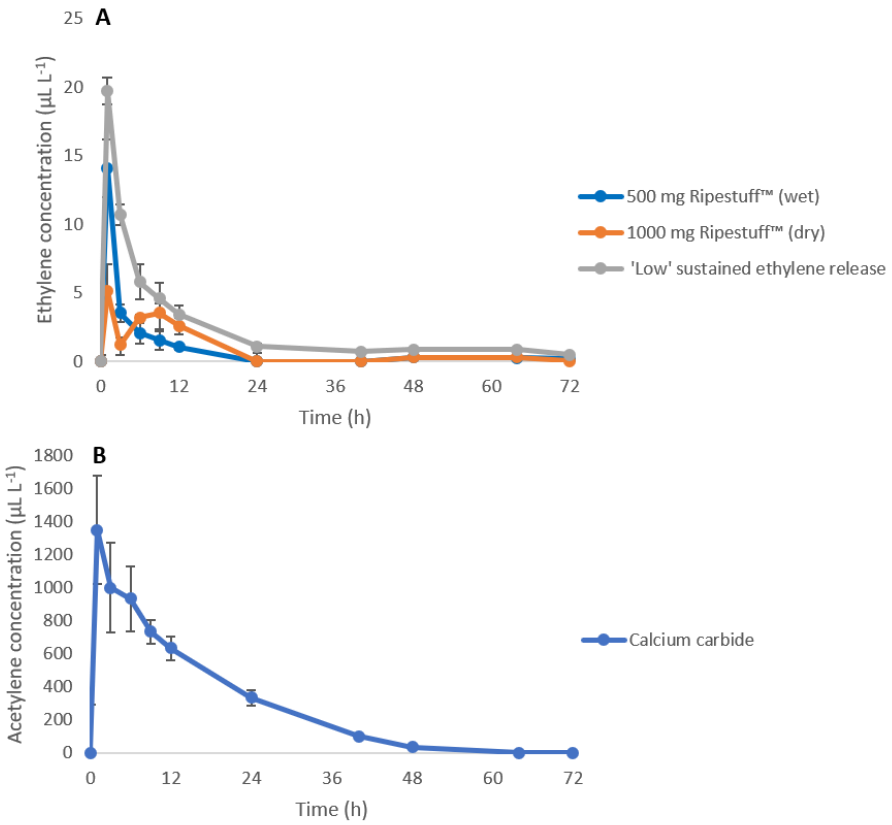

Figure 69. Concentrations of ethylene from Ripestuff™ (A) and acetylene from calcium carbide (B) during treatment of 'Carabao' mangoes inside bamboo baskets for 72 h. Error bars= SEM.

In terms of mango fruit quality, the treatment of 'low' sustained ethylene release was able to effectively ripen the fruit evenly which was similar to the effect of calcium carbide (Figures 70). Mangoes treated with calcium carbide or 'low' sustained ethylene had the same proportion of fully ripe mangoes (35-40%) after 72 h treatment in bamboo baskets (Figure 71). Moreover, fruit treated with 'low' sustained ethylene resulted in mangoes with more advanced peel and flesh (Figure 72) color compared to those treated with calcium carbide. Interestingly, mangoes treated with 1000 mg dry Ripestuff™ had similar proportion of mangoes with different peel color stages ranging from more green than yellow to fully yellow. Meanwhile, the development of ripe attributes in mango showed that Ripestuff™, whether at 1000 mg (dry) or through 'low' sustained ethylene release, had similar effect with calcium carbide in terms of peel (Figure 73) and flesh color (Figure 74), firmness (Figure 75B), visual quality, degree of blotchiness, and anthracnose (Figure 76). Mangoes treated with calcium carbide had higher weight loss (Figure 75A) probably due to increased rate of transpiration during treatment when heat was produced during reaction of calcium carbide with water.

Mangoes treated with 'low' sustained ethylene from Ripestuff™ resulted in more uniform ripening which took only 3.1 d to reach the saleable stage (Table 13). This result did not differ significantly from those treated with calcium carbide or 1000 mg dry Ripestuff™. The time to reach saleability of the mangoes was affected by maturity in favour of the more mature (sinker) fruit. Saleable days and shelf life did not differ among treatments and between two maturities (sinker or floater).

Figure 70. Peel and flesh color of 'Carabao' mango after treatment with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets. Each basket contained 5 kg fruit.

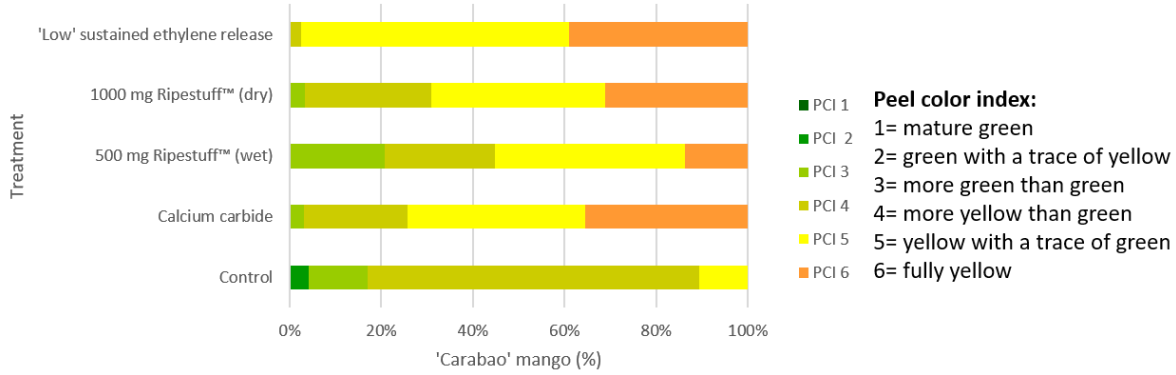

Figure 71. Percentage of 'Carabao' mango exhibiting a particular peel color index (PCI) after treatment with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets. Each basket contained 5 kg fruit.

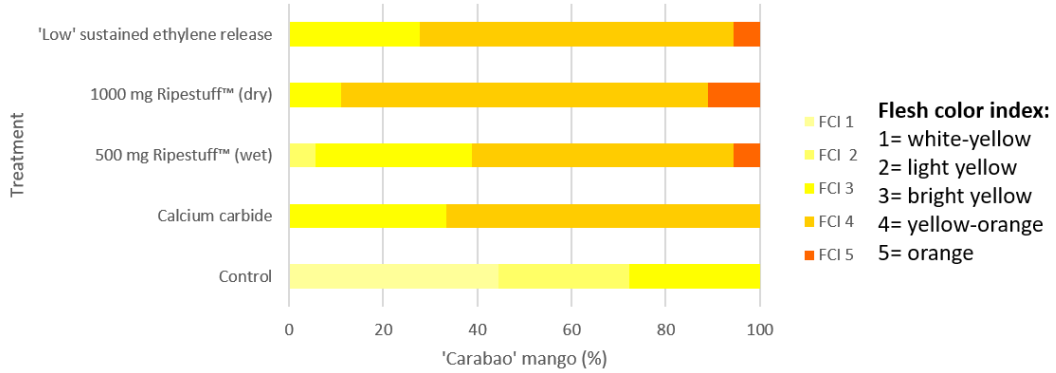

Figure 72. Percentage of 'Carabao' mango exhibiting a particular flesh color index (PCI) after treatment with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets. Each basket contained 5 kg fruit.

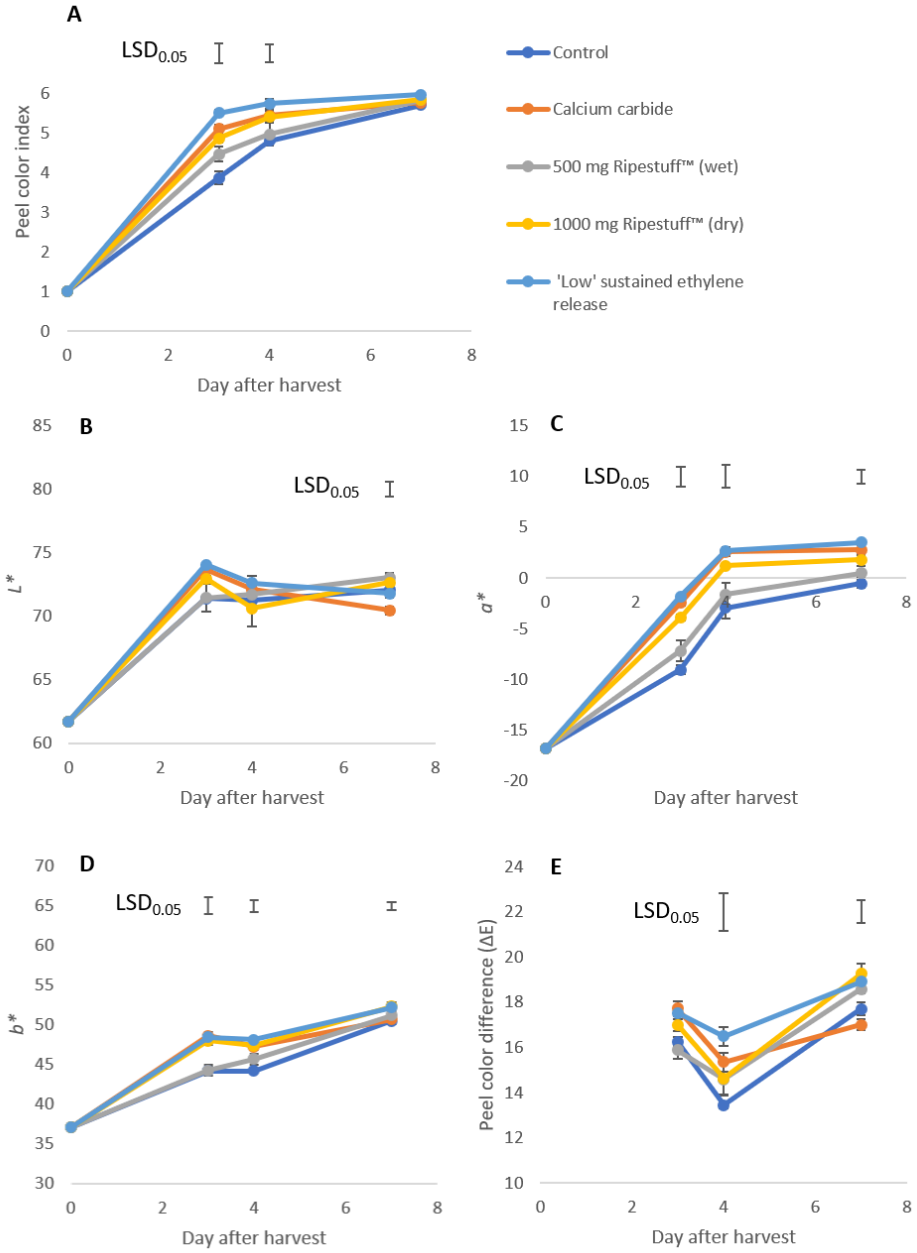

Figure 73. Peel color index (A), L* (B), a* (C), b* (D), and color difference (E) in 'Carabao' mango treated with calcium carbide, or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h, and stored in an air-conditioned room (26.4±0.5°C, 85.6±5.3% RH). Each basket contained 5 kg fruit. Peel color index: 1= mature green; 2= green with trace of yellow; 3= more green than yellow; 4= more yellow than green; 5= yellow with trace of green; 6= fully yellow. Data points with LSD bars are significantly different at P≤0.05. Error bars= SEM.

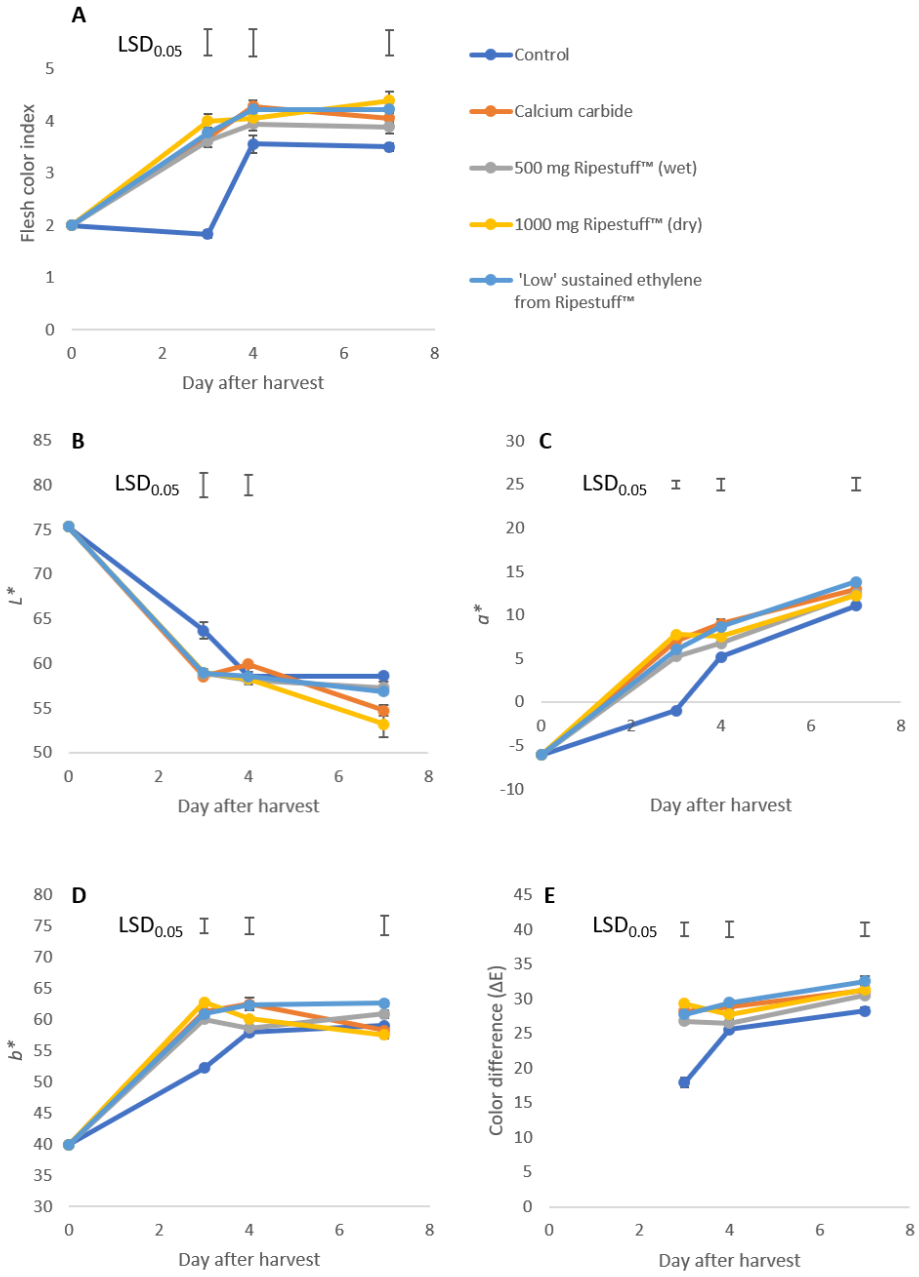

Figure 74. Flesh color index (A), *L** (B), *a** (C), *b** (D), and color difference (E) in 'Carabao' mango treated with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets, and stored in an air-conditioned room (26.4±0.5°C, 85.6±5.3% RH). Each basket contained 5 kg fruit. Flesh color index: 1= white-yellow; 2= light yellow; 3= bright yellow; 4= yellow-orange; 5= orange. Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

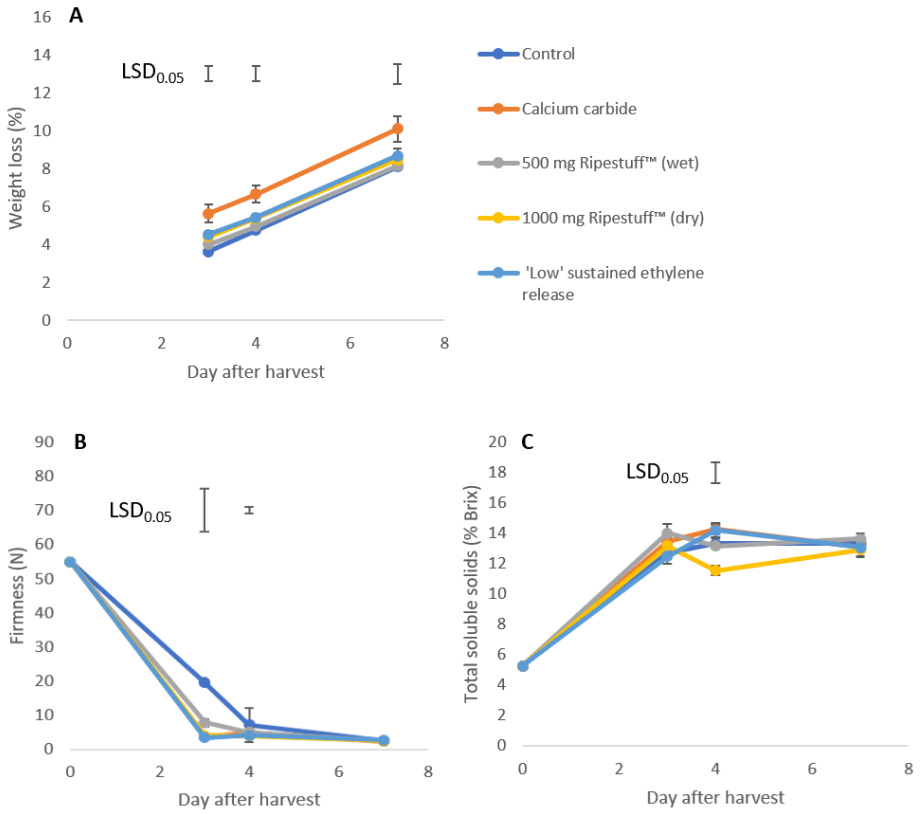

Figure 75. Weight loss (A), firmness (B), and total soluble solids (C) of 'Carabao' mango treated with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets, and stored in an air-conditioned room (26.4±0.5°C, 85.6±5.3% RH). Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

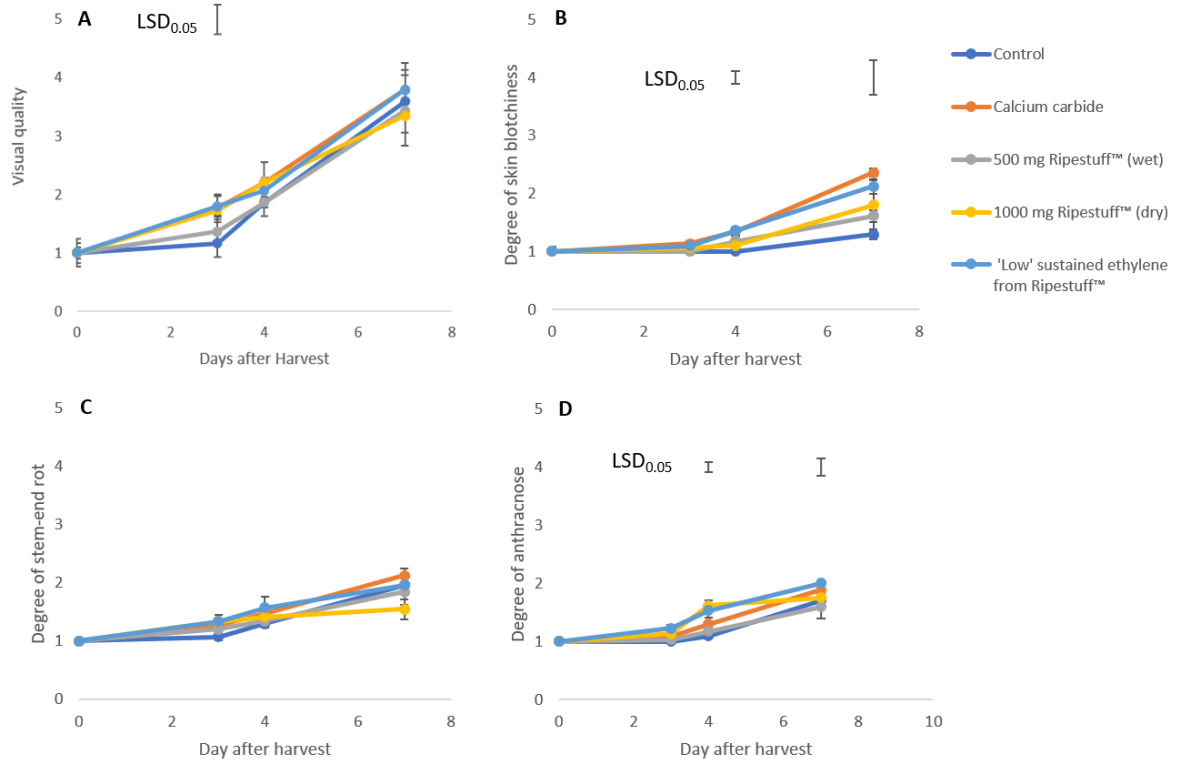

Figure 76. Visual quality (A), degree of skin blotchiness (B), stem-end rot (C) and anthracnose (D) in 'Carabao' mango treated with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets, and stored in an air-conditioned room (26.4±0.5°C, 85.6±5.3% RH). Each basket contained 5 kg fruit. Visual quality rating: 1= excellent; 2= good; 3= fair, limit of saleability; 4= poor; 5= extremely poor. Degree of skin blotchiness/ stem-end rot/ anthracnose: 1= none; 2= slight; 3= moderate; 4= moderately severe; 5= severe. Data points with LSD bars are significantly different at $P \leq 0.05$. Error bars= SEM.

Table 13. Saleability and shelf life of 'Carabao' mango treated with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h inside bamboo baskets[z], and stored in an air-conditioned room (26.4±0.5°C, 85.6±5.3% RH).

Treatment	Days to saleability[x]	Saleable days[NS]	Shelf life[NS] (d)
Control	4.5[a]	3.0	7.5
Calcium carbide	3.6[bc]	3.0	6.6
500 mg Ripestuff™ (wet)	4.0[ab]	3.8	7.8
1000 mg Ripestuff™ (dry)	3.6[bc]	2.9	6.5
'Low' sustained ethylene release	3.1[c]	3.4	6.4
Maturity			
Floater	4.1[A]	2.9	6.9
Sinker	3.4[B]	3.6	7.0

[z]Each basket contained 5 kg fruit.
[x]Per factor, means in a column with common letter/s are not significantly different using LSD at $P \leq 0.05$.
[NS]Not significant

10.4 Conclusion

This experiment revealed a prototype of Ripestuff™ application that could be used to ripen mangoes evenly inside baskets. Although leakage of ethylene was immense in a basket configuration, combining several Ripestuff™ treatments to attain a ‘low’ sustained ethylene release resulted in a relatively higher ethylene concentration that was sustained during the 72 h treatment period. The ‘low’ sustained ethylene release from Ripestuff™ resulted in faster and more uniform ripening that was comparable to the effect of calcium carbide. Furthermore, the effect of ‘low’ sustained ethylene release did not vary significantly with the effect of 1000 mg dry Ripestuff™ with 64 holes in the lid of its vessel. Since the former requires 5 containers to achieve a ‘low’ sustained ethylene release, it would be convenient to use the latter as an alternative ripening agent against calcium carbide.

11 Effect of Ripestuff™ on other crops

In addition to 'Carabao' mango, Ripestuff™ was also tested in other crops such as 'Solo' papaya and 'Lakatan' banana. For both crops, calcium carbide is also used as a 'traditional' ripening method for the local market in the Philippines. These experiments aimed to explore the effect of Ripestuff™ on other tropical horticultural crops and its possible application in the commercial value chains of other climacteric fruits.

11.1 Effect of Ripestuff™ in 'Solo' papaya

This experiment was conducted following the methods described in Experiment 8. A total of 60 kg (5 kg per basket) 'Solo' papaya at mature green stage was treated with calcium carbide (25 g) or Ripestuff™ (250 or 500 mg with added water in the vessel together with Ripestuff™) inside bamboo baskets for 72 h. Results showed that 'Solo' papaya ripened uniformly in response to the 500 mg Ripestuff™ treatment as compared to those treated with only 250 mg Ripestuff™ and the control (Figure 77). Papaya fruit treated with higher amount of Ripestuff™ resulted in ripening that was similar to the effect of calcium carbide.

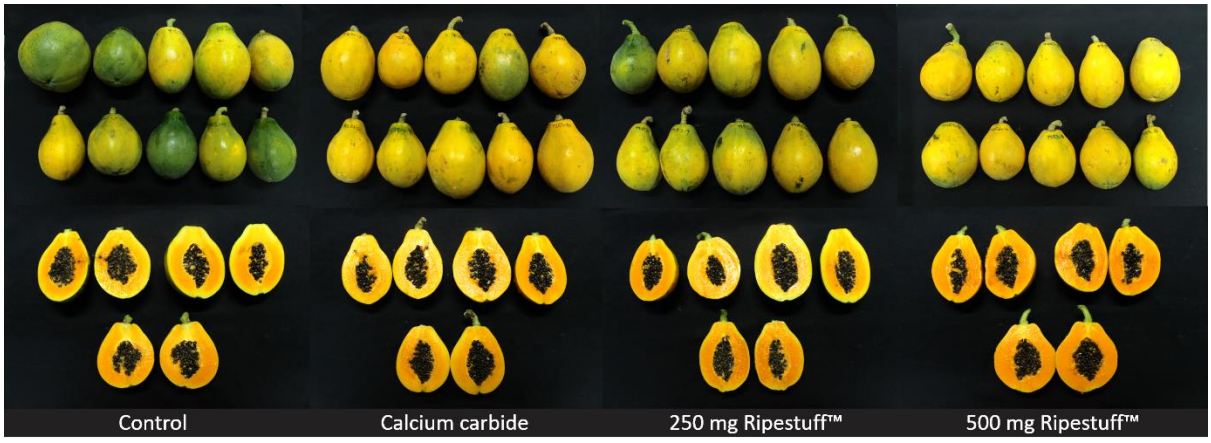

Figure 77. Peel and flesh appearance of 'Solo' papaya after treatment with calcium carbide or Ripestuff™ (250 or 500 mg with added water in the vessel) inside bamboo baskets for 72 h. Each basket contained 5 kg fruit.

11.2 Effect of Ripestuff™ in 'Lakatan' banana

This experiment was conducted as a demonstration of the effect of different ripening agents in 'Lakatan' banana during one of the Postharvest workshop series conducted in March 2018. 'Lakatan' banana (5 kg per basket) was treated with calcium carbide (5 g kg^{-1}), ethephon (1000 μL L^{-1}), *Gliricidia sepium* leaves (20% w/w), or Ripestuff™ (0.75 g kg^{-1}) inside bamboo baskets for 72 h. Results showed that the treatment of 3.75 g Ripestuff™ (0.75 g kg^{-1}) was comparable to the results of other ripening agents including calcium carbide, ethephon and *G. sepium* which were all different from the control (Figure 78). This shows that Ripestuff™ could also be a possible alternative ripening agent against calcium carbide for 'Lakatan' banana.

Figure 78. Appearance of 'Lakatan' banana after treatment with calcium carbide (5 g kg^{-1}), ethephon (1000 µL L^{-1}), *Gliricidia sepium* leaves (20% w/w), or Ripestuff™ (0.75 g kg^{-1}) inside bamboo baskets for 72 h. Each basket contained 5 kg fruit.

12 Conclusion

The use of Ripestuff™ demonstrated its ability to initiate ripening in 'Carabao' mango whether in an enclosed chamber or in basket. However, the use of Ripestuff™ powder alone was not able to turn the fruit in its readily saleable stage after 72 h of treatment due to insufficient release of ethylene from the cyclodextrin inclusion complex powder; thus, methods of application was optimized. Increased airflow and relative humidity outside the Ripestuff™ vessel, and amounts of Ripestuff™ powder (dry) did not fully release the ethylene from Ripestuff™. Through research collaboration with UQ, it was found that the addition of water into the Ripestuff™ vessel resulted in a fast release of ethylene into the headspace with full release within 12 h. The use of 250 mg Ripestuff™ (with added water in the vessel) per 5 kg mango in chamber (aimed to release 30 µL L^{-1} headspace ethylene) or in basket triggered ripening in fruit but not to its readily saleable stage after 72 h of treatment due to leakage of ethylene. Increasing the mass of Ripestuff™ at 500 mg (with added water in the vessel) per 5 kg fruit inside baskets resulted in more advanced mango ripening however, ethylene concentration was not sustained inside the basket due to its very leaky characteristic.

Collaborators in UQ came up with a prototype that could theoretically sustain ethylene in the basket through a combination of containers with different number of holes on lid, Ripestuff™ mass and whether the Ripestuff™ powder was wet or dry. The use of a 'low' sustained ethylene release from Ripestuff™ resulted in mangoes with more uniform ripe attributes such as peel and flesh color, firmness, and total soluble solids, which did not differ with those treated with calcium carbide or 1000 mg dry Ripestuff™ with 64 holes in the lid. The slow but relatively higher release of ethylene from Ripestuff™ through the 64 holes also facilitated a uniform ripening in mangoes than the effect of 500 mg wet Ripestuff™. Therefore, the use of 1000 mg dry Ripestuff™ contained in a vessel with 64 holes in the lid could be a more convenient way to achieve even ripening in 'Carabao' mango instead of using five containers to achieve a sustained ethylene release from Ripestuff™ in the basket.

Testing the effect of Ripestuff™ in other fruit crops also showed that it could also ripen other tropical fruits such as 'Solo' papaya and 'Lakatan' banana. The experiments conducted in this project could potentially lead to developing protocols for application of Ripestuff™ in the commercial value chains of climacteric fruits.

13 References

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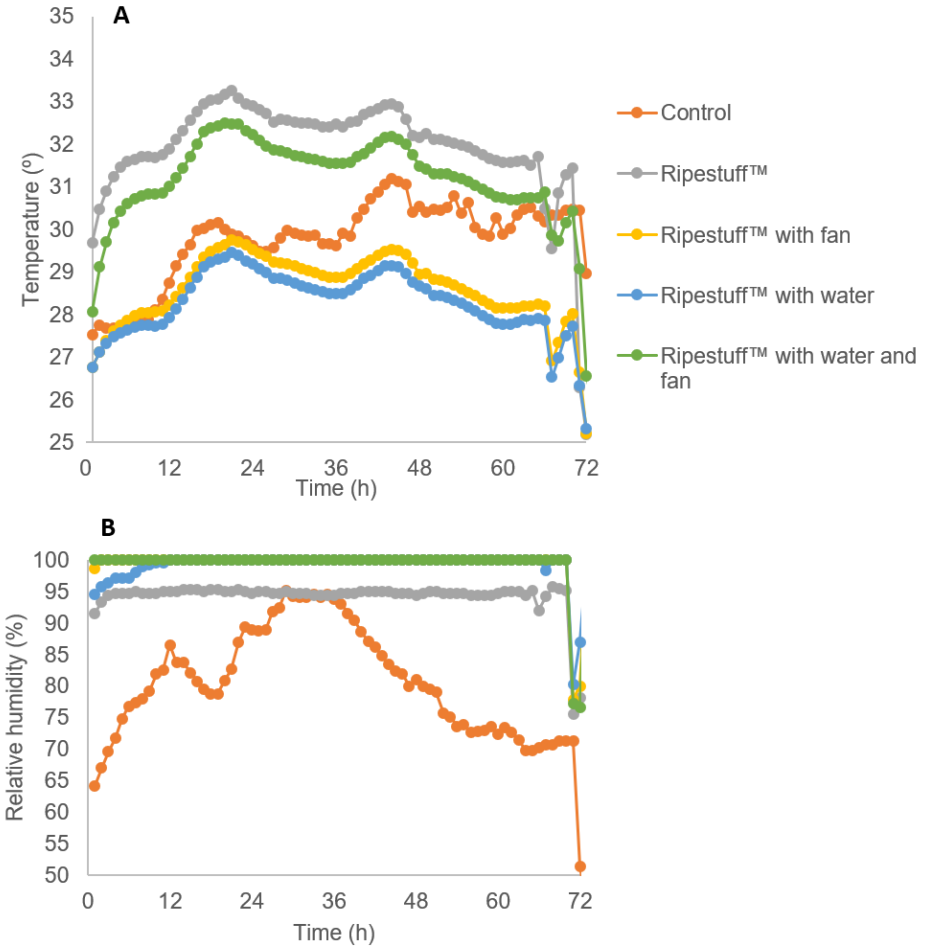
14 Appendices

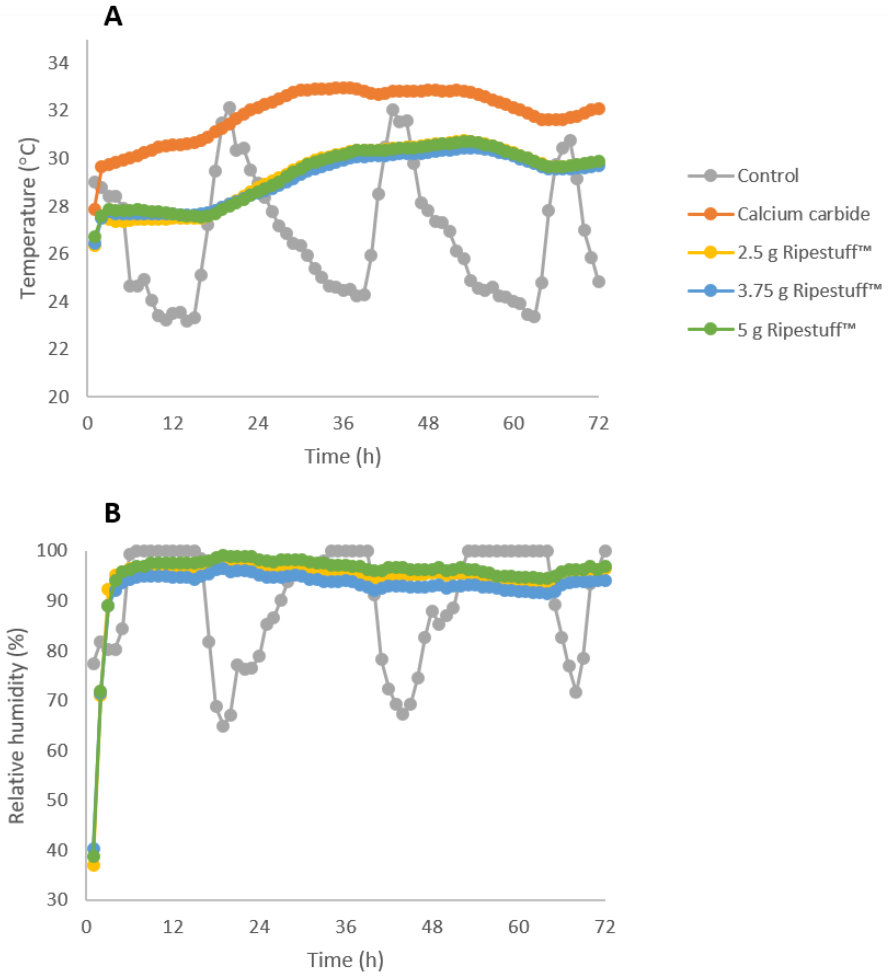
14.1 Temperature and relative humidity

14.1.1 Experiment 1- Effect of Ripestuff™ facilitated by increased RH and airflow on the ripening of 'Carabao' mango inside enclosed chambers

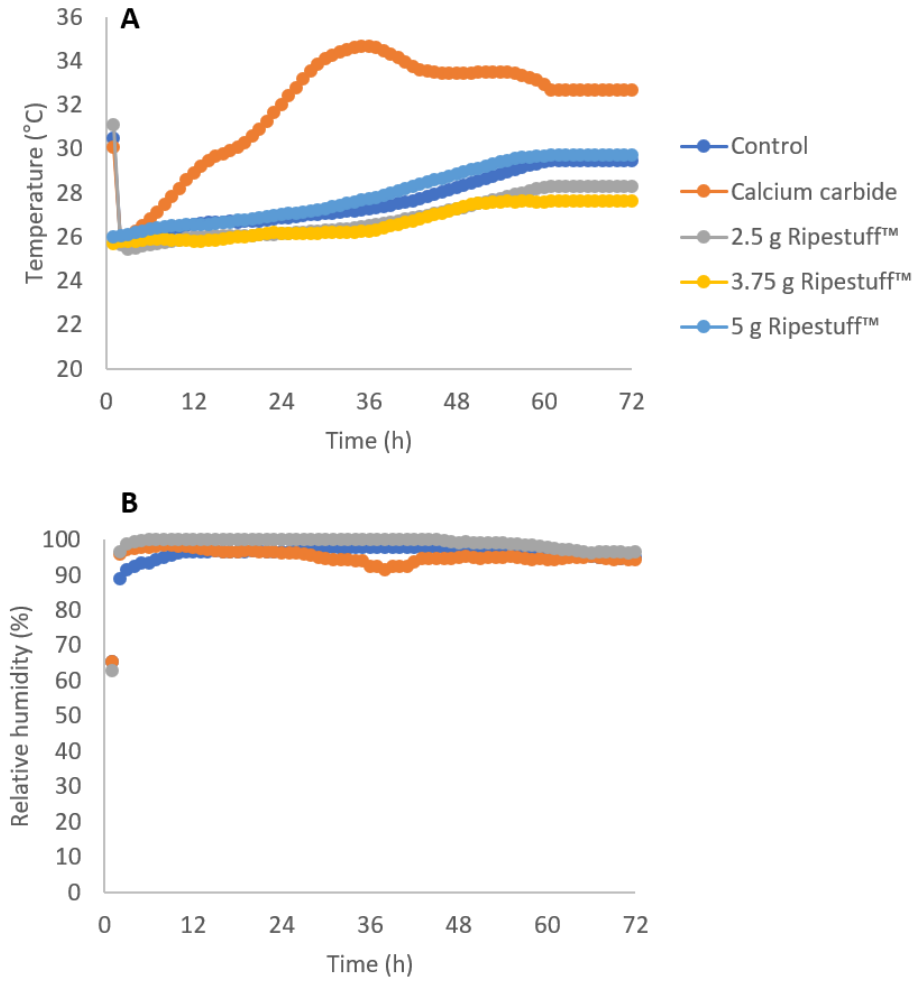


Appendix Figure 1. Temperature (A) and relative humidity (B) inside chambers during treatment of Ripestuff™ with presence of fan and/or water in the surrounding. Control= ambient

14.1.2 Experiment 2- Effect of different amounts of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets

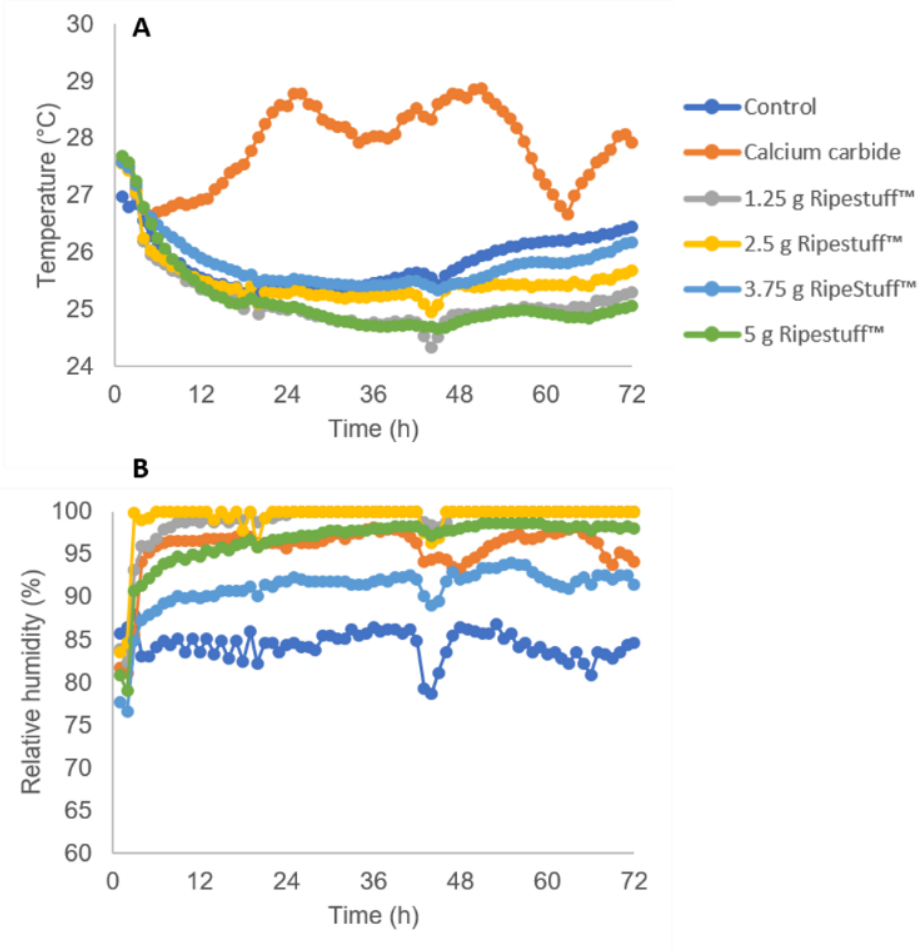


Appendix Figure 2.1. Temperature (A) and relative humidity (B) during treatment of 'Carabao' mango with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets for 72 h (Trial 1). The control was held in ambient.



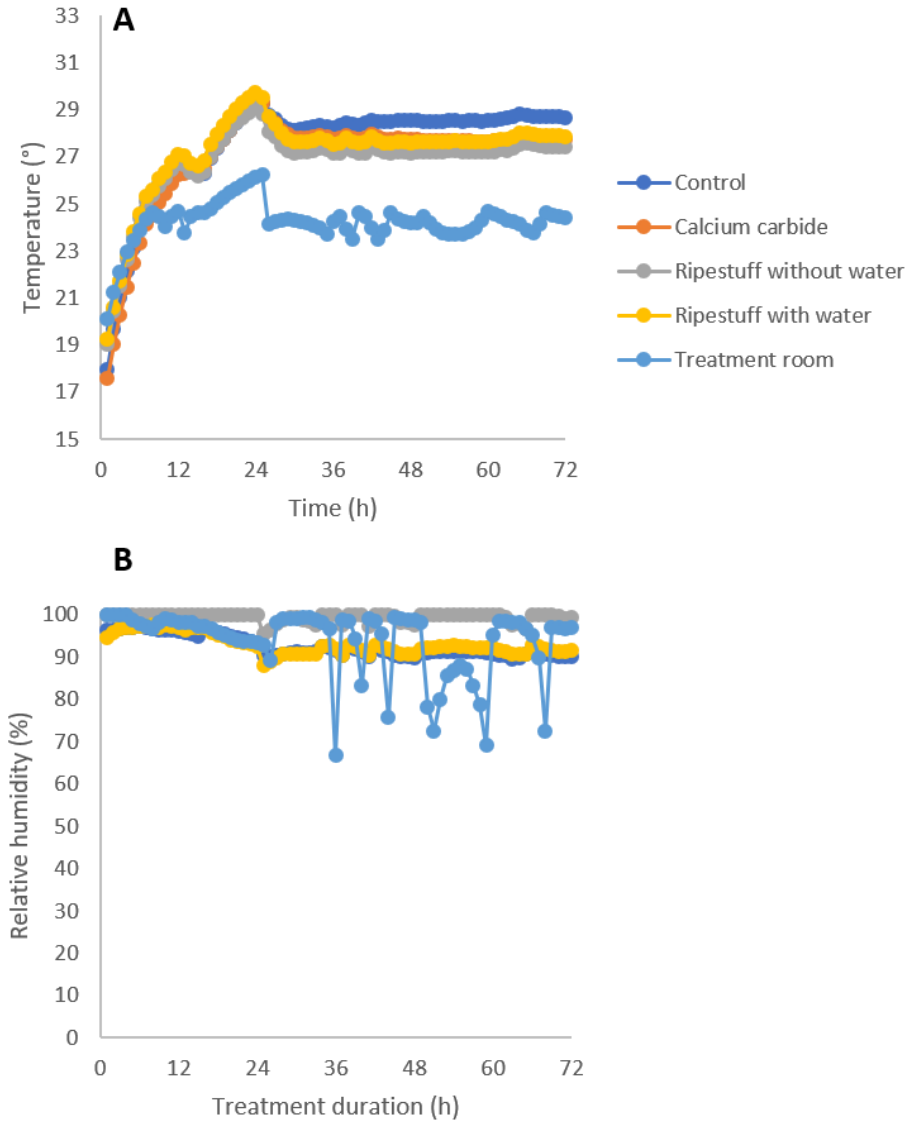
Appendix Figure 2.2. Temperature (A) and relative humidity (B) during treatment of 'Carabao' mango with different amounts of Ripestuff™ (2.5, 3.75, or 5 g) inside bamboo baskets for 72 h (Trial 2). The control fruit were inside bamboo baskets.

14.1.3 Experiment 3- Effect of different amounts of Ripestuff™ and the addition of water on the ripening of 'Carabao' mango inside bamboo baskets



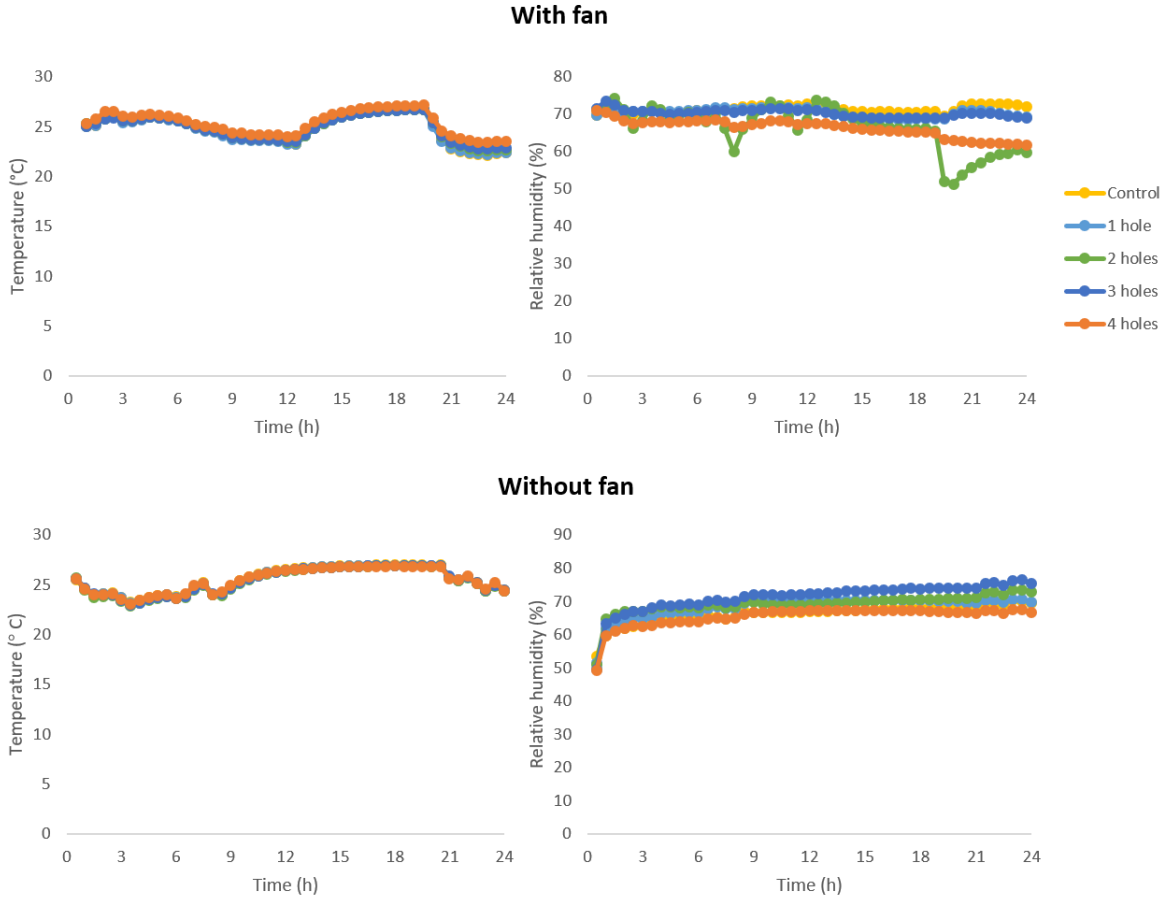
Appendix Figure 3. Temperature (A) and relative humidity (B) inside bamboo baskets during treatment of 'Carabao' mango with different amounts of Ripestuff™ (1.25, 2.5, 3.75, or 1 g) with addition of water in the surrounding.

14.1.4 Experiment 4- Effect of Ripestuff™ with added water in the vessel on the ripening of 'Carabao' mango inside enclosed chamber



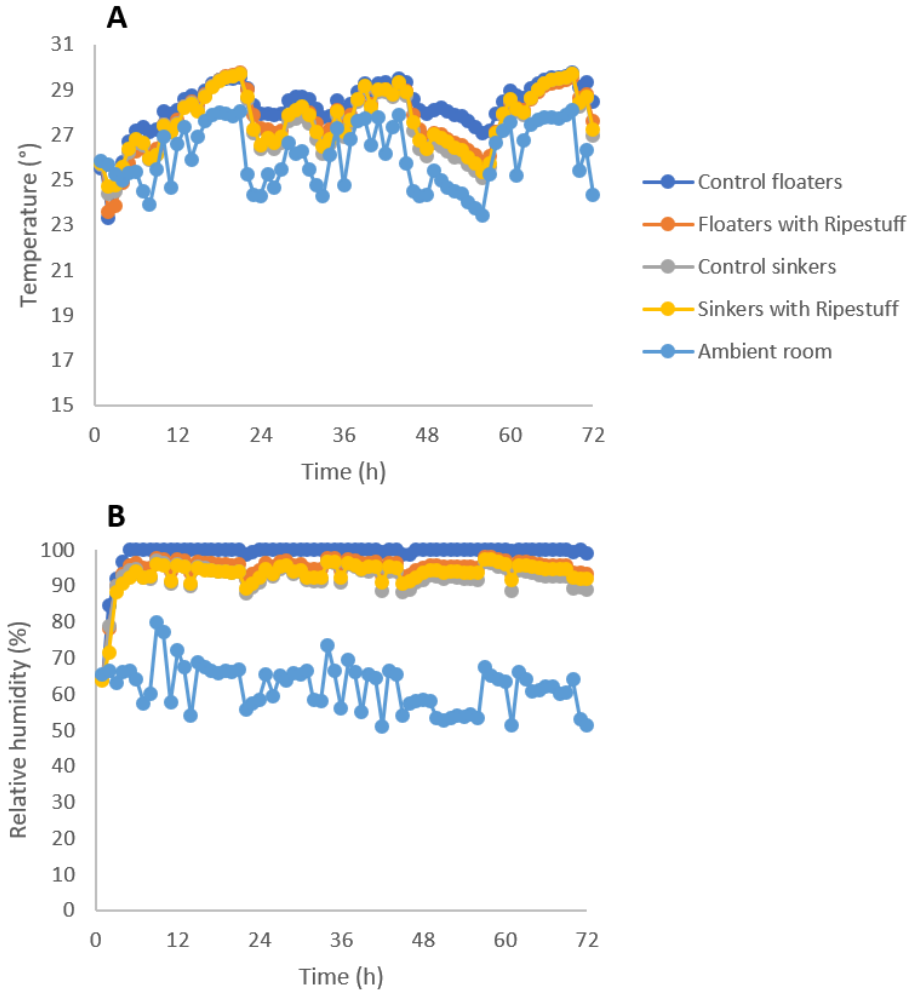
Appendix Figure 4. Temperature (A) and relative humidity (B) inside chambers with 'Carabao' mango treated with calcium carbide or Ripestuff™ with or without added water in the vessel.

14.1.5 Experiment 5- Release kinetics of ethylene from Ripestuff™ powder as influenced by the number of holes on the lid and air circulation



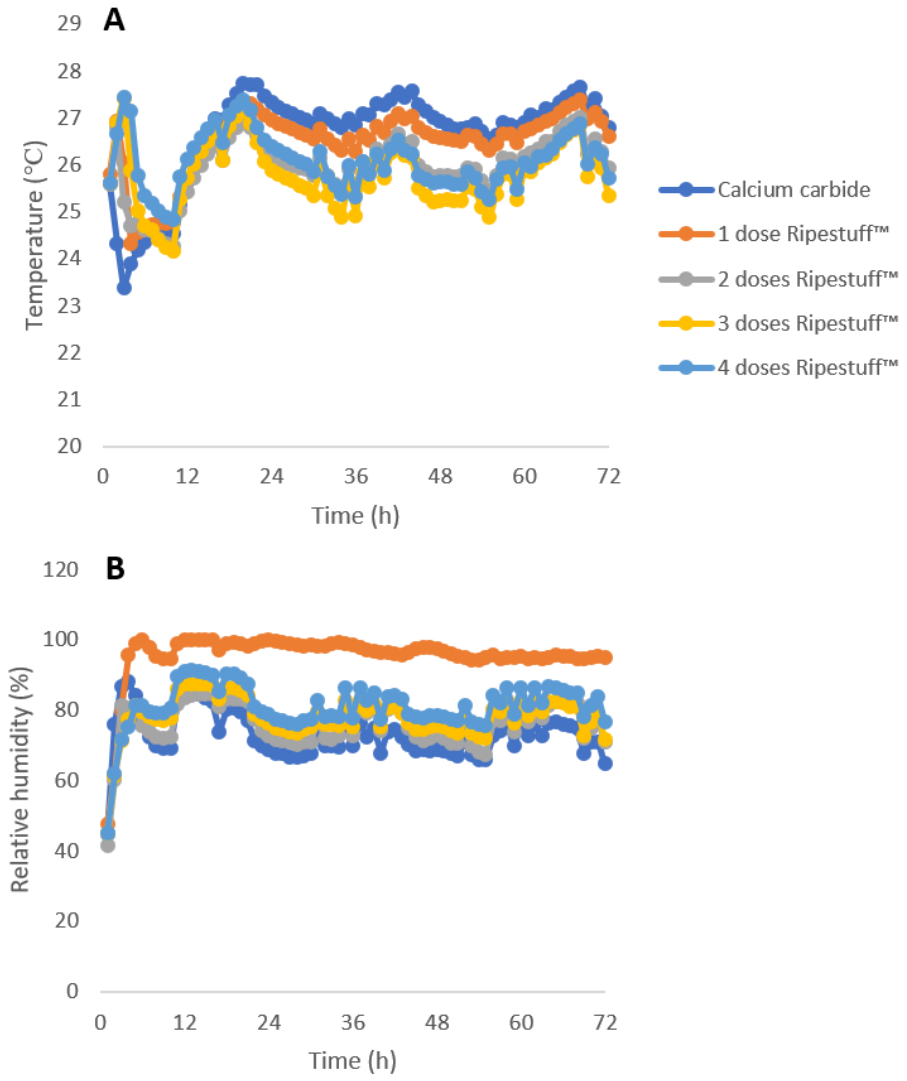
Appendix Figure 5. Temperature and relative humidity inside glass jars with or without fan containing Ripestuff™ in a vessel with different number of holes (0, 1, 2, 3, or 4) on the lid.

14.1.6 *Experiment 6- Effect of Ripestuff™ and harvest maturity on the ripening of 'Carabao' mango*



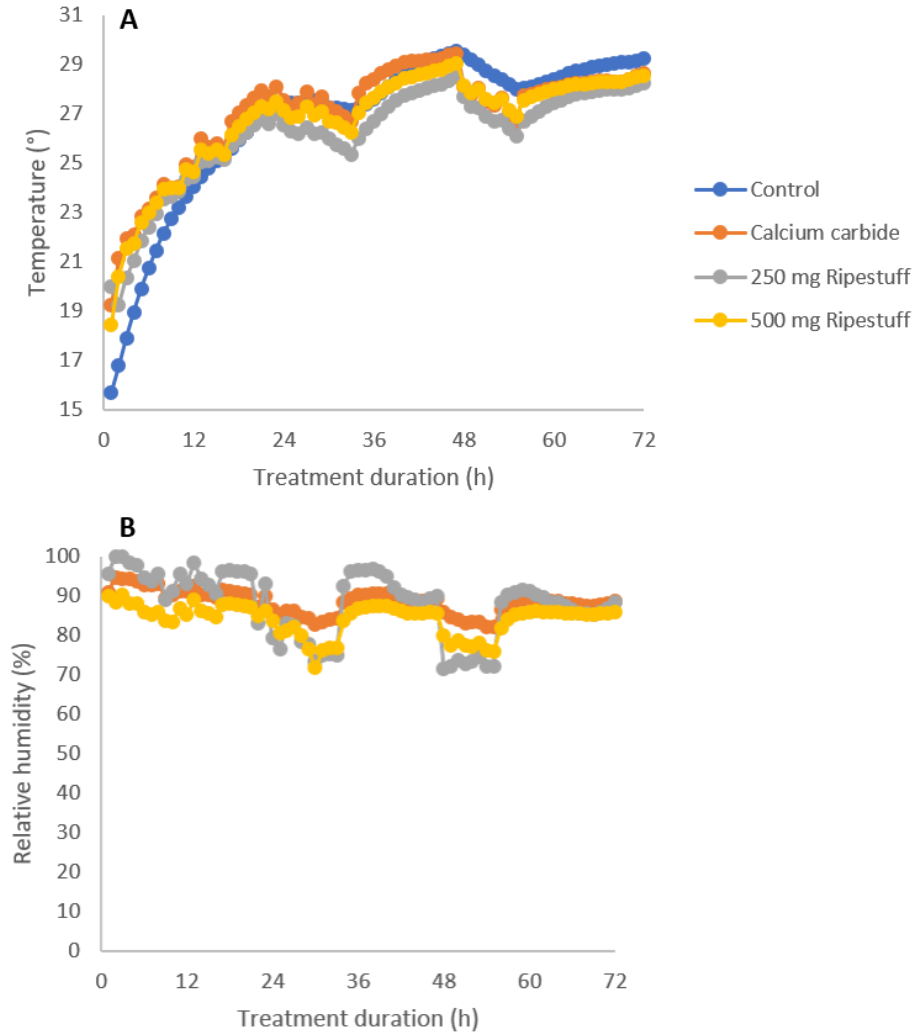
Appendix Figure 6. Temperature (A) and relative humidity (B) inside chambers during treatment of Ripestuff™ in 'Carabao' mango with different maturities (sinker or floater).

14.1.7 Experiment 7- Effect of different doses of Ripestuff™ on the ripening of 'Carabao' mango treated in bamboo baskets



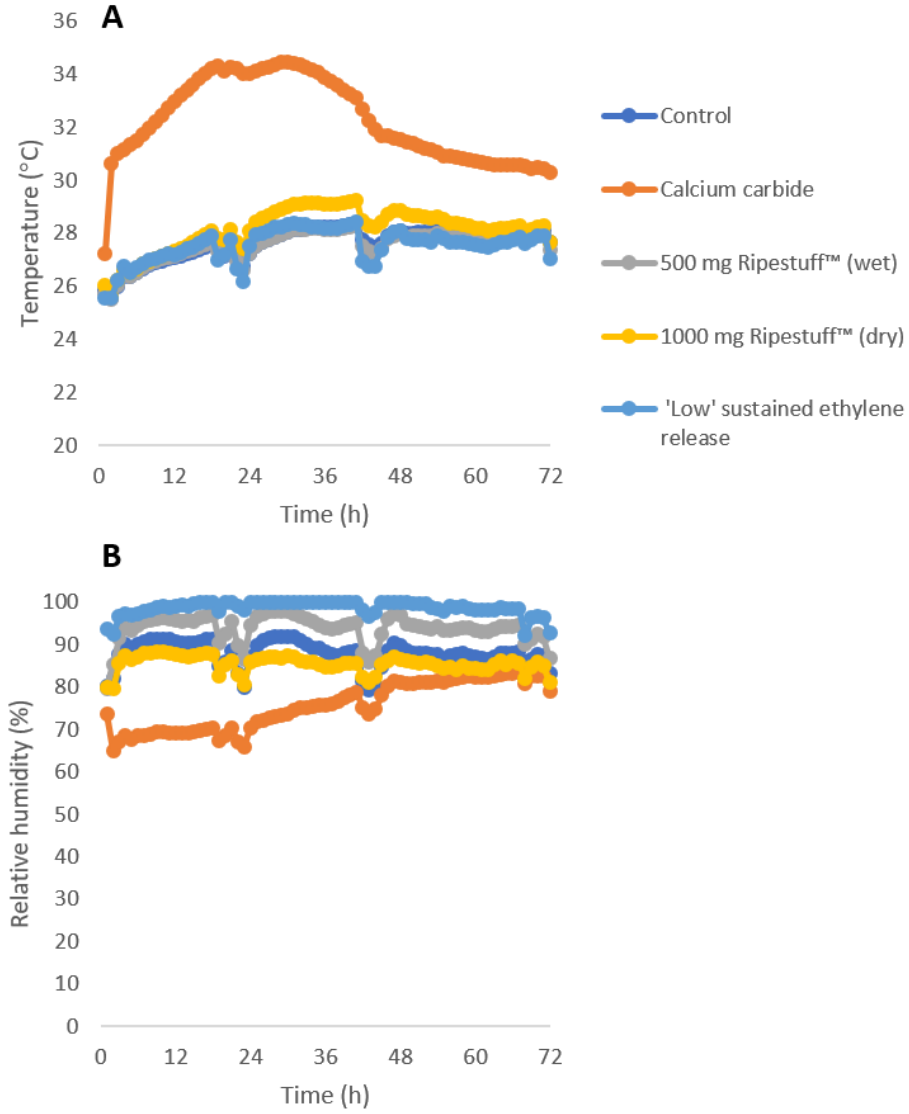
Appendix Figure 7. Temperature (A) and relative humidity (B) inside baskets containing 'Carabao' mango treated with calcium carbide or different doses of Ripestuff™ for 72 h.

14.1.8 Experiment 8- Effect of increased amount of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets



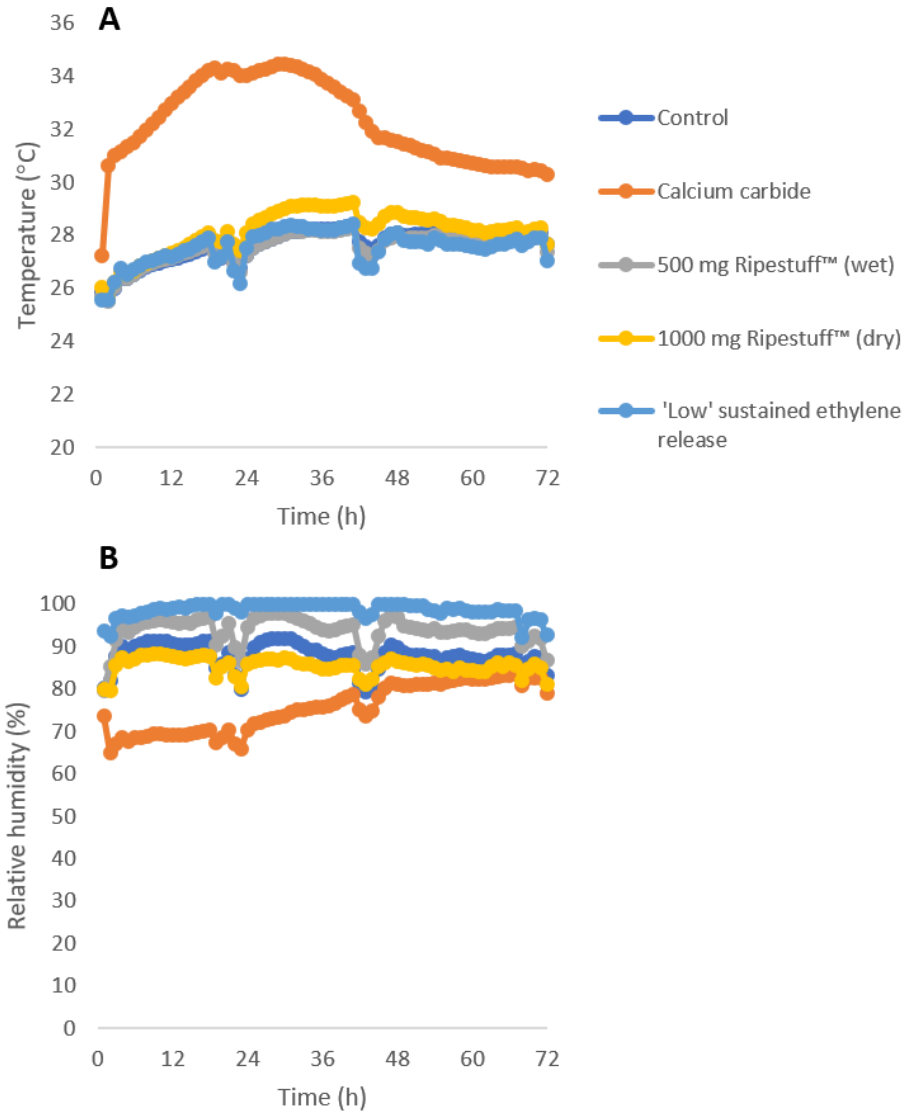
Appendix Figure 8. Temperature (A) and relative humidity (B) inside baskets with 'Carabao' mango treated with calcium carbide or Ripestuff™ (250 or 500 mg) for 72 h.

14.1.9 Experiment 9- Ethylene leakage from bamboo basket



Appendix Figure 9. Temperature (A) and relative humidity (B) inside baskets with 'Carabao' mango treated with 500 mg Ripestuff™ for 72 h inside bamboo basket.

14.1.10 *Experiment 10- Effect of sustained ethylene release from Ripestuff™ on the ripening of 'Carabao' mango*



Appendix Figure 10. Temperature (A) and relative humidity (B) inside baskets with 'Carabao' mango treated with calcium carbide or Ripestuff™ (500 mg (wet), 1000 mg (dry), or 'low' sustained ethylene release) for 72 h.

14.2 Quality indices

14.2.1 Mango peel color index

1	2	3	4	5	6
Mature green	Breaker; green with trace of yellow	Turning; more green than yellow	More yellow than green	Yellow with trace of green	Fully yellow

14.2.2 Mango flesh color index

1	2	3	4	5
White-yellow	Light yellow	Bright yellow	Yellow orange	Orange

14.2.3 Visual quality

1	2	3	4	5
Excellent, essentially no symptoms of deterioration	Good, minor symptoms of deterioration that are not objectionable	Fair, evident deterioration but not serious, limit of saleability	Poor, serious deterioration, limit of usability	Extremely poor, unusable

14.2.4 Degree of skin blotchiness

1	2	3	4	5
No blotch on the skin surface	Slight, 1-5% blotchy surface	Moderate, 6-10%	Moderately severe, 11-25%	Severe, >25%

14.2.5 Degree of stem-end rot

1	2	3	4	5
No visible spots	Slight infection, 1-5% of the surface	Moderate infection, 6-10%	Moderately severe infection, 11-25%	Severe infection, >25%

14.2.6 Degree of anthracnose

1	2	3	4	5
No visible spots	Slight infection, 1-5% of the surface	Moderate infection, 6-10%	Moderately severe infection, 11-25%	Severe infection, >25%

14.3 Activities over the course of the project

No.	Activity	Output/ milestones	Date	Person involved
1.	UP Min Experiment 1	Determined the effect of Ripestuff™ facilitated by increased RH and airflow on the ripening of 'Carabao' mango inside enclosed chambers	April 2018	UP Min Team
2.	UP Min Experiment 2 Trial 1	Determined the effect of different amounts of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets	May 2018	UP Min Team
3.	Introduction and planning of experiments (Skype meeting)	Preschedule drafted	31 May 2018	Sohail Mazhar Angelyn Lacap
4.	Discussion of preschedule (Phone discussion)	Comments on preschedule received	8 June 2018	Sohail Mazhar Angelyn Lacap
5.	Finalization of experimental plan (Skype meeting)	Updated experimental plan for UQ and UP Min	31 July 2018	Daryl Joyce Emma Ruth Bayogan Sohail Mazhar Angelyn Lacap
6.	Receipt of portable ethylene analyzer from Australia to the Philippines	Ethylene analyzer used for monitoring ethylene from Ripestuff™	01 August 2018	Jenny Ekman Angelyn Lacap
7.	UP Min Experiment 2 Trial 2	Determined the effect of different amounts of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets	August 2018	UP Min Team
8.	Melinda Perkins replaced Sohail Mazhar as Research Coordinator		08 October 2018	Sohail Mazhar Melinda Perkins
9.	UP Min Experiment 3	Determined the effect of different amounts of Ripestuff™ and the addition of water on the ripening of 'Carabao' mango inside bamboo baskets	November 2018	UP Min Team
10	Collaborative research in UQ Gatton	Conducted experiments on 1) Ripestuff™ release kinetics and 2) determination of ethylene concentration in old batches of Ripestuff™	10-14 December 2018	Angelyn Lacap Melinda Perkins
11	Research update (UQ Gatton)	Reviewed UP Min Experiments 1-3 and designed future experiments based	13 December 2018	Daryl Joyce Melinda Perkins Angelyn Lacap

No.	Activity	Output/ milestones	Date	Person involved
		on results from experiments conducted in UQ Brought Ripestuff™ powder with known amount of ethylene from Australia to the Philippines		
12	UP Min Experiment 4	Determined the effect of Ripestuff™ with added water in the vessel on the ripening of 'Carabao' mango inside enclosed chamber	January to February 2019	UP Min Team
13	UP Min Experiment 5	Determined the release kinetics of ethylene from Ripestuff™ powder as influenced by the number of holes on the lid and air circulation	March 2019	UP Min Team
14	Research update (Skype meeting)	Shared results of experiments in UQ (Experiments 1-4) and UP Min (Experiments 4-5), and discussed future Ripestuff™ experiments	13 March 2019	Daryl Joyce Emma Ruth Bayogan Melinda Perkins Angelyn Lacap
15	UP Min Experiment 6	Determined the effect of Ripestuff™ and harvest maturity on the ripening of 'Carabao' mango	March 2019	UP Min Team
16	UP Min Experiment 7	Determined the effect of different doses of Ripestuff™ on the ripening of 'Carabao' mango treated in bamboo baskets	April 2019	UP Min Team
17	Research update (Skype meeting)	Shared results of experiments in UQ (Experiments 5-6) and UP Min (Experiments 6-7)	07 May 2019	Daryl Joyce Emma Ruth Bayogan Melinda Perkins Angelyn Lacap
18	UP Min Experiment 8	Determined the effect of increased amount of Ripestuff™ on the ripening of 'Carabao' mango inside bamboo baskets	May 2019	UP Min Team
19	UP Min Experiment 9	Determined the ethylene leakage	June 2019	UP Min Team

No.	Activity	Output/ milestones	Date	Person involved
		from bamboo basket		
20	UP Min Experiment 10	Determined the effect of sustained ethylene release from Ripestuff™ on the ripening of 'Carabao' mango	June 2019	UP Min Team
21	Research update and wrap-up (Skype meeting)	Discussed results of experiments conducted in UP Min (Experiments 8-10)	2 July 2019	Daryl Joyce Melinda Perkins Angelyn Lacap
22	UP Min research report preparation	UP Min research report	8 July- 5 August 2019	Angelyn Lacap Emma Ruth Bayogan

14.4 Communication and dissemination activities

No.	Activity	Output/ milestones	Date	Person involved
1.	26th National Fruit Symposium	Presented a paper on "Bruise injury and its effects on 'Carabao' mango quality"	16-19 October 2018	Angelyn Lacap Emma Ruth Bayogan
2.	Visit of ACIAR delegates at the Postharvest research facility in UP Min	Shared information about postharvest studies conducted in UP Min including mango ripening by Ripestuff™	20 November 2018	Irene Kernot et al. Emma Ruth Bayogan Angelyn Lacap Leizel Secretaria Christine Diana Lubaton Marina Isabel Tac-an
3.	Tour of Philippine Science High School (PSHS) students at the Postharvest research facility in UP Min	Shared information about postharvest studies conducted in UP Min including mango ripening	5 December 2018	Angelyn Lacap Leizel Secretaria Marina Isabel Tac-an
4.	College of Science and Mathematics Research Colloquium (UP Min)	TED talk-style presentation on mango ripening	17 December 2018	Angelyn Lacap
5.	Tour of Central Mindanao University (CMU) faculty at the Postharvest research facility in UP Min	Shared information about postharvest studies conducted in UP Min including mango ripening	18 January 2019	Angelyn Lacap CMU Faculty members
6.	Tour of Senior High School Teachers (Molecular Biology Camp) at the Postharvest research facility in UP Min	Shared information about postharvest studies conducted in UP Min including mango ripening	14 May 2019	Angelyn Lacap Senior High School Teachers in Davao City
7.	Submitted abstract on the effect of Ripestuff™ on 'Carabao' mango for oral presentation at the Scientific Conference for the Federation of Plant Science Associations of the Philippines (FPSAP)	Abstract accepted for oral presentation	16-21 September 2019	Emma Ruth Bayogan Angelyn Lacap

20 November 2018 — Showing the results of Ripestuff™ experiment during the visit of ACIAR delegates led by the Research Program Manager for Horticulture, Irene Kernot, at the Postharvest research facility in UP Mindanao, Davao City.

10-14 December 2018— Sampling of ethylene and measuring it with a gas chromatograph during a collaborative research on release kinetics and maximum ethylene release in Ripestuff™ conducted in UQ Gatton with Dr. Melinda Perkins.

14 May 2019— Showing the Ripestuff™ experiment set up (left) and demonstrating how to measure respiration and ethylene production rates in 'Carabao' mango to selected Senior High School teachers in Davao City, Philippines (B).

14.5 Publications (August 2018- August 2019)

14.5.1 Published papers

Bayogan, E.V., Lacap, A.T., and Ekman, J.H. 2018. Sprouting of potato (*Solanum tuberosum* 'Granola') tubers as influenced by 1-methylcyclopropene. Acta Horticulturae. 1213:281-286.

Lacap, A.T., Bayogan, E.V., Secretaria, L.B., Lubaton, C.S., and Joyce, D.C. 2019. Responses of 'Carabao' mango to various ripening agents. Philippine Journal of Science. 148(3):513-523.

14.5.2 Papers under review

Lacap, A.T. and Bayogan, E.V. Responses of 'Carabao' mango to 1-methylcyclopropene. Tropical Agriculture. Under review.

Majomot, A.C., Secretaria, L.B., and Bayogan, E.V. Effect of hot water treatment and evaporative cooling on some postharvest characteristics of sweet pepper (*Capsicum annuum* cv. 'Sweet Cayenne'). Mindanao Journal of Science and Technology. Under review.

14.5.3 Newspaper features

Doquila, GA. "Study: Ethylene can be used to ripen mangoes". Sun Star Davao. Davao City, Philippines. 18 December 2018. <https://www.sunstar.com.ph/article/1779316>

Llemit, RL. "Safe ripening method for mangoes pushed". EDGE Davao. Davao City, Philippines. 18 December 2018. <http://edgedavao.net/latest-news/2018/12/18/safe-ripening-method-for-mangoes-pushed/>

14.6 Acknowledgment

The authors would like to thank ACIAR for funding this research, and acknowledge the assistance of Marina Isabel A. Tac-an, Leizel B. Secretaria, and Christine Diana S. Lubaton in setting up the experiments, collection of data, and reviewing this report.

Appendix C
CHARACTERISATION OF RIPESTUFF™ ETHYLENE RELEASE IN A 'MODEL' STATIC CHAMBER CONFIGURATION

UQ RESEARCH REPORT 1

prepared for
ACIAR Project HORT/2012/098

M.L. PERKINS AND D.C. JOYCE

SCHOOL OF AGRICULTURE AND FOOD SCIENCES
THE UNIVERSITY OF QUEENSLAND
GATTON QLD, AUSTRALIA

Summary

Ethylene release kinetics of Ripestuff™ powder during batch ripening of fruit in baskets is poorly understood. To assist knowledge gain in this area, a model static chamber system to test prototype Ripestuff™ delivery systems on a small scale was developed. Ethylene release from a delivery system comprising 10 mg Ripestuff™ in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid was investigated over a 72 h period. Chambers containing Ripestuff™ in aqueous solution exhibited a rapid increase in headspace ethylene concentration and had achieved complete ethylene release within 12 h at 23°C. Removal of the lid caused only a slight increase in the rate at which ethylene entered the chamber headspace, but resulted in a considerably faster increase in chamber relative humidity. These findings indicated that a lid with four holes substantially impedes moisture diffusion, but not ethylene diffusion. Almost no ethylene release was observed from Ripestuff™ in powder form, irrespective of whether the delivery system was lidded or unlidded. It was concluded that the prototype Ripestuff™ delivery system would be suitable for ripening fruit in baskets, provided sufficient moisture is present to trigger ethylene release. Furthermore, the static chamber configuration proved fit for purpose and was recommended for future laboratory-scale testing of prototype Ripestuff™ delivery systems.

Contents

1 Introduction

Ripestuff™ is a potential alternative to calcium carbide for batch-ripening of fruit in baskets. However, limited knowledge of Ripestuff™ ethylene release kinetics under such conditions is hindering development of a suitable delivery system. A simple static chamber configuration in which to test prototype delivery systems on a small scale would help fast-track our understanding of the factors governing ethylene release from Ripestuff™. Requirements of a static chamber configuration include: (1) construction from relatively inert materials, (2) ability to maintain a hermetically sealed environment, (3) easy access for repeat headspace sampling, and (4) a large enough capacity to contain the delivery system and provide sufficient headspace volume, but small enough that multiple chambers can be accommodated in the one experiment.

A static chamber configuration comprising a 2 L glass preserving jar with two sampling ports incorporated in the lid was devised. Previous laboratory experiments involving a similar (but more complex) configuration showed little ethylene release at ambient temperature and relative humidity (RH) from a delivery system comprising 10 mg Ripestuff™ in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid (Islam, unpublished data). Ethylene concentration in the chamber headspace reached a maximum of ~4 $\mu L.L^{-1}$ after 10 d. When RH inside the specimen container was increased to 94% by the inclusion of saturated potassium nitrate (KNO_3) aqueous solution, chamber headspace ethylene concentration rapidly increased within 24 h and reached a peak concentration of ~60 $\mu L.L^{-1}$ after 48 h. These findings are consistent with those of Ho et al. (2011), who found that Ripestuff™ at 25°C was very stable at 53% RH, but underwent rapid ethylene release at 94% RH.

The current investigation determined whether the revised static chamber configuration produced similar results to the original chamber configuration. Furthermore, the extent to which ethylene release is governed by the physical restriction to diffusion imposed by four holes in the specimen container lid was determined. Specimen containers either with no lid or a lid with four holes were used to hold 10 mg Ripestuff™ in either powder form or as an aqueous solution. Chamber headspace ethylene concentration and RH under each set of conditions was monitored at regular intervals for 72 h.

2 Methodology

2.1 Ripestuff™ powder

The experiment used a batch of Ripestuff™ powder prepared in 2017 at The University of Queensland (St Lucia, Australia) by encapsulation of ethylene into amorphous α-CD. Moisture content was 6.16% (wet-weight basis) and ethylene concentration was 0.554 $mol.mol^{-1}$ α-CD when assessed at the time of the study (November 2018). The powder was passed through a metal sieve (0.7 mm mesh size) to remove clumps that had formed during storage.

2.2 Ripestuff™ delivery system

Four delivery systems were employed in this experiment. In the first system, Ripestuff powder (10 mg) was weighed into 70 mL polypropylene specimen containers with yellow polyethylene screw caps (Techno Plas Pty Ltd, St Marys, Australia) and sealed with screw-top lids. These lids had previously been pierced with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm) to produce 4 evenly spaced holes of 0.5 mm ∅, 12.5 mm from the lid's centre. The second system was the same

as the first, but involved addition of 5 mL deionised water to the Ripestuff™ powder to produce a 0.02% (w/v) aqueous solution. Once prepared, the delivery system was immediately sealed inside a static chamber as described in the following section. The third and fourth delivery systems involved removal of the lid from the two previously described systems.

2.3 Chamber configuration

Static chambers were comprised of 2 L glass jars with screw-top metal lids incorporating a rubber seal (Ball Wide Mouth Half Gallon Glass Preserving Jars; Ball Mason Australia, South Kempsey, NSW, Australia). Headspace volume of the chambers was 1970 mL (measured gravimetrically by filling with deionised water at 25°C). Headspace sampling ports were prepared by making two holes in each lid using a 4 mm pin punch. The holes were positioned 20 mm from the lid's centre and directly opposite from each other. Clear multipurpose sealant (Sikaflex® Crystal Clear; Sika Australia Pty Ltd, Wetherill Park, NSW, Australia) was applied over the holes, on both sides of the lid, and smoothed to ~10 mm diameter using a finger dipped in an aqueous solution of 0.2% Tween 20. The resultant hermetic seal was allowed to cure for a minimum of 3 d. Lids were placed in a high humidity environment (desiccator with water in base) for 24 h prior to the experiment to ensure their rubber seals were supple. A thin film of petroleum jelly was applied to the rim of each jar to provide a good seal with the lid.

2.4 Treatments

The experiment was comprised of five treatments (Figure 1) which included: specimen jars with a lid and containing no Ripestuff™ (control), Ripestuff™ in powder form with and without a lid, and Ripestuff™ in 0.02% (w/v) aqueous solution with and without a lid.

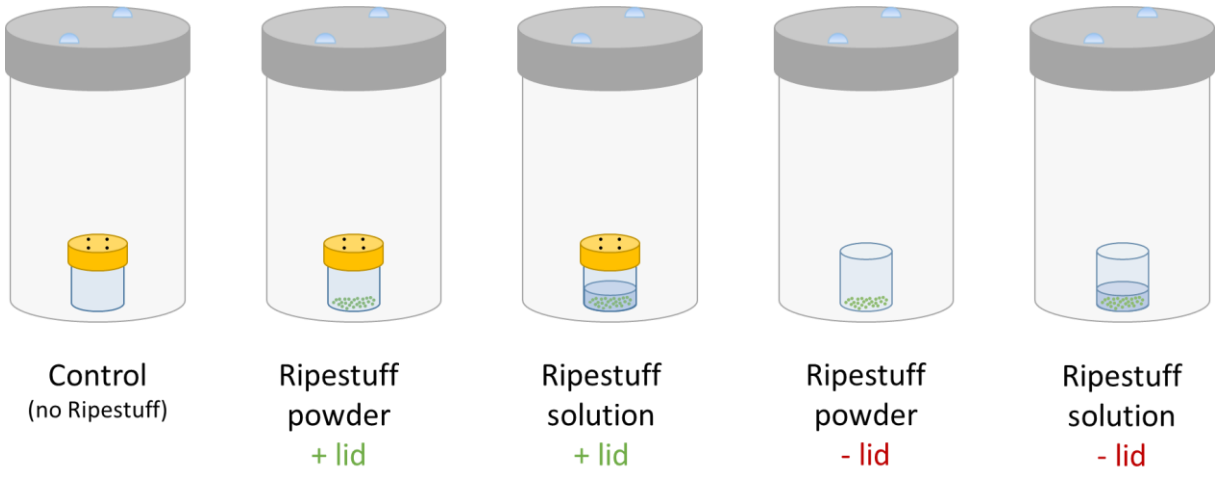


Figure 1. Treatments investigated for characterisation of Ripestuff™ ethylene release in a model static chamber configuration.

2.5 Data collection

2.5.1 Headspace ethylene concentration

A BD 5 mL disposable syringe with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm) was used to sample 2 mL of chamber headspace via one of the sampling ports at 1, 3, 6, 9, 12, 24, 48 and 72 h. During sampling, another syringe was used to introduce 2 mL of ethylene-free air into the chamber via the second sampling port to maintain pressure equilibrium. Ethylene-free air was

prepared by placing 80 g Purafil® media (Airepure® Australia, Mulgrave, Australia) in the base of a 2 L preserving jar fitted with a modified lid (as per the sample chamber lids) and allowing it to equilibrate at ambient conditions for 24 h prior to use.

Headspace samples were immediately injected into a 500 μL stainless steel sample loop[1] of a Shimadzu GC-2010 Plus gas chromatograph fitted with a SH-Rtx-BAC PLUS 1 capillary column (30 m x 0.32mm ID, 1.8 um film thickness; Shimadzu, Rydalmere, Australia) and a flame ionisation detector. Carrier gas was helium at a flow rate of 2.5 $mL.min^{-1}$. Injector and column temperatures were 120°C, and detector temperature was 260°C. Retention time of ethylene was ~1.8 min. Injection-to-injection time was ~3 min.

Ethylene quantification was based on external calibration with ethylene gas standards (BOC, Rocklea, Australia) using Lab Solutions Version 5.6 software (Shimadzu, Tokyo, Japan). Headspace ethylene concentration was multiplied by headspace volume to determine the total quantity (μmol) of ethylene in the headspace. This quantity was divided by the total amount of ethylene initially present in the Ripestuff™ and expressed as a percentage referred to as ethylene release (%). For treatments employing Ripestuff™ in aqueous solution, the calculation took into account the amount of ethylene remaining in the aqueous phase. For a system at equilibrium at 25°C, theoretical values based on Henry's Law estimate the concentration of dissolved ethylene to be 0.119 times that of the headspace concentration (Bassi et al. 1981).

2.5.2 Headspace temperature and relative humidity

Each chamber in Replicate 1 was fitted with an EasyLog data logger (EL-USB-2; Lascar Electronics, Wiltshire, UK) to record headspace temperature and RH at 15 min intervals for the duration of the experiment.

2.6 Experimental design and data analysis

A complete randomised design comprising three replicate chambers for each treatment was employed. Staggered commencement times were used, with a chamber being prepared every 3 min to accommodate the time required for subsequent GC analyses. Doing so ensured that the headspace of each chamber was sampled at consistent intervals and able to be immediately analysed.

Chamber headspace ethylene concentration and ethylene release (%) data were subjected to a two-factor (treatment x sampling time) analysis of variance using Minitab®, Version 17.3.1 (Minitab Pty Ltd, Sydney, Australia). Means were compared using Fisher's LSD test at a significance level of 0.01.

3 Results

Chambers containing Ripestuff™ in solution exhibited a rapid increase in headspace ethylene concentration (Figure 2) and had achieved complete ethylene release within 12 h (Figure 3). For these treatments, the presence of a lid with four holes had only a minor effect on slowing the rate of ethylene diffusion into the chamber. Significantly lower ($P < 0.01$) headspace ethylene

[1] Manufactured by Valco Instruments Co. Inc. (Houston, USA) and comprised of a 6 port valve with micro-electric actuator (product no. ED4C6UWE) with a 500 mL stainless steel sample loop (product no. SL500CUW). Sample loop was fitted with a Luer Lok valve adaptor (product no. REST-21283) supplied by Shimadzu (Rydalmere, Australia).

concentrations in lidded as opposed to unlidded Ripestuff™ solution treatments were observed at 1, 2 and 3 h, but not at later evaluation times.

Treatments involving Ripestuff™ in powder form released < 1% of their total ethylene after 72 h, irrespective of whether the specimen container was lidded or not (Figure 3). Chamber headspace ethylene concentration for these treatments did not differ from the control for the first 24 h. At 48 and 72 h, the concentrations for both the lidded and unlidded treatments were slightly higher ($P < 0.01$) than that of the control, but did not significantly differ from each other.

Chamber headspace temperature during the experiment was 23.4 ± 0.4°C (mean ± SD) for all treatments. Chamber RH varied between treatments (Figure 4). Ripestuff™ solution in an unlidded container produced >90% RH in the chamber headspace within 9 h. The presence of a lid with four holes impeded diffusion of water vapour to such an extent that chamber RH reached only ~70% after 72 h for this treatment. Chamber RH of the control and those treatments containing Ripestuff™ in powder form were similar and remained below 60% for the duration of the experiment.

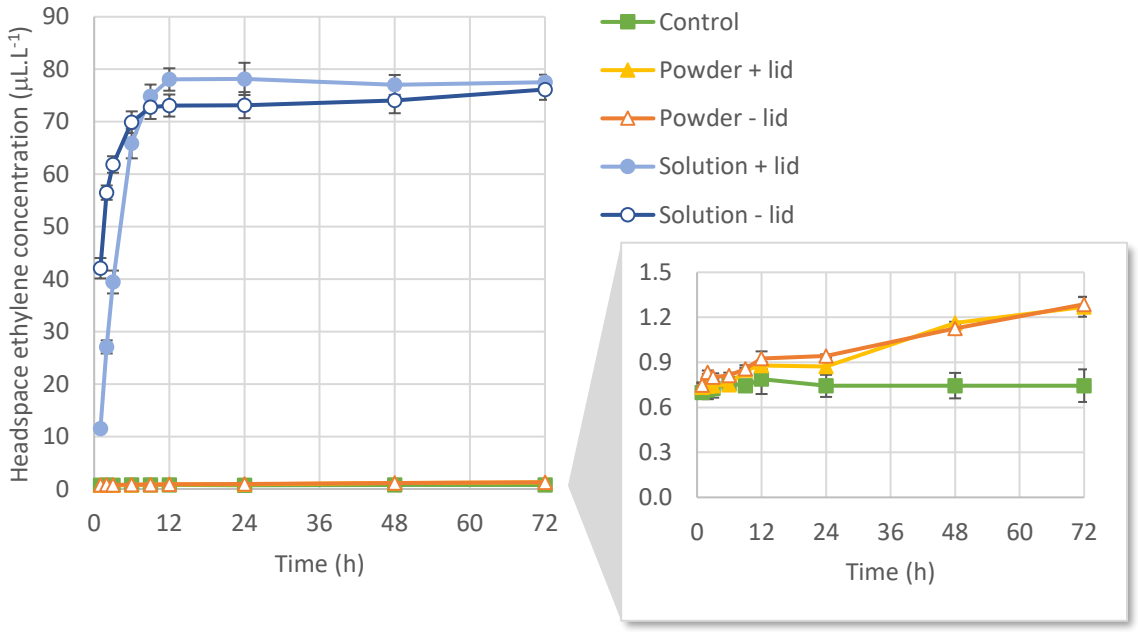


Figure 2. Headspace ethylene concentration in a 2 L chamber containing 10 mg Ripestuff™ either as a powder or 0.02% aqueous solution in 70 mL specimen containers with or without a lid pierced with four holes of 0.5 mm diameter. Chambers containing lidded specimen containers with no Ripestuff™ served as the control. Error bars represent standard error of the mean ($n = 3$).

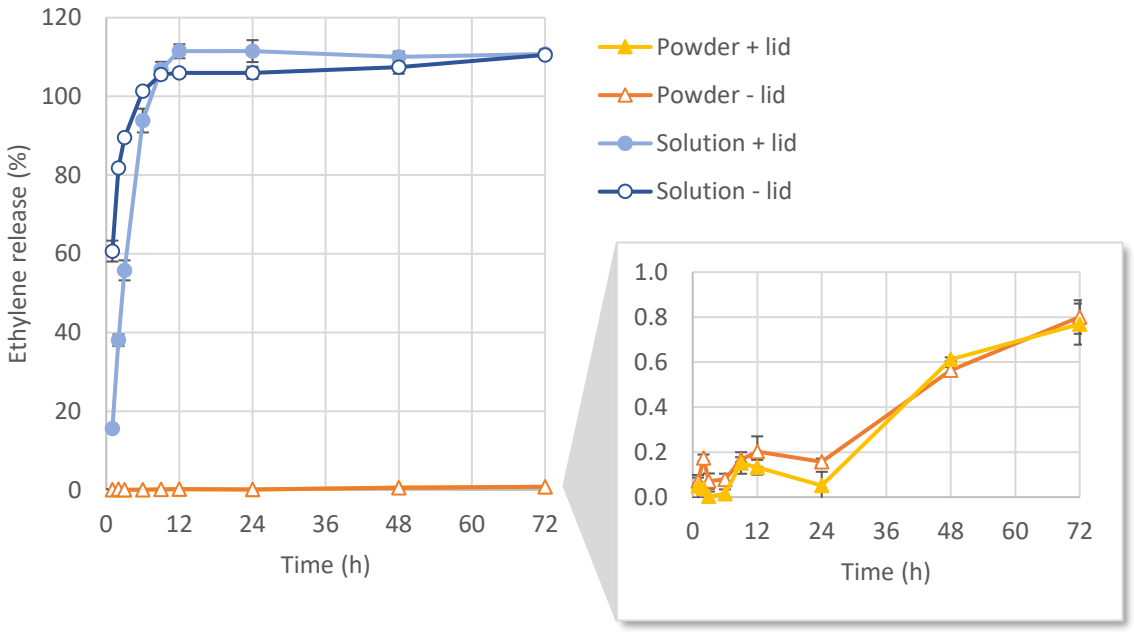


Figure 3. Ethylene release (expressed as a percentage of total ethylene in the system) in a 2 L chamber containing 10 mg Ripestuff™ either as a powder or 0.02% aqueous solution in specimen containers with or without a lid pierced with four holes of 0.5 mm diameter. Error bars represent standard error of the mean (*n* = 3).

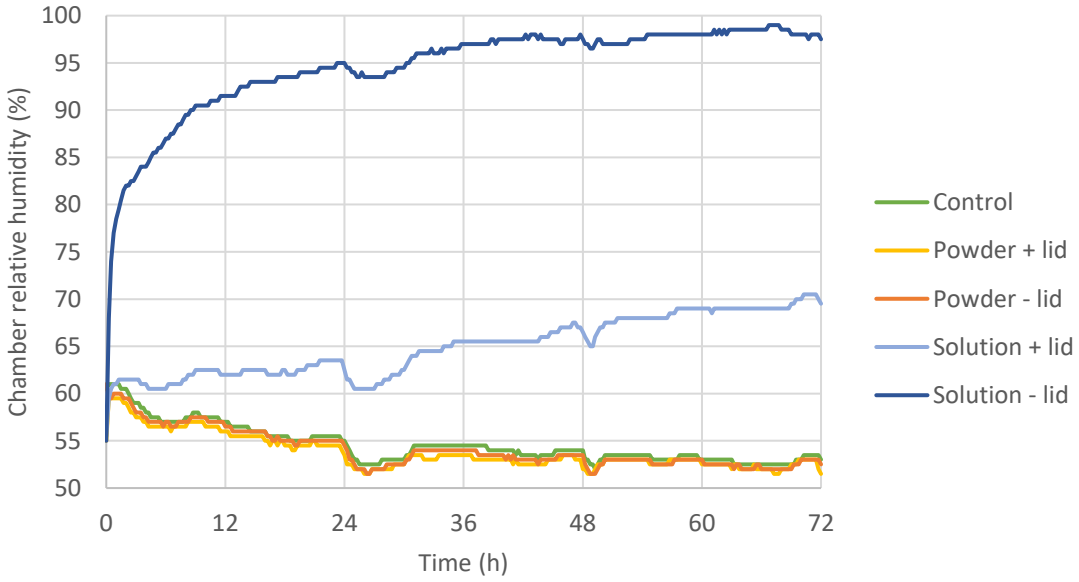


Figure 4. Relative humidity in a 2 L chamber containing 10 mg Ripestuff™ either as a powder or 0.02% aqueous solution in 70 mL specimen containers with or without a lid pierced with four holes of 0.5 mm diameter.

4 Discussion

The static chamber configuration proved suitable for testing prototype Ripestuff™ delivery systems on a small scale. A stable chamber headspace ethylene concentration was maintained once full ethylene release was achieved, which suggests no loss of ethylene, either via leakage from the chamber or ab/adsorption to chamber components. Results obtained using this new chamber configuration were similar to those obtained using its predecessor – almost no ethylene release was observed from Ripestuff™ unless water was included in the delivery system.

It is likely that under-estimation of the initial Ripestuff™ ethylene concentration was responsible for ethylene release values above 100% being reported. Diffusion of ethylene in water is very slow, as evidenced by a diffusion coefficient of 1.87×10^{-5} $cm^2.s^{-1}$ at 25°C (Cussler, 2009). The current protocol for assessing maximum ethylene release from Ripestuff™ involves dissolution of the powder in water, 30 min agitation at 35°C, 30 min equilibration at ambient temperature and subsequent sampling of the headspace. This process may not allow sufficient time for ethylene to reach equilibrium between the aqueous and gaseous phases in the sample vial. Hence, a revision of the protocol may be warranted.

The presence of a lid with four holes had little effect on ethylene diffusion from the headspace of the specimen container to the chamber, but it substantially impeded moisture diffusion. The diffusivity of ethylene in air is approximately half that of water vapour in air[2], which would suggest faster diffusion of water vapour than ethylene. Hence, another variable appeared to be influencing diffusion rate.

Fick's law states that rate of diffusion is directly proportional to the surface area and concentration gradient, and inversely proportional to the distance (Berk, 2018). In this case, both the surface area and distance are fixed by the number of holes in the lid, their cross-sectional area and the thickness of the lid. A small concentration gradient is therefore likely to be the factor responsible for the relatively low rate of moisture diffusion.

Chamber RH for the unlidded Ripestuff™ solution treatment reached 98%. It may be assumed that a lidded container of Ripestuff™ solution would also have a headspace RH of 98%, higher than the ~60% RH initially observed in the chamber. Conversion of these two RH values to absolute humidity shows that the water vapour concentration inside the lidded Ripestuff™ solution container (20.16 $g.m^{-3}$) would have been 1.6 times higher than that of the chamber (12.34 $g.m^{-3}$) at the beginning of the experiment.

In comparison, headspace ethylene concentrations of the chamber and the Ripestuff™ container from this treatment may have differed by two orders of magnitude or more. The baseline ethylene concentration of ~0.74 $\mu L.L^{-1}$ in the chamber headspace underwent a 100-fold increase to 76 $\mu L.L^{-1}$ after 72 h in the presence of aqueous Ripestuff™ solution. It is likely that headspace concentrations in the Ripestuff™ container were higher still (if only for a brief time) when the rate of ethylene release from the Ripestuff™ solution exceeded the rate of ethylene diffusion into the chamber.

This study showed that a 70 mL specimen container with four 0.5 mm ∅ holes in the lid provides minimal resistance to ethylene diffusion and may be considered a suitable delivery system for

[2] At normal temperature and pressure (NTP; 20°C, 1 atm), the diffusion coefficient of ethylene in air is 0.137 $cm^2.s^{-1}$ (Pritchard and Currie, 1982), as compared with 0.242 $cm^2.s^{-1}$ for water in air (Monteith and Unsworth, 2013).

Ripestuff™, provided sufficient moisture is present to trigger ethylene release. The new static chamber configuration proved fit for purpose and should be adopted for future laboratory-scale testing of prototype Ripestuff™ delivery systems.

5 References

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Appendix D

RELATIVE HUMIDITY EFFECTS ON RIPESTUFF™ ETHYLENE RELEASE FROM A PROTOTYPE DELIVERY SYSTEM

UQ RESEARCH REPORT 2

prepared for
ACIAR Project HORT/2012/098

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Summary

Variable success in ripening of mangoes has previously been achieved using a prototype Ripestuff™ delivery system incorporated into newspaper lined baskets of fruit. Exposure to moisture is known to trigger ethylene release from Ripestuff™ powder, but little is known regarding moisture diffusion between the basket headspace and the delivery system. This study emulated the high humidity environment created by respiring fruit and determined its effect on relative humidity inside a prototype Ripestuff™ delivery system (i.e. 10 mg Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid) and the subsequent rate of ethylene release at ambient temperature. Moisture diffusion into the delivery system under these conditions was sufficient to achieve full ethylene release within 72 h when tested in 'model' 2 L static chambers. This finding suggested that the delivery system should trigger uniform fruit ripening in a 'real world' basket configuration. However, as this has not been the case in the past, a close examination of ethylene headspace concentrations occurring in baskets during fruit ripening was recommended.

Contents

1 Introduction

It has been established that ethylene release from Ripestuff™ is triggered by exposure to moisture (Ho et al., 2011; Islam, unpublished data; Perkins and Joyce, 2019). Fruit respire during postharvest ripening, thereby increasing the relative humidity (RH) of the surrounding air. Early experiments conducted at UPMin showed that headspace RH in an enclosed newspaper-lined basket of 'Carabao' mango fruit exceeded 90% RH within 4 h at ambient temperature (Lacap and Bayogan, 2019; Experiments 2.1 and 2.2).

There is a need to determine whether such a high humidity environment provides sufficient moisture to trigger ethylene release from the prototype Ripestuff™ delivery system (i.e. a 70 mL specimen container with four 0.5 mm ∅ holes in the lid). The evidence to date is contradictory, with UPMin experiments showing variable success in ripening of mangoes when the delivery system was incorporated into newspaper lined baskets of the fruit for 72 h.

The aim of the current study was to emulate the high humidity environment created by respiring fruit and determine its effect on: (1) relative humidity inside the Ripestuff™ delivery system, and (2) the subsequent rate of ethylene release. Two experiments were conducted in which water or saturated salt solutions were used to manipulate RH of the static chamber configuration devised in UQ Research Report 1 (Perkins and Joyce, 2019). RH of Ripestuff™ delivery systems within these chambers was monitored by placing a small logger (about the size of a watch battery) inside the specimen container.

The first experiment involved repeat headspace sampling from the same chambers over a 72 h period. This approach allowed headspace ethylene concentration inside the chamber to be monitored, but prohibited regular measurement of ethylene concentration inside the Ripestuff™ delivery system. An understanding of ethylene movement from Ripestuff™ powder to the specimen container headspace, and from there to the chamber headspace would aid design of better delivery systems. Hence, the second experiment employed a 'destructive' headspace sampling approach whereby each chamber was sampled once only and then immediately opened to allow the Ripestuff™ delivery system to be sampled at the same time.

2 Methodology

2.1 Ripestuff powder

Both experiments used a batch of Ripestuff™ powder prepared in 2017 at The University of Queensland (St Lucia, Australia) by encapsulation of ethylene into amorphous α-CD. Moisture content was 6.16% (wet-weight basis) and ethylene concentration was 0.539 mol.mol^{-1} α-CD when assessed at the time of the second experiment (March 2019). The powder was passed through a metal sieve (0.7 mm mesh size) to remove clumps that had formed during storage.

2.2 Ripestuff delivery system

Both experiments employed the first delivery system described in UQ Research Report 1 (Perkins and Joyce, 2019), which was 10 mg Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid. In the first experiment, the inner wall of all specimen containers was also lined with a quarter sheet of Whatman™ chromatography paper (11 x 3.5 cm, grade 3MM CHR)

either with or without the addition of 1.25 mL deionised water (respectively referred to as ‘wet’ and ‘dry’ paper).

2.3 Chamber configuration

Both experiments employed the 2 L static chamber configuration described in UQ Research Report 1 (Perkins and Joyce, 2019), with slight modification. In the first experiment (Figure 1), a single full sheet of Whatman™ chromatography paper (11 x 14 cm, grade 3MM CHR) either with or without the addition of 5 mL deionised water (respectively referred to as ‘wet’ and ‘dry’ paper) was used to line the inner wall of the chamber. All chambers in the first experiment also contained an unlidded 15 mL Falcon tube affixed to the interior wall. These tubes were either empty or contained 5 mL deionised water. In the second experiment, chambers contained 10 mL of either saturated NaCl or KNO_3 solution in an unlidded 70 mL specimen container to maintain 76 or 94% RH in the chamber headspace, respectively.

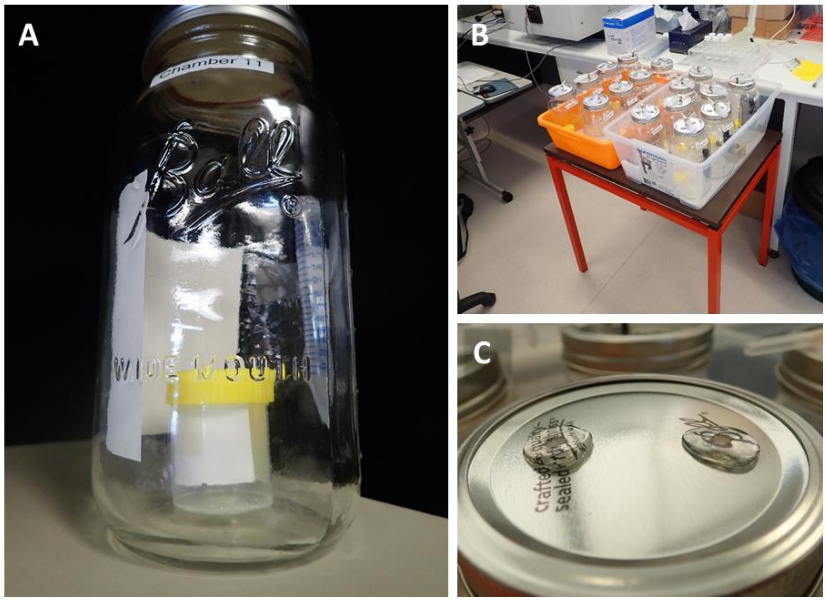

Figure 1. Photographs depicting (A) the chamber configuration used in Experiment 1, (B) the environment in which the chambers were held during each experiment and (C) a chamber lid with two sampling ports, each comprising a 4 mm ∅ hole filled with clear silicone sealant.

2.4 Treatments

The first experiment was comprised of six treatments (Figure 2) in which the headspace of the Ripestuff™ container (i.e. source), the chamber (i.e. sink) or neither/both were humidified by inclusion of wet chromatography paper as described above. As an additional treatment, humidification of the sink was achieved by inclusion of 5 mL deionised water contained in a 15 mL Falcon tube, which had an internal diameter of 14 mm. This treatment provided an available surface area for moisture diffusion that was 100 times less (1.54 cm^2) than that provided by a full sheet of wet chromatography paper (154 cm^2). A treatment without Ripestuff™ (and containing dry paper in both the source and sink) was used as a control. The second experiment was comprised of four treatments (Figure 3) in which no Ripestuff™ (controls) or 10 mg Ripestuff™ was placed in a chamber (i.e. sink) maintained at either 76 or 94% RH by inclusion of saturated salt solutions as described previously.

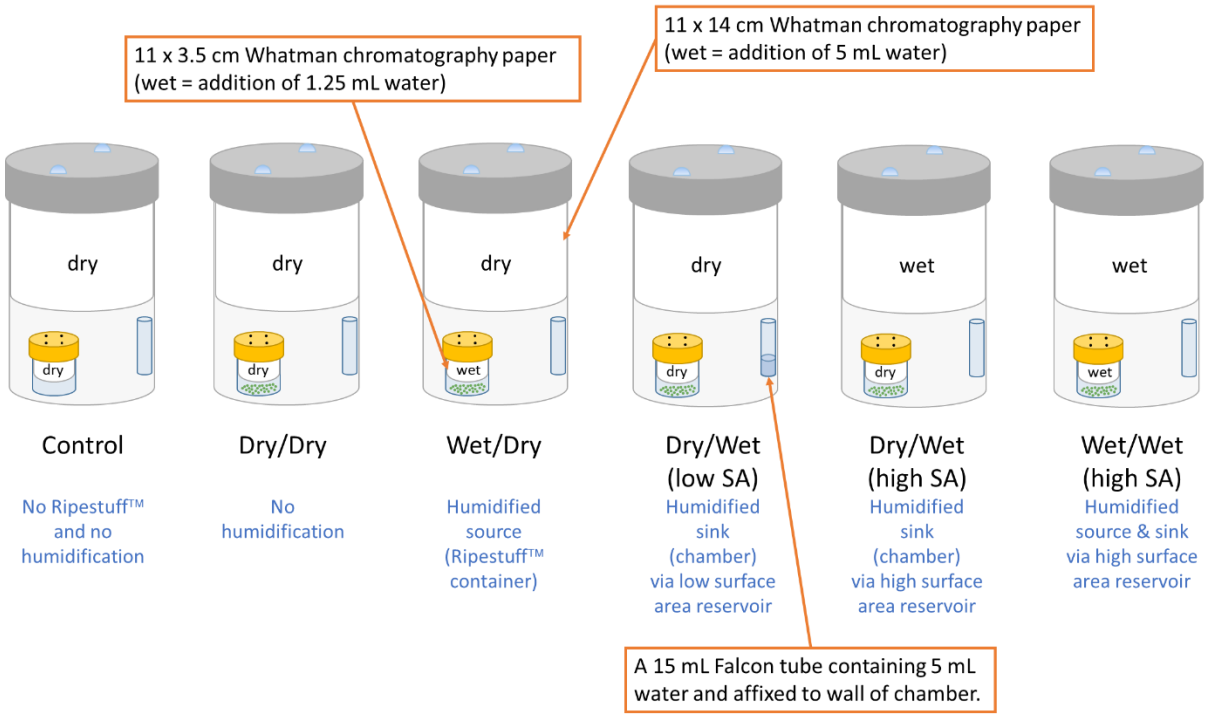

Figure 2. Treatments investigated in the first experiment to determine humidity effects on ethylene release from a protoype delivery system comprising 10 mg Ripestuff™ in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid. Variables included ± humidification of the Ripestuff™ container (i.e. source) in combination with ± humidification of the chamber (i.e. sink).

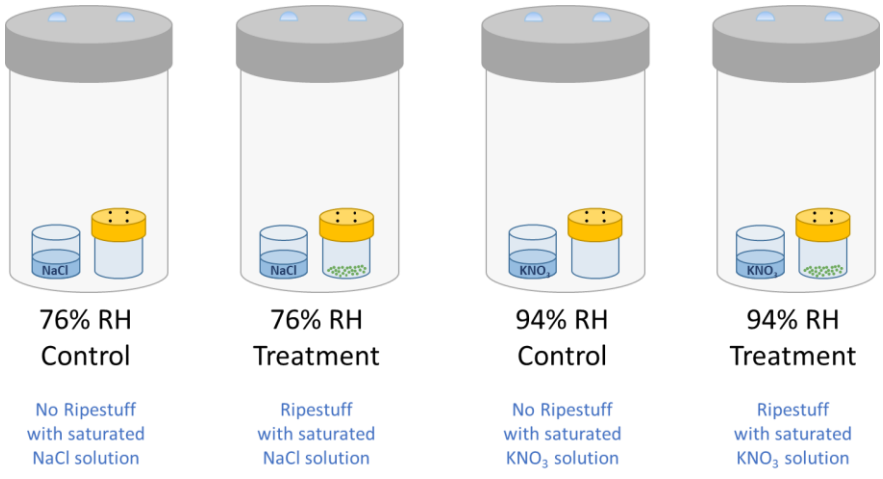

Figure 3. Treatments investigated in the second experiment to determine humidity effects on ethylene release from a protoype delivery system comprising 10 mg Ripestuff in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid. Variables included humidification of the chamber (i.e. sink) to 76 or 94% RH using saturated NaCl or KNO_3 solution, respectively.

2.5 Data collection

2.5.1 Headspace ethylene concentration

Sampling and analysis of headspace ethylene concentration was conducted using the procedure described in UQ Research Report 1 (Perkins and Joyce, 2019). In the first experiment, repeat sampling from each chamber was conducted at 1, 2, 3, 6, 9, 12 and 24 h, and subsequently at 24 h intervals until 240 h (10 d). For Experiment 2, each chamber was sampled once only at 3, 6, 9, 12, 24, 48 or 72 h.

At the end of Experiment 1 (i.e. 240 h), a single headspace sample of 2 mL was immediately taken from each Ripestuff™ container (via one of the four holes in the lid) to determine whether ethylene partitioning between the headspace inside and outside the container had reached equilibrium. For Experiment 2, sampling of Ripestuff™ container headspace was conducted at each evaluation time.

2.5.2 Headspace temperature and relative humidity

In Experiment 1, each chamber in Replicate 1 (excluding the control) was fitted with an EasyLog data logger (EL-USB-2; Lascar Electronics, Wiltshire, UK) affixed to the inner wall with self-adhesive Velcro (Figure 4a). Ripestuff™ containers in these same chambers were fitted with a Hygrochron HC data logger (DS1923; Thermochron Australia, Castle Hill, Australia) affixed to the inner surface of the lid with self-adhesive Velcro (Figure 4b). In Experiment 2, two chambers from each of the 76% RH and 94% RH treatments (excluding controls) were fitted with an EasyLog data logger loosely placed in the chamber base. Ripestuff™ containers in these same chambers were fitted with a Hygrochron HC data logger (DS1923; Thermochron Australia, Castle Hill, Australia) placed sensor-side up in the base of the container. All loggers were configured to record headspace temperature and relative humidity at 15 min intervals for the duration of the experiments.

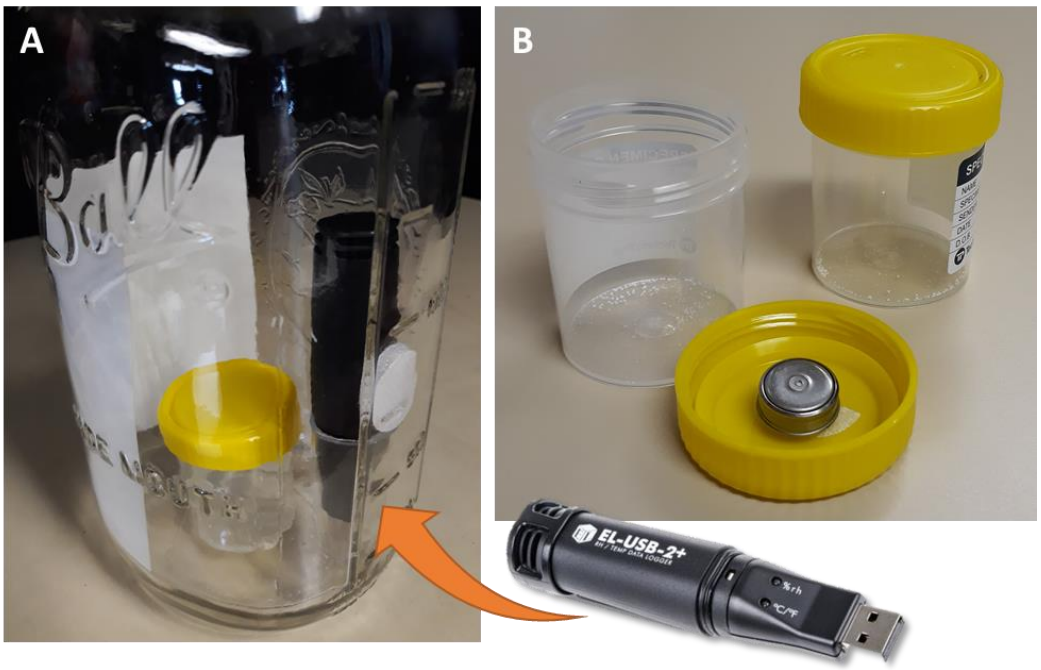

Figure 4. Headspace temperature and relative humidity were monitored using (A) EasyLog USB loggers and (B) Hygrochron HC data loggers incorporated into chambers and Ripestuff™ containers, respectively. Photographs show the loggers affixed with Velcro, as used in Experiment 1.

2.6 Experimental design and analysis

A complete randomised design comprising three replicate chambers for each treatment was employed. Staggered commencement times were used, with a chamber being prepared every 3 min (Experiment 1) or 6 min (Experiment 2) to accommodate the time required for subsequent GC analyses. Doing so ensured that the headspace of each chamber (and Ripestuff™ container in Experiment 2) was sampled at consistent intervals and able to be immediately analysed.

Headspace ethylene concentration data from Experiment 1 were subjected to a two-factor (treatment x sampling time) analysis of variance using Minitab®, Version 17.3.1 (Minitab Pty Ltd, Sydney, Australia). For Experiment 2, a two-factor (treatment x vessel) analysis of variance was conducted at each sampling time. Means were compared using Fisher's LSD test at a significance level of 0.01. Data obtained for 76% RH and 94% RH control treatments in Experiment 2 did not significantly differ ($P > 0.01$) and were therefore grouped to provide a single control dataset for each vessel (chamber or Ripestuff™ container) at each evaluation time.

Ratios of source to sink headspace ethylene concentration were subjected to a one-way analysis of variance for treatment effects in Experiment 1 and a two-way (treatment x sampling time) analysis of variance in Experiment 2. For both experiments, means were compared using Fisher's LSD test at a significance level of 0.05.

3 Results

3.1 Experiment 1

Humidification of the Ripestuff™ container (source) caused a rapid increase in chamber headspace ethylene concentration to ~70 $\mu L.L^{-1}$ within the first 9 h, irrespective of whether the chamber (sink) was also humidified (Figure 5). This concentration corresponded to near complete release of ethylene from the Ripestuff™ powder (Figure 6). Conversely, almost no ethylene release was observed in the dry/dry treatment for which there was no source or sink humidification. After 10 d (240 h) the sink headspace ethylene concentration for this treatment was 2.4 $\mu L.L^{-1}$ (Figure 5), which was slightly but significantly ($P < 0.01$) higher than the 1.8 $\mu L.L^{-1}$ exhibited by the control treatment.

Humidification of the sink only (i.e. dry/wet treatments) also resulted in very low sink headspace ethylene concentrations (Figure 5) which accounted for < 4% ethylene release after 10 d (Figure 6). However, by 5 d (120 h) the high SA dry/wet treatment had begun to exhibit significantly higher ($P < 0.01$) concentrations than the low SA dry/wet treatment.

A comparison of source and sink headspace ethylene concentrations at 240 h revealed that equilibrium had been reached (i.e. source to sink ratio was ~1) in both treatments involving humidification of the source (Table 1). A similar ratio was observed in the dry/dry treatment. Humidification of the sink produced higher ratios, more so for the high than the low SA treatment.

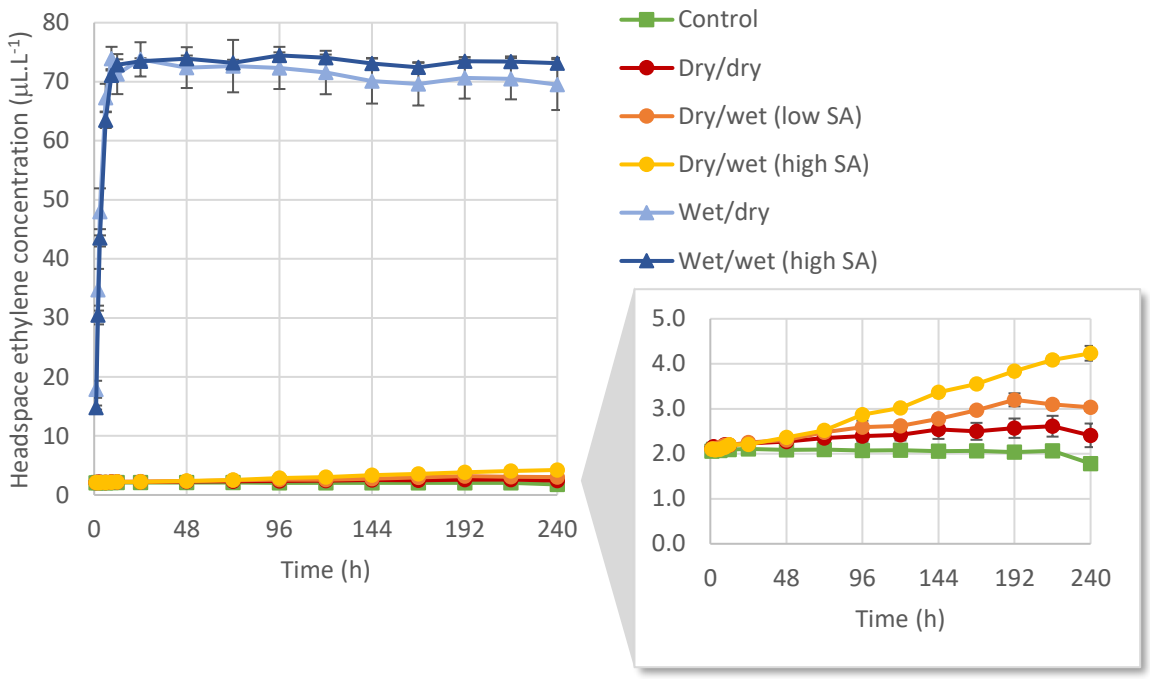

Figure 5. Headspace ethylene concentration in a 2 L chamber (sink) containing 10 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Treatments were humidification (wet/--) or no humidification (dry/--) of the source in combination with humidification (--/wet) or no humidification (--/dry) of the sink. Humidification of the sink was achieved by inclusion of 5 mL deionised water with a surface area of 1.54 (low SA) or 154 cm^2 (high SA). Chambers with no Ripestuff™ and no sink or source humidification served as the control. Error bars represent standard error of the mean (n = 3).

Table 1. Ethylene distribution at 240 h between sink (chamber) and source (Ripestuff™ container) headspace for treatments with (wet/--) or without (dry/--) humidification of the source in combination with humidification (--/wet) or no humidification (--/dry) of the sink. Humidification of the sink was achieved by inclusion of 5 mL deionised water with a surface area of 1.54 (low SA) or 154 cm^2 (high SA). A system in equilibrium is indicated by a source to sink ratio of 1. Means followed by the same letter do not significantly differ according to Fisher's LSD test ($P > 0.05$).

Treatment	Headspace ethylene concentration (µL.L^{-1})		Source to sink ratio
	Sink	Source	
Dry/dry	2.4	2.7	1.13 bc
Dry/wet (low SA)	3.0	3.6	1.20 b
Dry/wet (high SA)	4.2	5.7	1.33 a
Wet/dry	69.5	72.1	1.04 cd
Wet/wet	73.2	72.2	0.99 d

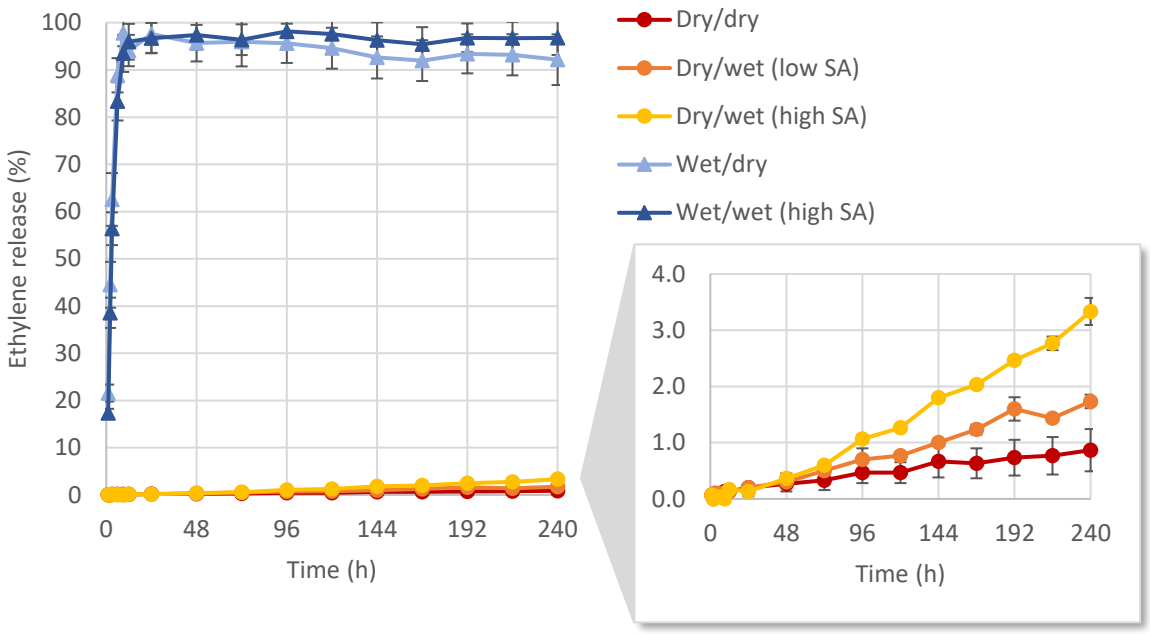

Figure 6. Ethylene release (expressed as a percentage of total ethylene in the system) in a 2 L chamber (sink) containing 10 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Treatments were humidification (wet/--) or no humidification (dry/--) of the source in combination with humidification (--/wet) or no humidification (--/dry) of the sink. Humidification of the sink was achieved by inclusion of 5 mL deionised water with a surface area of 1.54 (low SA) or 154 cm^2 (high SA). Error bars represent standard error of the mean (n = 3).

Sink headspace temperature during the experiment was 23.2 ± 0.4°C (mean ± SD) for all treatments. Figure 7b shows that sink RH remained below 60% when there was no humidification of source or sink, and that it gradually increased to 72% after 10 d (240 h) when source humidification was used. Sink RH increased to > 90% within 2 h when humidified with wet chromatography paper (i.e. high SA), but increased at a much slower rate to a maximum of 86% RH after 240 d when humidified with water in a 15 mL Falcon tube (i.e. low SA).

RH of the source rapidly increased to > 90% within 15 min when source humidification was used (Figure 7a). In the absence of source humidification, 90% RH in the source was achieved only when the sink was humidified with wet chromatography paper (i.e. high SA treatment) and only after 9 d. Source RH increased even more slowly in the dry/wet (low SA) treatment, reaching a maximum of 77% RH after 10 d (240 h).

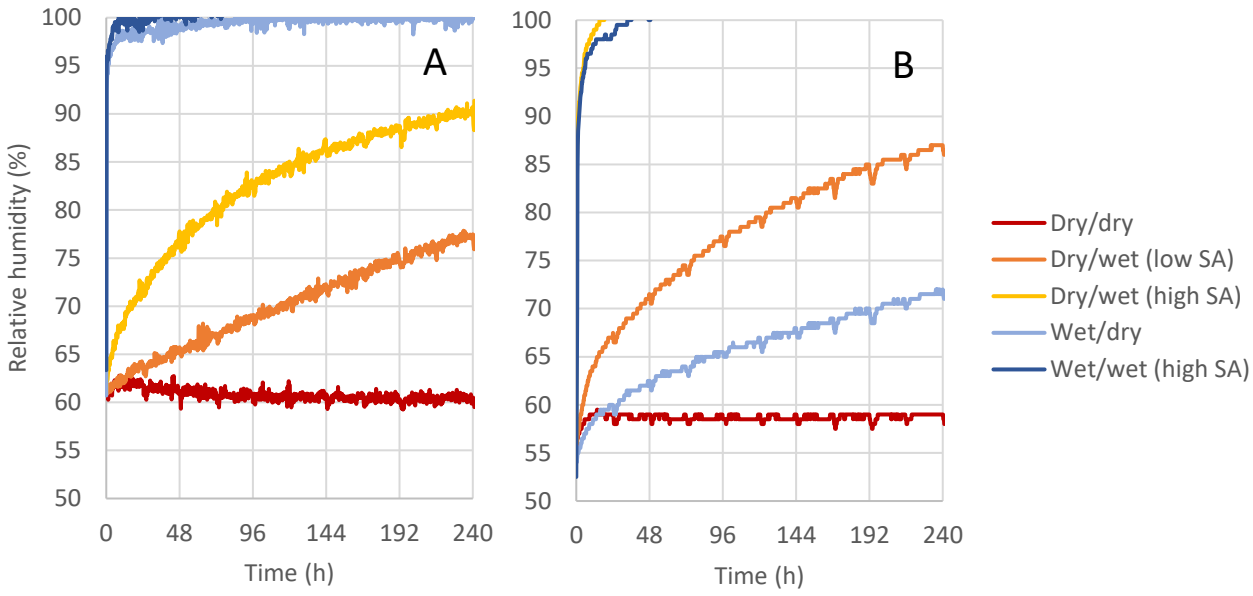

Figure 7. Headspace relative humidity in (A) a 70 mL specimen container with four holes in the lid and containing 10 mg Ripestuff (source), and (B) the 2 L chamber (sink) within which the source is contained. Treatments were humidification (wet/--) or no humidification (dry/--) of the source in combination with humidification (--/wet) or no humidification (--/dry) of the sink. Humidification of the sink was achieved by inclusion of 5 mL deionised water with a surface area of 1.54 (low SA) or 154 cm^2 (high SA).

3.2 Experiment 2

No ethylene release occurred within the first 3 h of the experiment (Figure 8), as indicated by a lack of significant difference ($P > 0.01$) in headspace ethylene concentration observed at this time between the sink and source of any treatment, or between treatments. By 6 h, the concentration in the 94% RH source was significantly higher ($P < 0.01$) than concentrations found in the source of the control or 76% RH treatment. Headspace ethylene concentration in the 94% RH source increased rapidly thereafter until 24 h, then gradually declined to 98 $\mu L.L^{-1}$ by 72 h. A corresponding concentration increase in the 94% RH sink commenced at 12 h and continued throughout the experiment to reach a maximum of 67 $\mu L.L^{-1}$ at 72 h.

Ethylene release occurred slowly and to a limited extent in the 76% RH treatment (Figure 8). Source headspace ethylene concentration for this treatment did not differ from that of the control until 12 h, and increased only slightly thereafter to a maximum of 11 $\mu L.L^{-1}$. The increase was insufficient to produce a corresponding increase in sink concentration – no difference in sink headspace ethylene concentration between the control and 76% RH treatments was observed at any time.

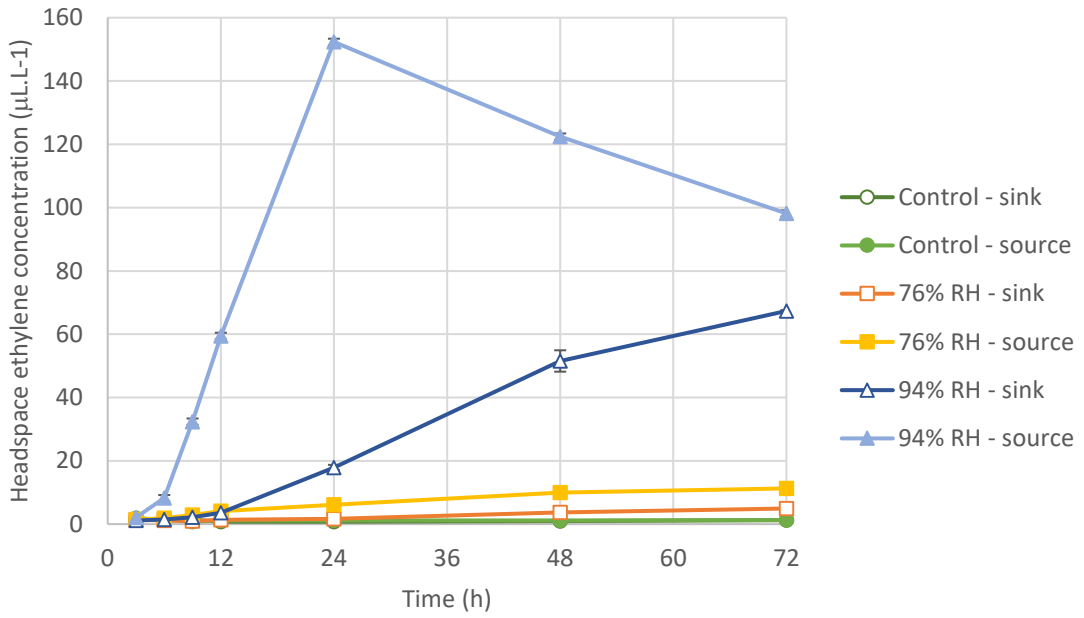

Figure 8. Sink and source headspace ethylene concentration for a 2 L chamber (sink) containing 10 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Sink headspace was maintained at either 76% or 94% RH by inclusion of 10 mL saturated solution of NaCl or KNO_3, respectively. Chambers containing no Ripestuff™ powder and maintained at either 76% or 94% RH served as the control. (Data from both RH levels were grouped to provide a single control treatment). Error bars represent standard error of the mean (n = 3).

Ethylene release from Ripestuff™ powder in the 94% RH treatment was faster than the rate of ethylene diffusion through the four holes in the lid, as evidenced by the high ratios of source to sink headspace ethylene concentration recorded for this treatment (Figure 9). The highest ratio occurred at 12 h, when source concentration was 16 times higher than sink concentration. Source to sink ratios as high as four were observed in the 76% RH treatment, indicating that ethylene build-up in the source occurred in this treatment also, but to a much lesser degree.

For the 94% RH treatment, almost all (~99%) ethylene in the system had been released into the sink or source headspace by 72 h (Figure 10). Conversely, only ~8% of ethylene from the 76% RH treatment had been released into the sink or source headspace by this time.

Sink headspace temperature during the experiment was 22.7 ± 0.3°C (mean ± SD) for all treatments. For the 76% RH treatment, sink RH increased faster than source RH but the two were closely matched from 10 h onwards (Figure 11). Sink RH also increased faster than source RH in the 94% RH treatment, but a slight difference between the two was maintained throughout the experiment – sink RH was generally higher than source RH by ~3 percentage points.

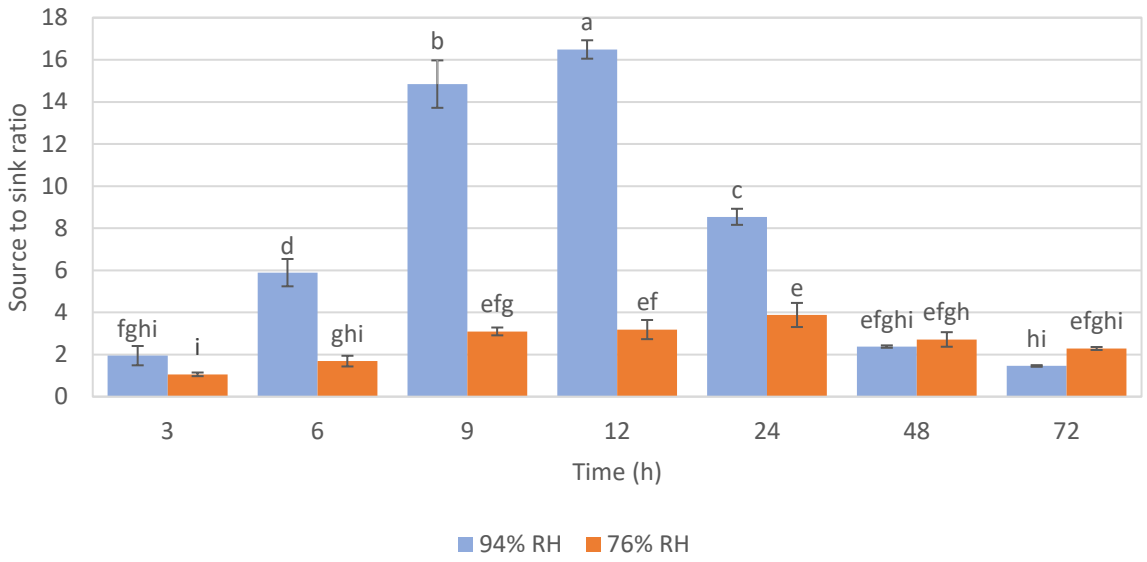

Figure 9. Ratio of source to sink headspace ethylene concentration at 72 h for a 2 L chamber (sink) containing 10 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Sink headspace was maintained at either 76% or 94% RH by inclusion of 10 mL saturated solution of NaCl or KNO_3, respectively. A system in equilibrium is indicated by a source to sink ratio of 1. Error bars represent standard error of the mean (*n* = 3). Means followed by the same letter do not significantly differ according to Fisher's LSD test (P > 0.05).

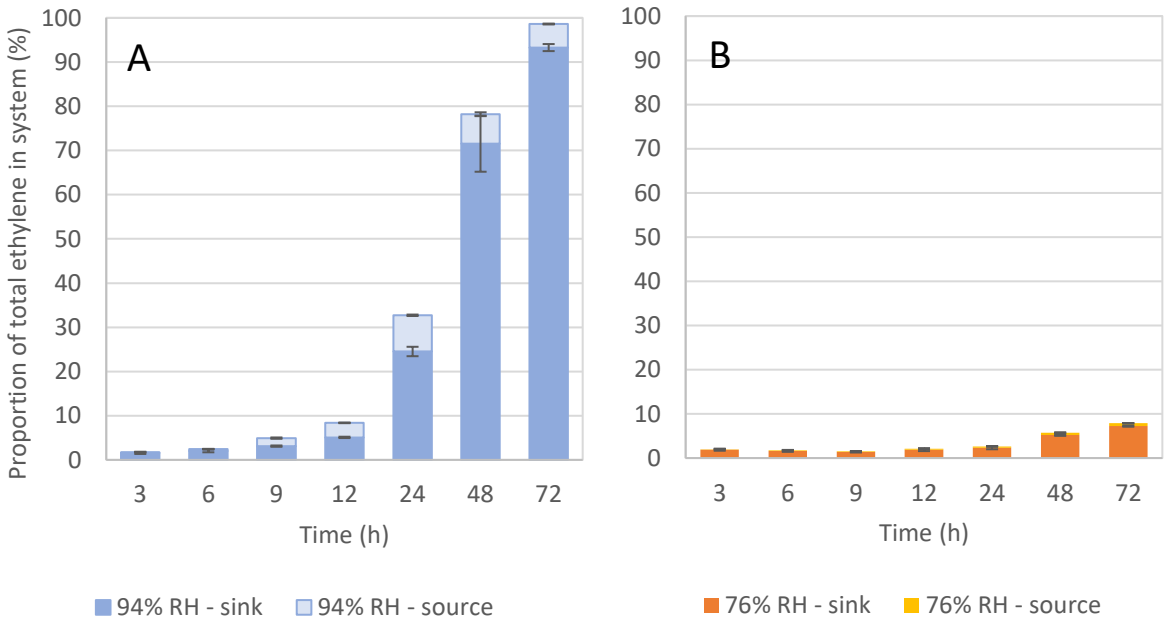

Figure 10. Ethylene distribution between sink and source headspace (expressed as a percentage of total ethylene in the system) for a 2 L chamber (sink) containing 10 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Chamber headspace was maintained at either (A) 94% or (B) 76% RH by inclusion of 10 mL saturated solution of KNO_3 or NaCl, respectively. Error bars represent standard error of the mean (*n* = 3).

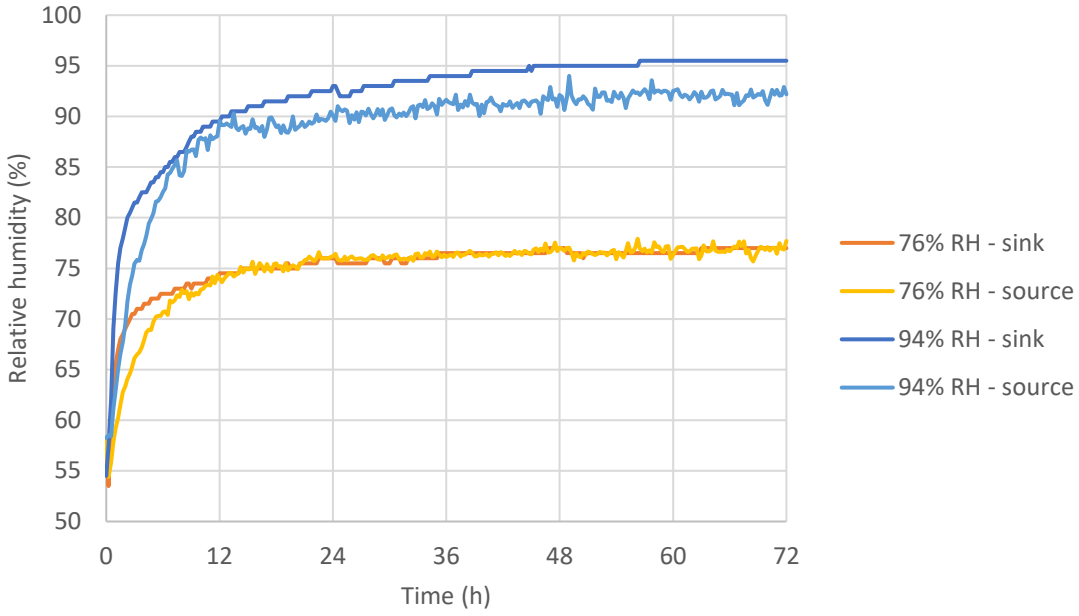

Figure 11. Sink and source headspace relative humidity for a 2 L chamber (sink) containing 10 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Sink headspace was maintained at either 76% or 94% RH by inclusion of 10 mL saturated solution of NaCl or KNO_3, respectively.

4 Discussion

Experiment 1 showed that Ripestuff™ at ambient temperature (~23 °C) and humidity (~60% RH) was relatively stable, exhibiting < 1% ethylene release after 10 d. However, humidification of the source headspace to > 90% RH resulted in near complete ethylene release from Ripestuff™ powder within 9 h. These findings confirm those of Ho et al. (2011) who reported accelerated ethylene release from Ripestuff™ powder exposed to 94% RH as opposed to 76% RH, and almost no ethylene release from powder exposed to 53% RH.

In the absence of source humidification, ethylene release in a high humidity sink environment was virtually non-existent in Experiment 1 (dry/wet treatments) but proceeded rapidly in Experiment 2 (94% RH treatment). The likely reason for this discrepancy is the inclusion of dry chromatography paper in the Ripestuff™ containers from Experiment 1. Moisture diffusing into these containers may have been preferentially ab/adsorbed by the paper rather than the Ripestuff™ powder, thus limiting ethylene release. This theory is supported by source RH data, which increased to >90% RH within 24 h in Experiment 2 (94% RH treatment) yet remained below 80% RH for the first 72 h in Experiment 1 (dry/wet high SA treatment).

On the basis of the results from Experiment 2, it may be concluded that the prototype delivery system containing Ripestuff™ powder without humidification is appropriate for use in situations where respiring fruit create a high humidity environment. Our findings suggest that moisture diffusion into the delivery system under these conditions is sufficient to achieve full ethylene release within 72 h at ambient temperature. However, early UPMin experiments that tested this delivery

system in newspaper-lined baskets of mango fruit reported inconsistent ripening responses which, in some instances, were no different from those exhibited by untreated control fruit (Lacap and Bayogan, 2019; Experiments 2.1 and 2.2). At the time of this study, there had not been a close examination of ethylene headspace concentrations occurring in baskets during mango ripening. Such information is needed to confirm whether Ripestuff™ ethylene release is being triggered in this situation and, if so, whether fruit are being exposed to a sufficient ethylene dosage to initiate ripening.

In conclusion, this study has shown that ethylene release from the prototype Ripestuff™ delivery system can be achieved either by inclusion of water in the system itself or by placing the system in a high humidity environment. From a practical viewpoint, the latter would be preferable as it eliminates the step of having to add water to the system prior to use. However, inconsistencies in ethylene release observed between 'model' and 'real world' situations employing this approach warrant further investigation. It is recommended that an experiment be conducted in which Ripestuff™ ethylene release from the prototype delivery system is closely monitored during basket ripening of fruit.

5 References

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Lacap, A.T., Bayogan, E.V., 2019. Application of Ripestuff™ for safer ripening of 'Carabao' mango in the Philippines. UPMin Research Report.

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Appendix E
EFFICACY OF A PROTOTYPE RIPESTUFF™ DELIVERY SYSTEM FOR BATCH-RIPENING OF 'CAVENDISH' BANANA FRUIT

UQ RESEARCH REPORT 3

prepared for
ACIAR Project HORT/2012/098

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Summary

Ripestuff™ has the potential to be a safe and effective alternative to calcium carbide for small-scale ripening of fruit. However, previous experiments to compare the mango ripening efficacy of Ripestuff™ versus calcium carbide have had inconsistent results. Here we investigated banana ripening response when exposed for 72 h to a prototype Ripestuff™ delivery system in newspaper-lined baskets, similar to those used to ripen fruit in the Philippines. Delivery systems with and without inclusion of wet paper were employed. Monitoring of headspace ethylene concentration inside the basket revealed that peak ethylene release occurred at 24 h when water was included in the system (High RH treatment), resulting in uniform fruit ripening within the 72 h treatment period. Without the addition of water (Low RH treatment), the delivery system was incapable of increasing the ethylene concentration in the basket headspace and the fruit remained unripe. Moisture diffusion into the Low RH delivery system from the surrounding high humidity environment created by the respiring fruit appeared insufficient to trigger ethylene release within 72 h. It was speculated that modification of the Low RH delivery system in terms of reducing the amount of Ripestuff™ powder and/or increasing the surface area available for moisture diffusion would likely produce a system that can effectively ripen fruit, without the need for water addition.

Contents

1 Introduction

Small-scale mango and banana producers in developing countries rely on batch ripening with calcium carbide to achieve marketable fruit of uniform quality within a short time (around 2-3 days earlier than if fruit were to ripen naturally). Green mature fruit are generally packed into open-weave cane baskets lined with newspaper and containing a newspaper-wrapped bundle of calcium carbide in the base (Figure 1). Newspaper is wrapped over the top of the basket and held in place with string to fully enclose the fruit. Ripening is triggered when acetylene released from calcium carbide binds with ethylene receptors in the fruit. However, calcium carbide contains carcinogenic impurities (Asif, 2012) and can be explosive if improperly handled (Abat, 2013). These health and safety concerns have led to calcium carbide being banned for fruit ripening use in several countries including India (Vasdev, 2001; Vikram, 2015). Furthermore, anecdotal evidence suggests that calcium carbide produces uneven fruit ripening – treated fruit appear visually ripe in terms of peel colour but tend to have firm, unripened flesh (Hossain et al., 2015).

Figure 1. Preparation of mango fruit for batch-ripening with calcium carbide in the Philippines (photo credit: Angelyn Lacap).

Ripestuff™ has the potential to be a safe and effective alternative to calcium carbide. However, previous experiments to compare the mango ripening efficacy of Ripestuff™ versus calcium carbide have had inconsistent results. As at the time of this study, only one of the three UPMin experiments that used a basket configuration showed Ripestuff™ (in a prototype delivery system with no added water) was capable of ripening mango at a rate similar to calcium carbide (Lacap and Bayogan, 2019; Experiment 2.1). In the other two experiments, Ripestuff™ showed no difference in ripening rate as compared with untreated control fruit (Lacap and Bayogan, 2019; Experiments 2.2 and 3). None of these three experiments measured headspace ethylene in the basket.

In order to explain the inconsistent results between experiments, more information was needed with respect to the rate of moisture diffusion between the headspace of the basket and the

Ripestuff™ delivery system, as well as the rate of ethylene diffusion from the Ripestuff™ delivery system to the basket headspace.

The current experiment investigated banana ripening response when exposed to a prototype Ripestuff™ delivery system in a basket configuration. Banana was chosen for its ready availability at the time of the study (mango was not in season) and because the ripening efficacy of Ripestuff™ had not previously been investigated for this fruit crop. Our earlier findings using static chambers showed rapid ethylene release when wet paper was incorporated in the Ripestuff™ delivery system (Perkins and Joyce, 2019b). Hence, delivery systems with and without inclusion of wet paper were investigated. Ethylene concentration inside the basket was monitored during the 72 h treatment period, and fruit quality was assessed before and after treatment. Headspace temperature and RH of both the basket and the Ripestuff™ delivery system were also monitored during the treatment period.

2 Methodology

2.1 Ripestuff™ powder

The experiment used a batch of Ripestuff™ powder prepared in 2017 at The University of Queensland (St Lucia, Australia) by encapsulation of ethylene into amorphous α-CD. Moisture content was 6.18% (wet-weight basis) and ethylene concentration was 0.543 mol.mol^{-1} α-CD when assessed at the time of the study. The powder was passed through a metal sieve (0.7 mm mesh size) to remove clumps that had formed during storage.

2.2 Fruit

Banana fruit cv. 'Cavendish' at green mature stage were sourced from Murray Brothers (Brisbane Markets, Rocklea, Queensland) and transported in an air-conditioned vehicle to The University of Queensland Gatton Campus (UQG) within 2 h of collection. The fruit were held at 13°C until commencement of the experiment the following day. Hands of uniform quality and free from visible defects were used in the experiment. Hands of fruit were kept intact throughout the experiment to prevent wound-induced ethylene production.

2.3 Ripestuff™ delivery system

The experiment employed a delivery system comprising 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid. The inner wall of some specimen containers was also lined with a quarter sheet of Whatman™ chromatography paper (11 x 3.5 cm, grade 3MM CHR) to which 1.25 mL deionised water had been added.

2.4 Basket configuration

Baskets lined with newspaper (2-3 sheets thick) were used to contain ~5 kg fruit and the Ripestuff™ delivery system. Polypropylene twine was used to firmly secure the newspaper around the fruit, thereby creating an enclosed environment (Figure 2). Baskets were constructed of plastic mesh and were 32 cm high with an internal base diameter of 28 cm. To facilitate headspace sampling, a 5 cm length of silicone tubing (4 mm internal diameter) was inserted through a 5 mm hole in the top of the newspaper lining. The hole was fitted with a metal eyelet to firmly hold the tube in place without tearing the newspaper. A 19 mm fold back metal clip (Esselte No. 1) kept the tube pinched closed during the experiment.

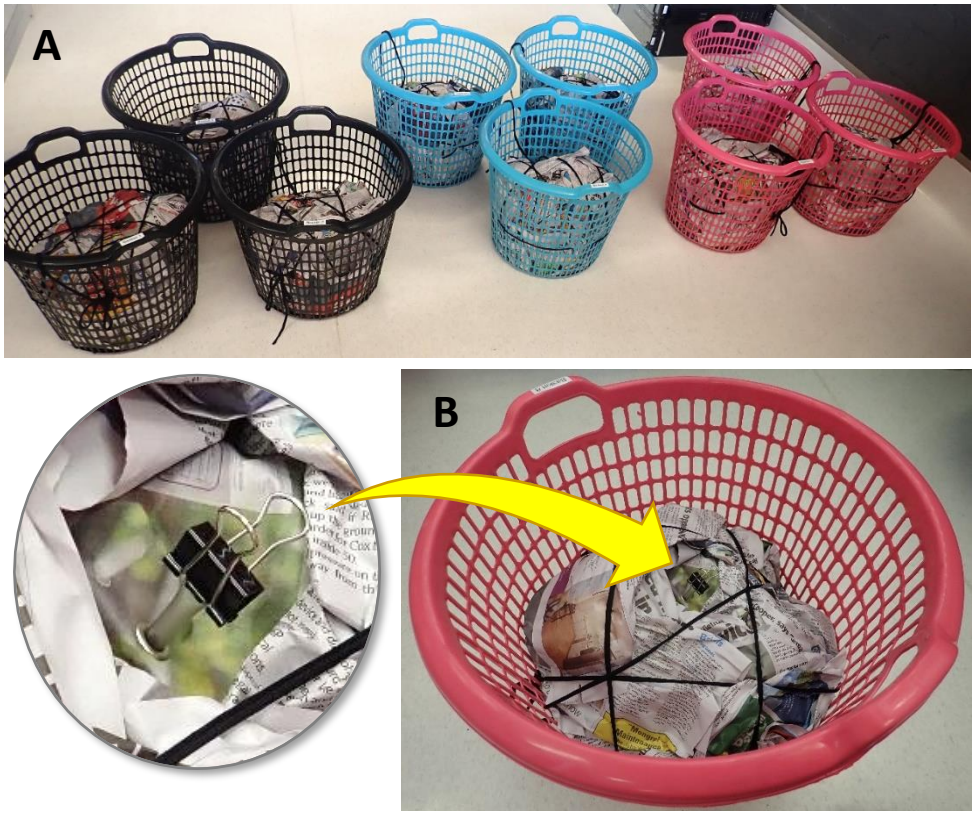

Figure 2. A Ripestuff™ delivery system and ~5 kg 'Cavendish' bananas were packed into (A) plastic baskets lined with newspaper which was (B) firmly held in place around the fruit with polypropylene twine. A short length of silicone tubing was inserted through the newspaper layer to allow for headspace sampling (inset).

2.5 Treatments

The experiment was comprised of three treatments (Figure 3) in which no Ripestuff™ (control) or 3.75 g Ripestuff™ was placed in a prototype delivery system comprising a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with a piece of wet chromatography paper. The mass of Ripestuff™ corresponded to that used in previous mango ripening experiments conducted at UPMin. One Ripestuff™ delivery system was placed centrally in the base of a newspaper-lined basket containing ~5 kg fruit (minimum of six hands per basket). The newspaper was immediately wrapped over the top of the fruit and secured with polypropylene twine. The prepared baskets of fruit were maintained at ambient laboratory conditions for 72 h.

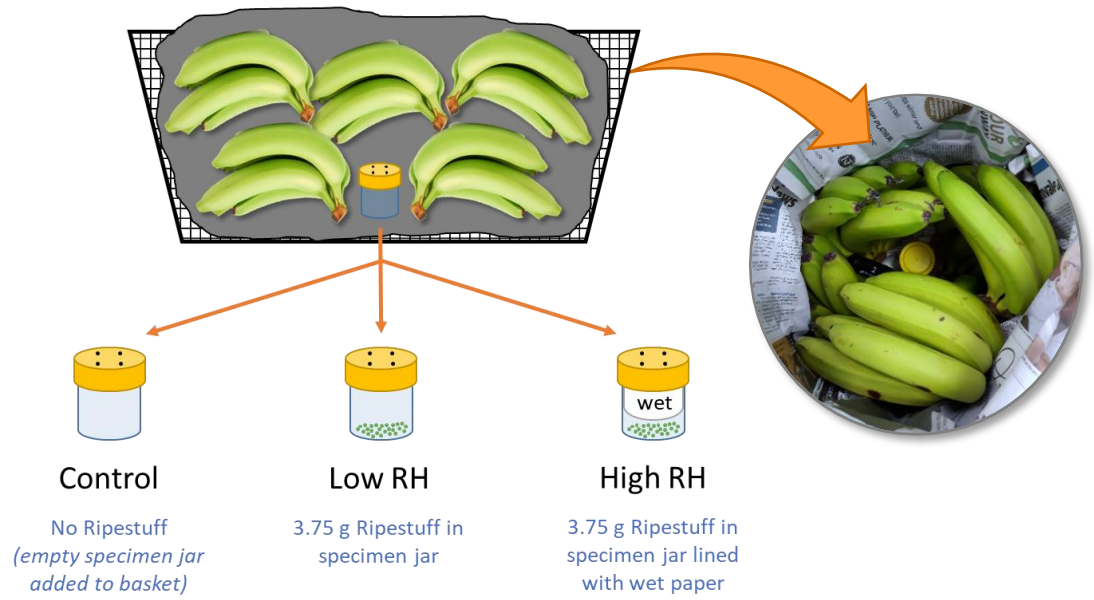

Figure 3. Treatments investigated in determining efficacy of a prototype Ripestuff™ delivery system for ripening of 'Cavendish' banana fruit in newspaper-lined baskets. The delivery system was comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control.

2.6 Data collection

2.6.1 Headspace ethylene concentration

Sampling and analysis of headspace ethylene concentration was conducted using the procedure described in UQ Research Report 1 (Perkins and Joyce, 2019a). Headspace sampling of baskets was achieved by temporarily removing the clip and attaching a BD 5 mL disposable syringe to the silicone tube (Figure 4). The syringe was pumped three times to evacuate the tube prior to sampling 2 mL of headspace at 3, 6, 9, 12, 24, 48 and 72 h. At the end of the treatment period (i.e. 72 h), a single headspace sample of 2 mL was taken from each delivery system (via one of the four holes in the lid) using a 5 mL syringe fitted with a with a BD PrecisionGlide™ needle (0.5 mm x 25 mm).

Figure 4. Headspace sampling of baskets was achieved via (A) a 5 cm length of 4 mm i.d. silicone tube fitted to a 5 mm i.d. eyelet in the newspaper lining. A metal clip was (B) used to pinch the tube closed during the experiment and (C) temporarily removed to facilitate sample collection with a 5 mL syringe.

2.6.2 Headspace temperature and relative humidity

Each basket and Ripestuff™ container in Replicate 1 was fitted with an EasyLog data logger (EL-USB-2; Lascar Electronics, Wiltshire, UK) and Hygrochron HC data logger (DS1923; Thermochron Australia, Castle Hill, Australia), respectively, to record headspace temperature and relative humidity at 15 min intervals for the duration of the experiment.

2.6.3 Fruit quality

Initial fruit quality was assessed on the day prior to commencement of treatments (Day 0). After the 72 h treatment period (Day 4), baskets were opened and the fruit transferred to a laboratory bench where they were held at 23.3 ± 0.7°C (mean ± SD) and 63.3 ± 4.6% RH (mean ± SD) for a further 6 d (Figure 5).

Figure 5. 'Cavendish' banana fruit on laboratory bench at Day 4 (0 d after treatment). Fruit were maintained in this environment for 6 d and regularly assessed for quality.

2.6.3.1 Non-destructive assessments

Daily assessment of ripeness stage, weight loss, peel colour and postharvest disease was conducted on three hands of fruit from each basket. Measurements were averaged to provide a mean value for each basket at each evaluation time. Fruit were also photographed at each assessment time. Ripeness stage was subjectively scored on a 7-point rating scale in accordance with the Dole® Retail Banana Ripening Guide (Figure 6). Weight loss (%) was calculated as the difference between initial hand mass and hand mass at time of evaluation, expressed as a percentage of initial hand mass. Peel colour at mid-length of three fruit per hand was measured using a Minolta CR400 chroma meter (Minolta Ltd, Osaka, Japan) and expressed as L*, a* and b* values of the CIELAB colour space. Body rot severity was scored on a 5-point rating scale (1= no visible black spots; 2 = slight infection, 1-5% of the surface affected; 3 = moderate infection, 6-10% of the surface affected; 4 = moderately severe infection, 11-25% of the surface affected; 5 = severe infection, >25% of the surface affected). Stem end rot severity was scored on a similar 5-point rating scale (1= no discoloration at the stem

end; 2 = slight infection, 1-5% of the surface affected; 3 = moderate infection, 6-10% of the surface affected; 4 = moderately severe infection, 11-25% of the surface affected; 5 = severe infection, >25% of the surface affected).

Figure 6. Dole® Retail Banana Ripening Guide (https://www.obecet.tk/8071/banana-color-chart.html).

2.6.3.2 Destructive assessments

A single hand of fruit from each basket was destructively assessed for total soluble solids (TSS), titratable acidity (TA), pulp firmness and peel firmness at 0, 2, 4 and 6 d after treatment. TSS was assessed in accordance with BS ISO 2173:2003 (British Standards, 2003), whereby 30 g of pulp obtained from the mid-length of three fruit (~10 g per fruit) was pureed and accurately weighed into a tared beaker to which ~100 mL of deionised water was then added. The mixture was stirred with a glass rod until homogenous and placed in a boiling waterbath for 15 min, with occasional stirring. Once cooled to 20°C, the beaker and its contents were weighed to the nearest 0.01 g. TSS of the filtered mixture was measured using a handheld refractometer (Atago N1, 0-32%, Japan). TSS of the pulp was calculated as follows:

$$Pulp\ TSS\ (\%) = \frac{Filtrate\ TSS\ (\%) \times mass\ of\ sample\ after\ dilution\ (g)}{mass\ of\ sample\ before\ dilution\ (g)}$$

TA was assessed using a procedure adapted from Buguad et al. (2013), whereby 6 g of pulp obtained from the mid-length of three fruit (~2 g per fruit) was pureed and accurately weighed into a beaker to which 60 mL deionised water was then added. The mixture was stirred with a glass rod until homogenous and allowed to stand for 20 min prior to titration with 0.1 N NaOH to an endpoint of pH 8.2 using 1% phenolphthalein indicator in 70% ethanol. Pulp TA (%) was calculated using following equation:

$$Pulp\ TA\ (\%) = \frac{concentration\ NaOH\ solution\ (N) \times volume\ NaOH\ solution\ (mL) \times 67}{pulp\ mass\ (g) \times 10}$$

where 67 is the malic acid factor (malic acid being the predominant organic acid in banana). The value of 10 in the denominator converts the units of measurement from grams of acid per 1000 g of pulp, to grams of acid per 100 g of pulp (i.e. %).

Peel firmness at the mid-length of three fruit per hand was measured using a FruitFirm Meter (TR Turoni, Forli, Italy) and reported in arbitrary FruitFirm units on a scale of 0 (very soft) to 100 (very firm). Pulp firmness was measured using a stand-mounted penetrometer (TR Turoni, Forli, Italy) fitted with an 8 mm diameter probe. Unpeeled cross-sections of 1 cm thickness were excised from mid-length of three fruit per hand. The force (N) required for the probe to penetrate the pulp of these cross-sections to a depth of 8 mm at an approximate speed of 4 $mm.s^{-1}$ was recorded. Firmness measurements were averaged to provide a mean value for each basket at each evaluation time.

2.7 Experimental design and data analysis

A complete randomised design comprising three replicate baskets for each treatment was employed. Staggered commencement times were used, with a basket being prepared every 3 min to accommodate the time required for subsequent GC analyses. Doing so ensured that the headspace of each basket was sampled at consistent intervals and able to be immediately analysed.

Data for Ripestuff™ ethylene concentration and moisture content, and all fruit quality parameters were subjected to a two-factor (treatment x sampling time) analysis of variance using Minitab®, Version 17.3.1 (Minitab Pty Ltd, Sydney, Australia). Treatment effects on source to sink headspace ethylene ratio were determined by one-way analysis of variance. Means were compared using Fisher's LSD test at a significance level of 0.05.

3 Results

3.1 Headspace ethylene concentration

Ethylene release from the High RH delivery system was gradual in the first 9 h, but rapidly increased between 9 and 24 h to reach a mean peak basket headspace ethylene concentration of 17 $\mu L.L^{-1}$ (Figure 7a). A large variation between replicates was observed in ethylene concentrations for this treatment at 12 h. Closer examination revealed that Replicate 3 of the High RH treatment exhibited an earlier and higher peak ethylene concentration (23 $\mu L.L^{-1}$) in the basket headspace than either of the other two replicates, both of which peaked at 15 $\mu L.L^{-1}$ (Figure 7b). By 32 h, all replicates exhibited similarly low ethylene concentrations and by 48 h they were no different to those observed in the control and Low RH treatments.

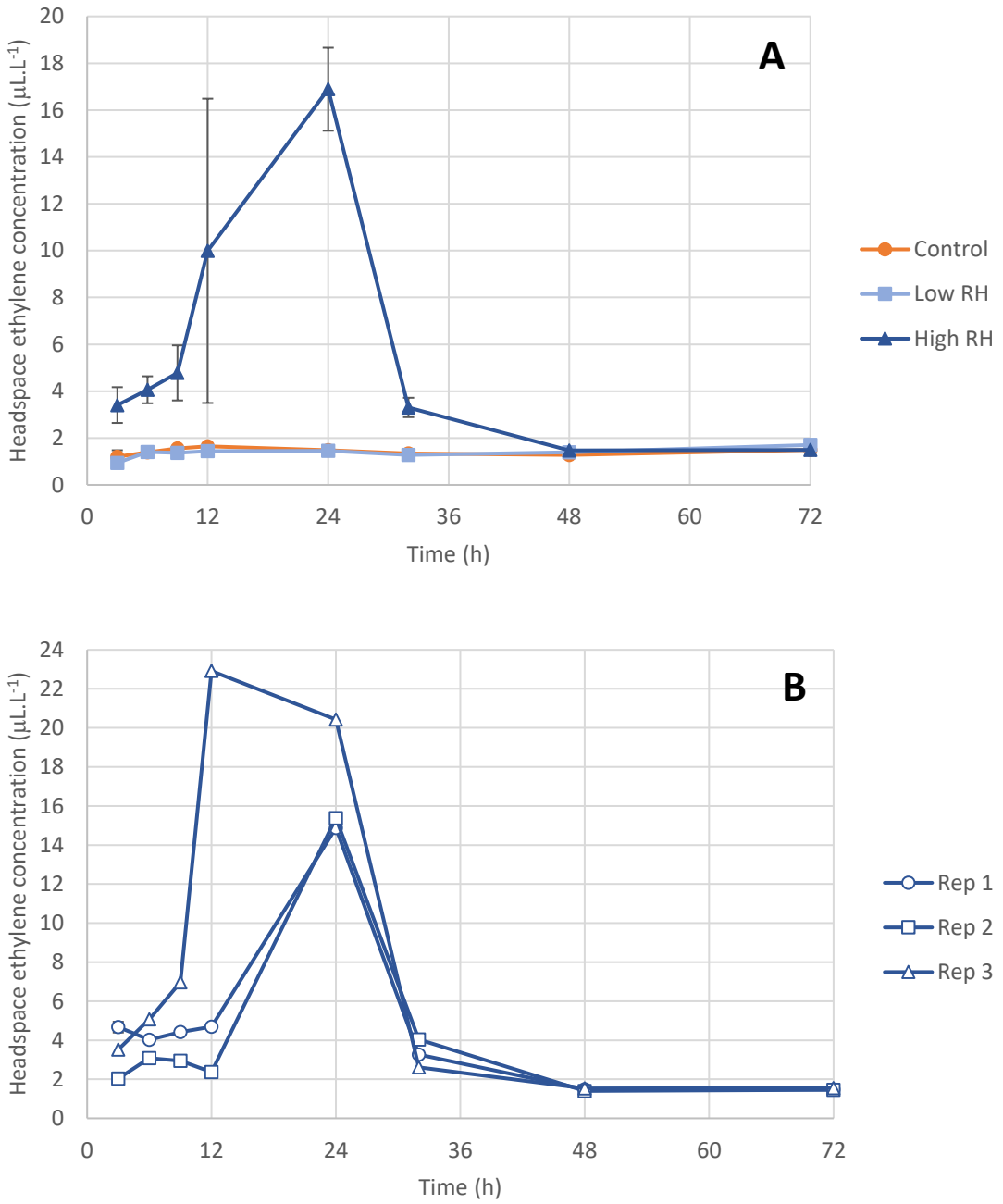

Figure 7. (A) Headspace ethylene concentration inside a newspaper-lined basket containing 5 kg 'Cavendish' banana fruit and a prototype Ripestuff™ delivery system comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control. Error bars represent SEM (*n* = 3). (B) Headspace ethylene concentration inside individual baskets (replicates) from High RH treatment.

The Low RH delivery system produced ethylene concentrations in the basket headspace that were below 2 $\mu L.L^{-1}$ (similar to the control) throughout the 72 h treatment period (Figure 7a). Comparison of source (Ripestuff™ container) and sink (basket) headspace ethylene concentrations at 72 h suggested that ethylene release from the Low RH treatment had commenced, as evidenced by a very high source to sink ratio, but that ethylene diffusion into the basket headspace had yet to occur (Table 1).

Almost no residual ethylene was detected in Ripestuff™ powder from the High RH treatment at 72 h (Table 2). Ripestuff™ powder from the Low RH treatment retained most of its ethylene at 72 h, but this concentration was significantly lower than the initial level. Moisture content of Ripestuff™ powder at 72 h showed no difference between initial and final levels for the Low RH treatment. However, final moisture content of Ripestuff™ from the High RH treatment was 2.6 times higher than the initial level (Table 2).

Table 1. Ethylene distribution between Ripestuff™ delivery system (source) and newspaper-lined basket containing 5 kg 'Cavendish' banana fruit (sink) after 72 h treatment period. Ripestuff™ delivery system was comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control. A system in equilibrium is indicated by a source to sink ratio of 1. Means followed by the same letter do not significantly differ according to Fisher's LSD test (P > 0.05).

Treatment	Headspace ethylene concentration ($\mu L.L^{-1}$)		Source to sink ratio
	Source	Sink	
Control	1.3	1.5	1 a
Low RH	1356.1	1.9	733 c
High RH	46.9	1.5	31 b

Table 2. Ethylene concentration and moisture content of Ripestuff powder™ before (initial) and after (final) 72 h treatment period. Ripestuff™ delivery system was comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. Means within a column and followed by the same letter do not significantly differ according to Fisher's LSD test (P > 0.05).

Treatment	Ripestuff™ ethylene concentration*		Moisture content (%)
	$mol.mol^{-1}$	% of initial	
Initial	0.543 a	100.0 a	6.18 b
Final			
Low RH	0.472 b	87.6 b	6.71 b
High RH	0.003 c	0.5 c	16.16 a

** dry weight basis*

3.2 Headspace temperature and relative humidity

Similar temperature profiles between the control and Low RH treatments were observed for both the source and sink headspaces during the 72 h treatment period (Figure 8). Headspace temperatures for the High RH treatment were initially similar to those of the other treatments, but underwent a marked increase at ~18 h to reach a temperature that was ~1.5°C higher. Sink headspace temperature was initially low (~19°C) for all treatments. The reason was that the fruit were not at room temperature when treatment commenced, despite having been removed from the cold room 3 h beforehand.

Source RH reached 90% within 1 h for the High RH treatment and 20 h for the control treatment, but remained below 70% for the Low RH treatment (Figure 9a). Sink RH profile was initially similar between all treatments and exceeded 90% RH within 1 h (Figure 9b). As headspace temperature increased, a corresponding decrease in sink RH was observed. Sink RH for control and Low RH treatments remained above 85% RH, whereas sink RH for the High RH treatment was slightly lower (minimum of 83% RH).

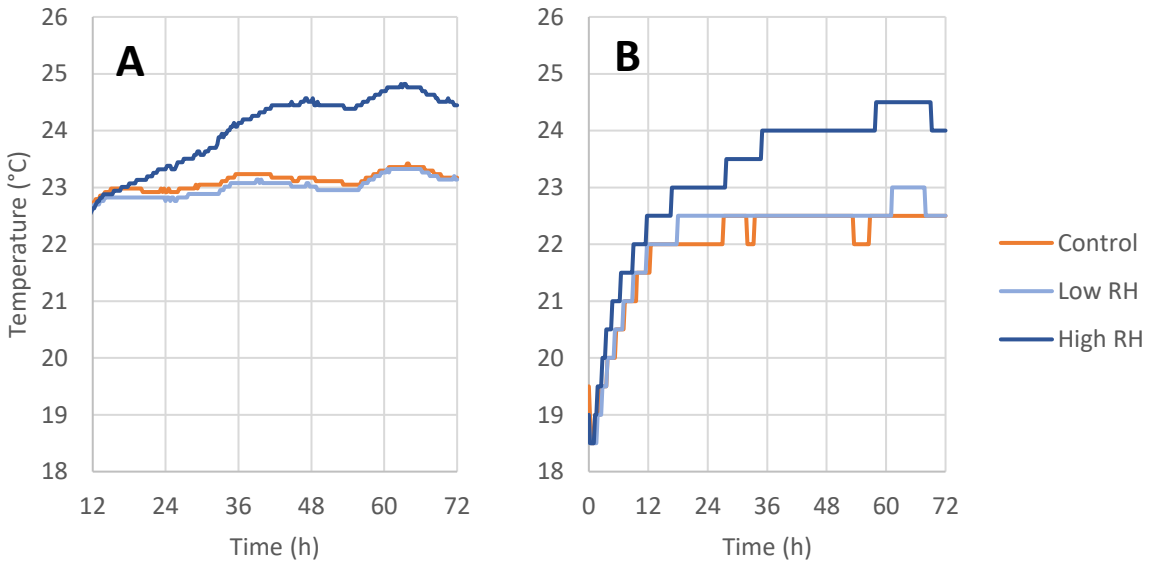

Figure 8. Headspace temperature inside (A) Ripestuff™ delivery system (source) and (B) newspaper-lined basket containing 5 kg 'Cavendish' banana fruit (sink) during 72 h treatment period. Ripestuff™ delivery system was comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control.

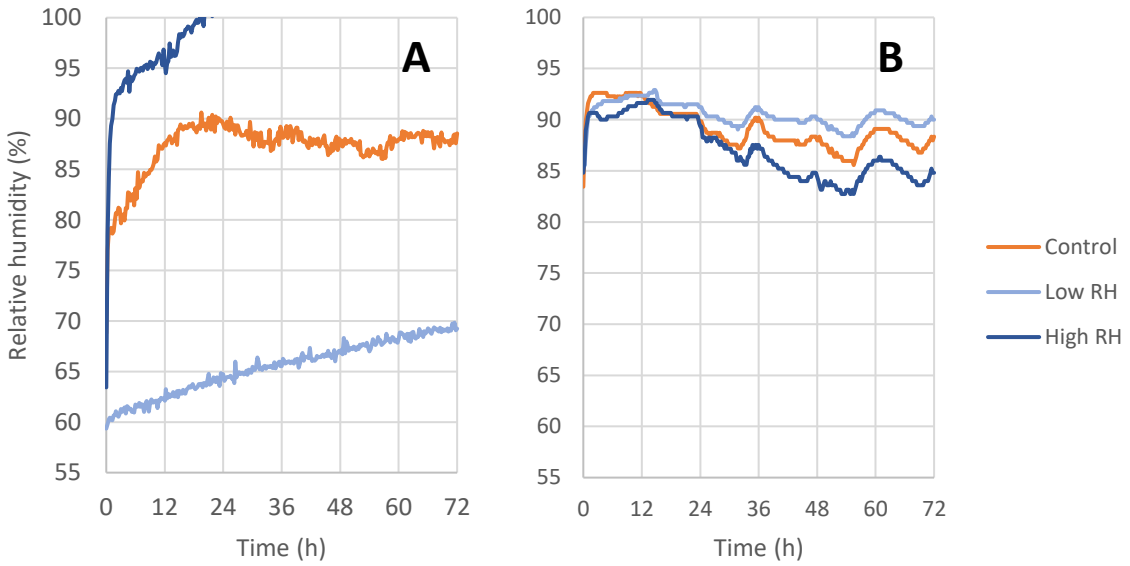

Figure 9. Headspace relative humidity inside (A) Ripestuff™ delivery system (source) and (B) newspaper lined basket containing 5 kg 'Cavendish' banana fruit (sink) during 72 h treatment period. Ripestuff™ delivery system was comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control.

3.3 Fruit quality

Differences in degree of ripening were immediately evident upon opening of the baskets at 72 h (Day 4 of the experiment; Figure 10). Fruit from the High RH treatment were uniformly yellow with green tips (i.e. ripeness stage 5) at this time, whereas fruit from the control and Low RH treatments remained unripe (Figure 11a). Objective peel colour measurements for L*, a* and b* were also higher on Day 4 in fruit from the High RH treatment, as compared with the other two treatments (Figure 11c-e). Fruit from the High RH treatment underwent more rapid weight loss from Day 4 onwards than fruit from the other treatments (Figure 11b).

Fruit from the High RH treatment exhibited higher TSS, higher TA and lower peel and pulp firmness than the control or Low RH treated fruit on Day 4 (Figure 12). These differences were maintained throughout the evaluation period, with the exception of TA. TA of fruit from the High RH treatment markedly declined from Day 4 to Day 10, whereas TA of fruit from the other treatments gradually increased during this period. Postharvest disease was more pronounced in fruit from the High RH treatment, with higher body rot severity from Day 5 onwards and higher stem end rot severity from Day 6 onwards than observed in fruit from either of the other treatments (Figure 13).

Fruit from the control and Low RH treatments showed no difference in any fruit quality parameter between Day 4 and Day 7. However, the Low RH treated fruit showed more advanced ripening in terms of ripeness stage from Day 8 onwards, and a* peel colour value from Day 9 onwards (Figure 11). Low RH treated fruit also showed higher body rot severity on Day 10 than fruit from the control treatment (Figure 13).

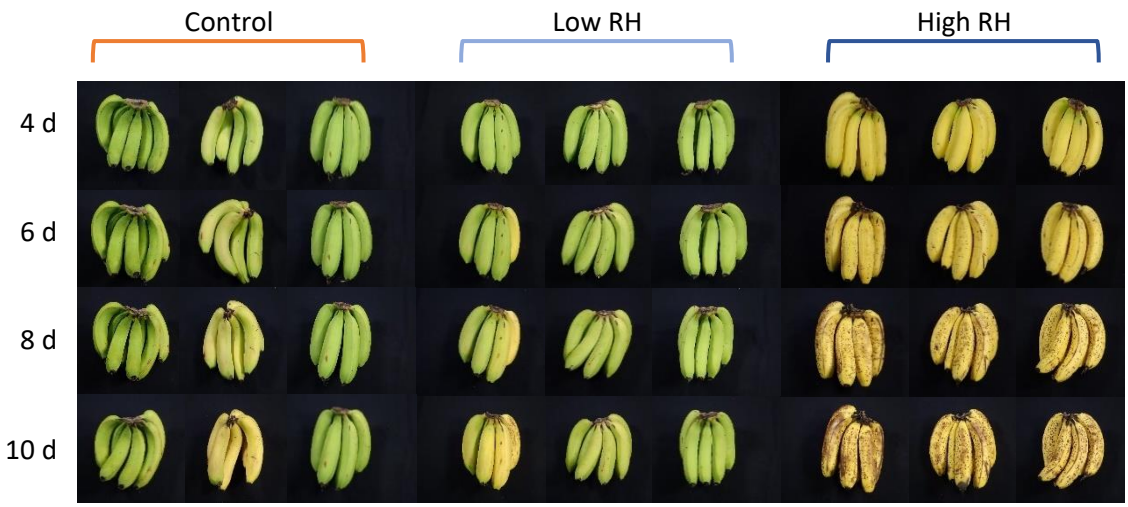

Figure 10. 'Cavendish' banana fruit photographed on Day 4, 6, 8 and 10 of the experiment (corresponding to 0, 2, 4 and 6 d after treatment). Treatment involved 72 h exposure of ~5 kg of fruit in a newspaper-lined basket to a prototype Ripestuff™ delivery system comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control.

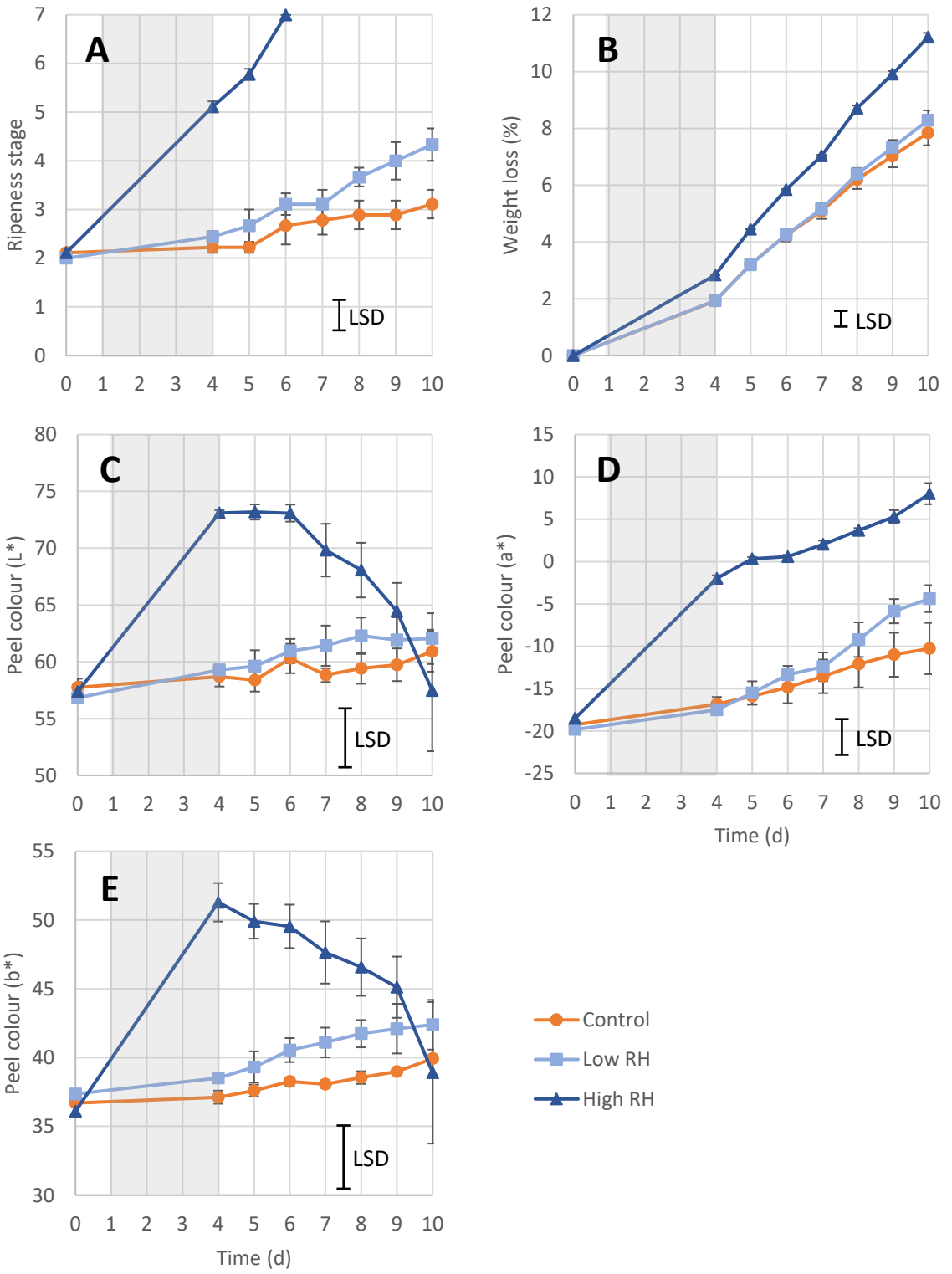

Figure 11. Non-destructive assessment of 'Cavendish' banana fruit (A) ripeness stage, (B) weight loss, and (C-E) peel colour in response to 72 h exposure (grey shading) to a prototype Ripestuff™ delivery system comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control. Treatment involved enclosing 5 kg of fruit in a newspaper-lined basket with the delivery system. Error bars represent SEM (*n* = 3).

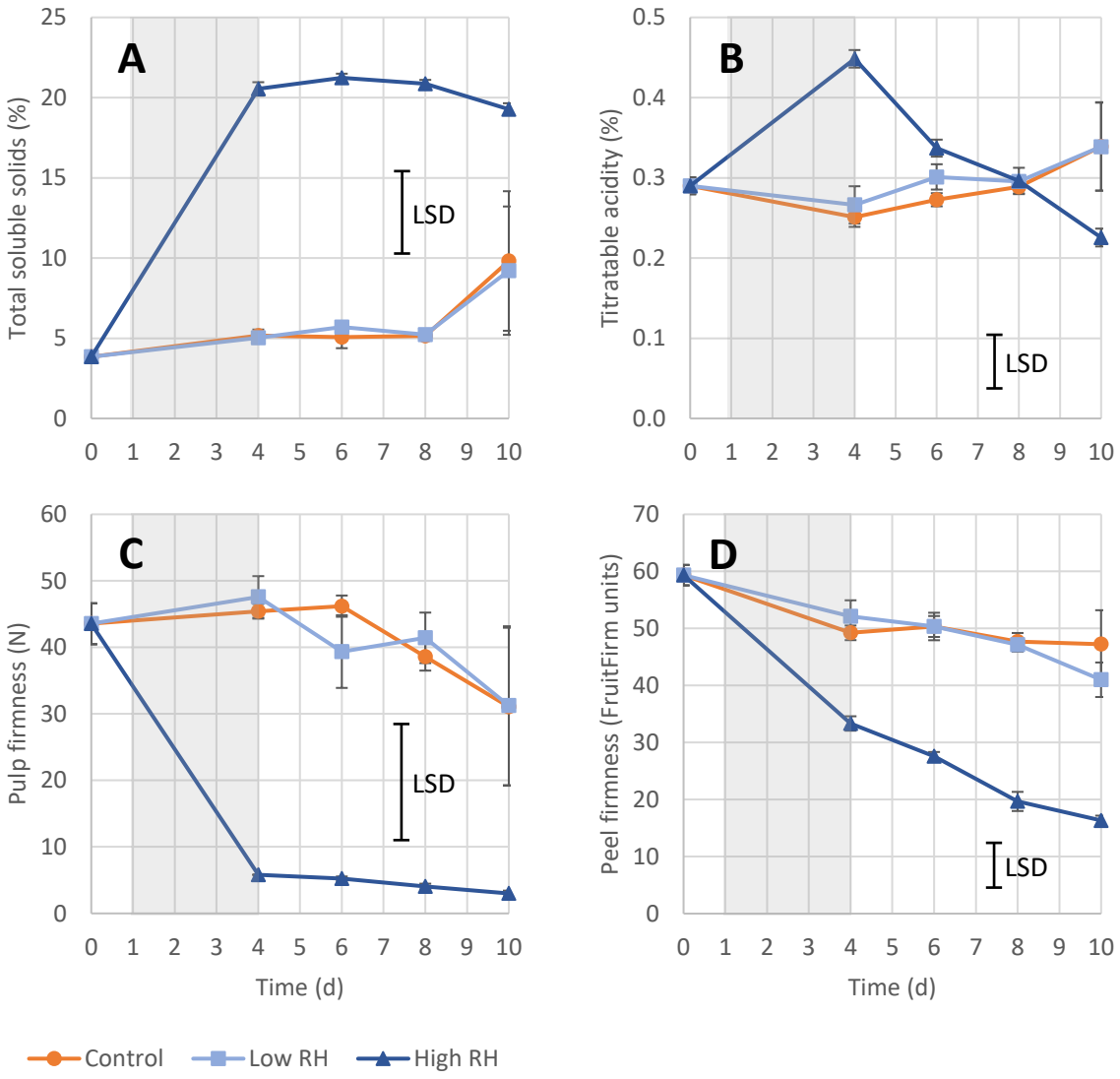

Figure 12. Destructive assessment of 'Cavendish' banana fruit (A) total soluble solids, (B) titratable acidity, (C) pulp firmness and (D) peel firmness in response to 72 h exposure (grey shading) to a prototype Ripestuff™ delivery system comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control. Treatment involved enclosing 5 kg of fruit in a newspaper-lined basket with the delivery system. Error bars represent SEM (*n* = 3).

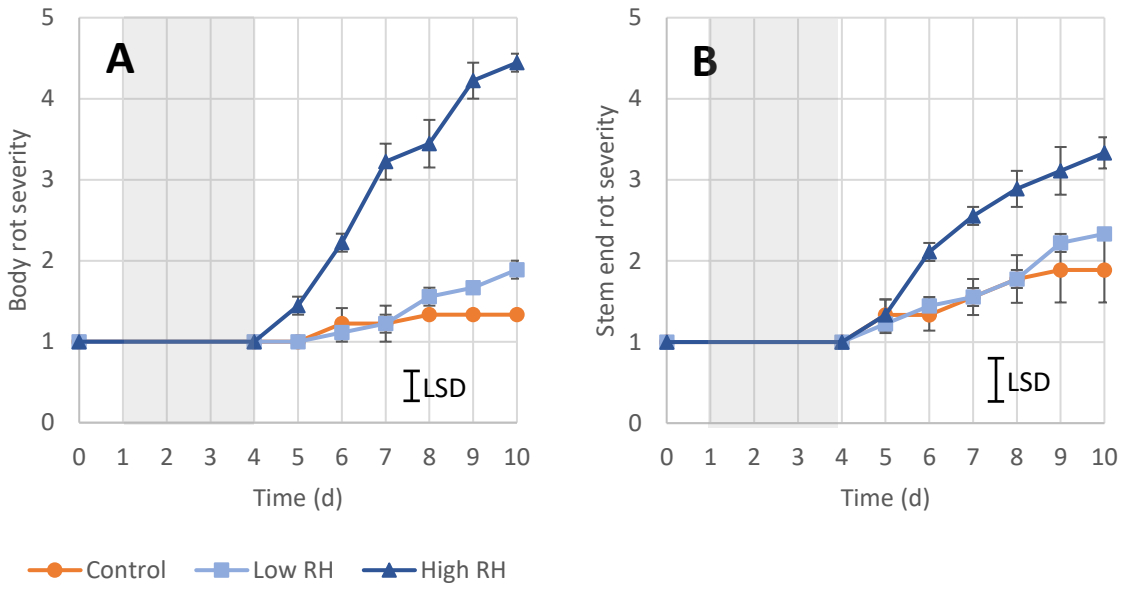

Figure 13. Severity of (A) body rot and (B) stem end rot in 'Cavendish' banana fruit exposed for 72 h (grey shading) to a prototype Ripestuff™ delivery system comprised of 3.75 g Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid, and lined (high RH) or not lined (low RH) with wet chromatography paper. A delivery system containing no Ripestuff™ or paper served as the control. Treatment involved enclosing 5 kg of fruit in a newspaper-lined basket with the delivery system. Error bars represent SEM (*n* = 3).

4 Discussion

The High RH Ripestuff™ delivery system investigated in the current study was capable of generating >10 $\mu L.L^{-1}$ ethylene in the basket headspace for up to 12 h and was subsequently shown to produce uniform fruit ripening. Commercial banana cultivars generally require 24-48 h exposure to 100-150 $\mu L.L^{-1}$ ethylene at 15-20°C and 90-95% relative humidity to induce uniform ripening (Kader, 1996). However, ethylene concentrations as low as 1 $\mu L.L^{-1}$ have been shown to trigger ripening in 'Cavendish' banana fruit after 10 h exposure at 20-25°C (Inaba and Nakamura, 1986). These authors also showed that a tenfold increase in ethylene concentration reduced the necessary exposure time for ripening by ~2 h.

The rise in basket headspace temperature observed for the High RH treatment at 18 h indicated that ripening had been initiated at this time. Furthermore, fruit ripening was well advanced upon opening the baskets from this treatment after 72 h. On the basis of this evidence, a shorter treatment period appears warranted when a High RH Ripestuff™ delivery system is used. Ethylene had mostly dissipated from the basket headspace by 32 h. Therefore, a treatment period of 24-32 h at 23°C would likely be sufficient to produce fruit at ripeness stage 4 – the stage at which the fruit is ready for retail display (Figure 6).

The large variation in basket headspace ethylene concentration between replicates of the High RH treatment may have resulted from slightly tighter wrapping of newspaper around fruit in Replicate 3. Better enclosure of the fruit would have minimised leakage of ethylene from the basket and maintained higher concentrations in the basket headspace for a longer period. The basket for Replicate 3 was prepared last and may have been better wrapped, despite efforts to use a consistent

wrapping technique. Inspection of the delivery systems used in the High RH treatment revealed no obvious differences in the amount or distribution of the Ripestuff™ powder within the container, the size of the holes in the container lid, or the tightness with which the lid was secured. Furthermore, there were no obvious differences in the mass of fruit or the dimensions of the wrapped bundle of fruit from each replicate that might account for the higher basket headspace ethylene concentrations in Replicate 3.

The Low RH treatment used the same Ripestuff™ delivery system as was used by UPMin researchers in their mango ripening experiments (Lacap and Bayogan, 2019; Experiments 2.1, 2.2 and 3). The current study found that this delivery system does not release ethylene into the basket headspace within 72 h. This finding explains why previous attempts to ripen mango fruit with a Low RH delivery system were generally unsuccessful. However, when this delivery system was tested in 2 L static chambers almost full ethylene release was achieved in 72 h (Perkins and Joyce, 2019b). The only difference between the Low RH delivery systems used here and in the static chambers is the amount of Ripestuff™ powder included in each – 3750 and 10 mg, respectively.

Past experiments have been conducted on the assumption that incorporation of more Ripestuff™ in the delivery system would result in higher headspace ethylene concentrations. However, this does not appear to be true in situations where moisture is limiting. An experiment conducted at UPMin employing the Low RH delivery system showed that a lower Ripestuff™ quantity (1.25 as opposed to 5 g) seemed to promote mango ripening in terms of more rapid fruit softening (Lacap and Bayogan, 2019; Experiment 3). The current study revealed that 3.75 g Ripestuff™ powder in a Low RH delivery system absorbs moisture from the surrounding air (as evidenced by a substantially lower source RH as compared to the control treatment), but that it is not sufficient to trigger ethylene release within the 72 h treatment period. Inclusion of less powder in the delivery system might allow the critical moisture content for ethylene release to be reached earlier. Alternatively, the rate of moisture diffusion into the delivery system could be increased by placing more holes in the lid of the Ripestuff™ container.

In conclusion, this study has shown that banana fruit can be successfully ripened using a Ripestuff™ delivery system to which water has been added. Without the addition of water, the current delivery system is not suitable for fruit ripening in a basket configuration. Modification of the Low RH delivery system in terms of the amount of Ripestuff™ powder and/or the surface area available for moisture diffusion will likely produce a system that can effectively ripen fruit. Future experiments should therefore aim to optimise ethylene release from a Low RH delivery system by careful matching of Ripestuff™ quantity with an appropriate number of holes in the lid of the Ripestuff™ container.

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Appendix F
OVERLOADING INHIBITS ETHYLENE RELEASE FROM A PROTOTYPE RIPESTUFF™ DELIVERY SYSTEM

UQ RESEARCH REPORT 4

prepared for
ACIAR Project HORT/2012/098

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Summary

Inconsistent fruit ripening responses have been observed following exposure to the current prototype Ripestuff™ delivery system. A possible inverse relationship between Ripestuff™ quantity and the rate of ethylene release from this system is believed to exist and that inadvertent overloading of the system with Ripestuff™ powder is hindering ethylene release and, thereby, fruit ripening. To test this theory, ethylene release from delivery systems containing 10, 100, 500 or 3750 mg Ripestuff™ powder was investigated using 2 L humidified static chambers. An initial lag phase in ethylene release was identified, the duration of which become longer as the amount of Ripestuff™ in the delivery system increased. An intermediate Ripestuff™ quantity of 500 mg produced the highest ethylene headspace concentrations in the first 72 h, up to five times greater than those produced by the largest quantity of 3750 mg. It was postulated that as Ripestuff™ quantity increases, so too does the amount of water it needs to absorb before ethylene release is initiated. In the current delivery system, access to water is limited by the rate of moisture diffusion into the system from the external environment. Further refinement of the prototype delivery system in terms of increasing the number of holes in the lid to accommodate faster moisture diffusion and, thereby, faster ethylene release from large Ripestuff™ quantities was recommended.

Contents

1 Introduction

Previous efforts to ripen mango fruit using a prototype Ripestuff™ delivery system based on a 70 mL specimen container with four 0.5 mm holes in the lid have had inconsistent results. It has since been shown that ethylene is readily released from this system when used in conjunction with a small quantity of 10 mg Ripestuff™ powder (Perkins and Joyce, 2019b), but not a larger quantity of 3750 mg (Perkins and Joyce, 2019c). Furthermore, mango ripening in terms of fruit softening occurred earlier as Ripestuff™ quantity in the delivery system decreased from 5000 to 1250 mg (Lacap and Bayogan, 2019; Experiment 3). These findings suggest that an inverse relationship may exist between Ripestuff™ quantity and the rate of ethylene release from the current prototype delivery system. If true, then inadvertent overloading of the delivery system may be responsible for the inconsistent fruit ripening responses reported to date.

The aim of the current experiment was to firstly confirm the existence of this relationship and, secondly, identify an appropriate Ripestuff™ quantity to use in conjunction with the prototype delivery system. Ethylene release from delivery systems containing 10, 100, 500 or 3750 mg Ripestuff™ powder was investigated using 2 L humidified static chambers to emulate the environment created by respiring fruit.

2 Methodology

2.1 Ripestuff™ powder

The experiment used a batch of Ripestuff™ powder prepared in 2017 at The University of Queensland (St Lucia, Australia) by encapsulation of ethylene into amorphous α-CD. Moisture content was 6.18% (wet-weight basis) and ethylene concentration was 0.543 $mol.mol^{-1}$ α-CD when assessed at the time of the study. The powder was passed through a metal sieve (0.7 mm mesh size) to remove clumps that had formed during storage.

2.2 Ripestuff™ delivery system

The experiment employed a delivery system comprising 0, 10, 100, 500 or 3750 mg Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid.

2.3 Chamber configuration

Chambers were 2 L preserving jars fitted with two headspace sampling ports, as described in UQ Research Report 1 (Perkins and Joyce, 2019a). A 10 mL aliquot of saturated KNO_3 solution in an unlidded 70 mL specimen container was added to each chamber to create a high humidity environment of ~94% at ambient temperature (Winston and Bates, 1960).

2.4 Treatments

The experiment was comprised of five treatments (Figure 1) in which no Ripestuff™ (control) or Ripestuff™ quantities of 10, 100, 500 or 3750 mg were placed in the prototype delivery system described above. A Ripestuff™ quantity of 10 mg corresponded to that used in previous 2 L static chamber experiments (Perkins and Joyce, 2019a,b), whereas 3750 mg corresponded to the quantity used in previous fruit ripening experiments employing a basket configuration (Lacap and Bayogan, 2019; Perkins and Joyce, 2019c).

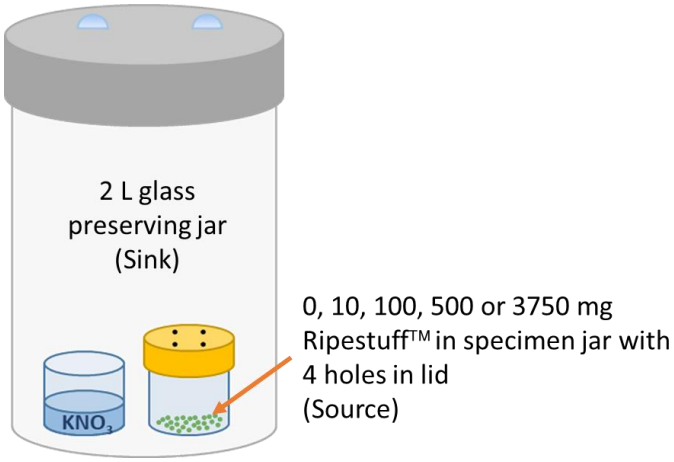

Figure 1. Treatments investigated in determining effects of Ripestuff™ quantity on ethylene release from a prototype delivery system.

2.5 Data collection

2.5.1 Headspace ethylene concentration

Sampling and analysis of headspace ethylene concentration was conducted at 3, 24, 48, 72 and 96 h and then every second or third day until 524 h (~22 d) using the procedure described in UQ Research Report 1 (Perkins and Joyce, 2019a). From 72 h onwards, headspace samples from the 500 and 3750 mg treatments were diluted prior to GC injection to accommodate the high ethylene concentrations generated by these treatments. The procedure involved using a BD 1 mL disposable syringe with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm) to accurately sample 1 mL of headspace via a sampling port of each chamber. Another syringe was used to introduce 1 mL of ethylene-free air into the chamber via the second sampling port to maintain pressure equilibrium. Headspace samples were immediately diluted by injection into a 40 mL headspace vial fitted with a PTFE/silicone septum screw cap (Macherey Nagel product code 702877, supplied by MicroAnalytix Pty Ltd, Taren Point, Australia). After ~3 min equilibration time, 2 mL diluted headspace was sampled using a BD 5 mL disposable syringe with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm) and immediately injected into the 500 µL sample loop of the GC-FID.

At the end of the experiment (i.e. 524 h), a single headspace sample was taken from each delivery system (via one of the four holes in the lid) for ethylene analysis. Samples from the 500 and 3750 mg treatments were diluted prior to injection into the GC-FID, as described above.

2.5.2 Residual Ripestuff™ ethylene concentration and moisture content

Upon opening the chambers at 524 h, Ripestuff™ samples were immediately collected from each delivery system. Samples were not collected from the 10 mg treatment since the quantity of Ripestuff™ powder was not enough for analysis. For residual ethylene determination, 15 mg powder was accurately weighed (to the nearest 0.1 mg) into 120 mL headspace vials to which 7.5 mL deionised water was then added and the vial immediately sealed. The vials were placed in a reciprocal shaking waterbath (Lab Companion™ BS-31) at 100 rpm for 30 min at 35°C then allowed to equilibrate with ambient laboratory conditions for 30 min. A headspace dilution step (as described in Section 2.5.1) was conducted prior to ethylene analysis by GC-FID.

For residual moisture determination, 80-100 mg Ripestuff™ powder from each chamber was accurately weighed (to the nearest 0.1 mg) and dried to constant mass at 60°C to gravimetrically determine dry matter content (%) as:

$$\frac{final\ Ripestuff\ ™mass}{initial\ Ripestuff\ ™mass} \times 100$$

Dry matter was considered to represent the α-CD component of Ripestuff™ powder. The mass of water present in the Ripestuff™ powder was obtained by subtracting the residual ethylene mass and the α-CD mass from the initial Ripestuff™ mass. Hence, moisture content (% wet-weight basis) was calculated as:

$$\frac{initial\ Ripestuff\ ™mass\ - aCD\ mass - residual\ ethylene\ mass}{initial\ Ripestuff\ ™mass} \times 100$$

2.5.3 Headspace temperature and relative humidity

Each Ripestuff™ container in Replicate 1 was fitted with Hygrochron HC data logger (DS1923; Thermochron Australia, Castle Hill, Australia) to record headspace temperature and relative humidity at 15 min intervals for the duration of the experiment. The same data were collected for the headspace of all chambers in Replicate 1 using EasyLog data loggers (EL-USB-2; Lascar Electronics, Wiltshire, UK).

2.6 Experimental design and data analysis

A complete randomised design comprising three replicate chambers for each treatment was employed. Staggered commencement times were used, with a chamber being prepared every 3 min to accommodate the time required for subsequent GC analyses. Doing so ensured that the headspace of each chamber was sampled at consistent intervals and able to be immediately analysed.

Treatment effects on headspace ethylene concentration (at each sampling time), residual Ripestuff™ ethylene concentration and moisture content, and source to sink ethylene ratio were determined by one-way analysis of variance using Minitab®, Version 17.3.1 (Minitab Pty Ltd, Sydney, Australia). Means were compared using Fisher's LSD test at a significance level of 0.01. Data from Replicate 1 were excluded from all statistical analyses, as the presence of loggers in this replicate was found to hamper ethylene release.

3 Results

3.1 Headspace ethylene concentration

Ethylene concentration in the sink headspace at 3 h was low (< 7 $\mu L.L^{-1}$) for all treatments. Between 24 and 72 h, the highest ethylene concentration in the chamber (sink) headspace was generated by delivery systems containing 500 mg Ripestuff™ powder (Figure 2). Concentrations during this period were up to five times higher (at 48 h) than those generated by the larger quantity of 3750 mg Ripestuff™ powder. Similarly, the sink headspace ethylene concentrations generated by the 100 mg treatment at 24 and 48 h were more than two times higher than those generated by the 3750 mg treatment. These differences resulted from a longer lag phase prior to ethylene release as

Ripestuff™ quantity increased (Figure 3). Ethylene release at 72 h was 5% from the 3750 mg treatment, as opposed to 54% from the 500 mg treatment and >85% from the 100 and 10 mg treatments.

Beyond 72 h, ethylene release from the 3750 mg treatment rapidly proceeded and produced higher sink headspace ethylene concentrations than any other treatment (Figure 2). Despite full ethylene release being achieved in the 10 and 100 mg treatments, ethylene release from the 500 and 3750 mg treatments plateaued at ~80% (Figure 3). A comparison of source and sink headspace ethylene concentrations at the end of the experiment (i.e. 524 h) revealed that all treatments had a source to sink ratio of one, which is indicative of a system in equilibrium (Table 1).

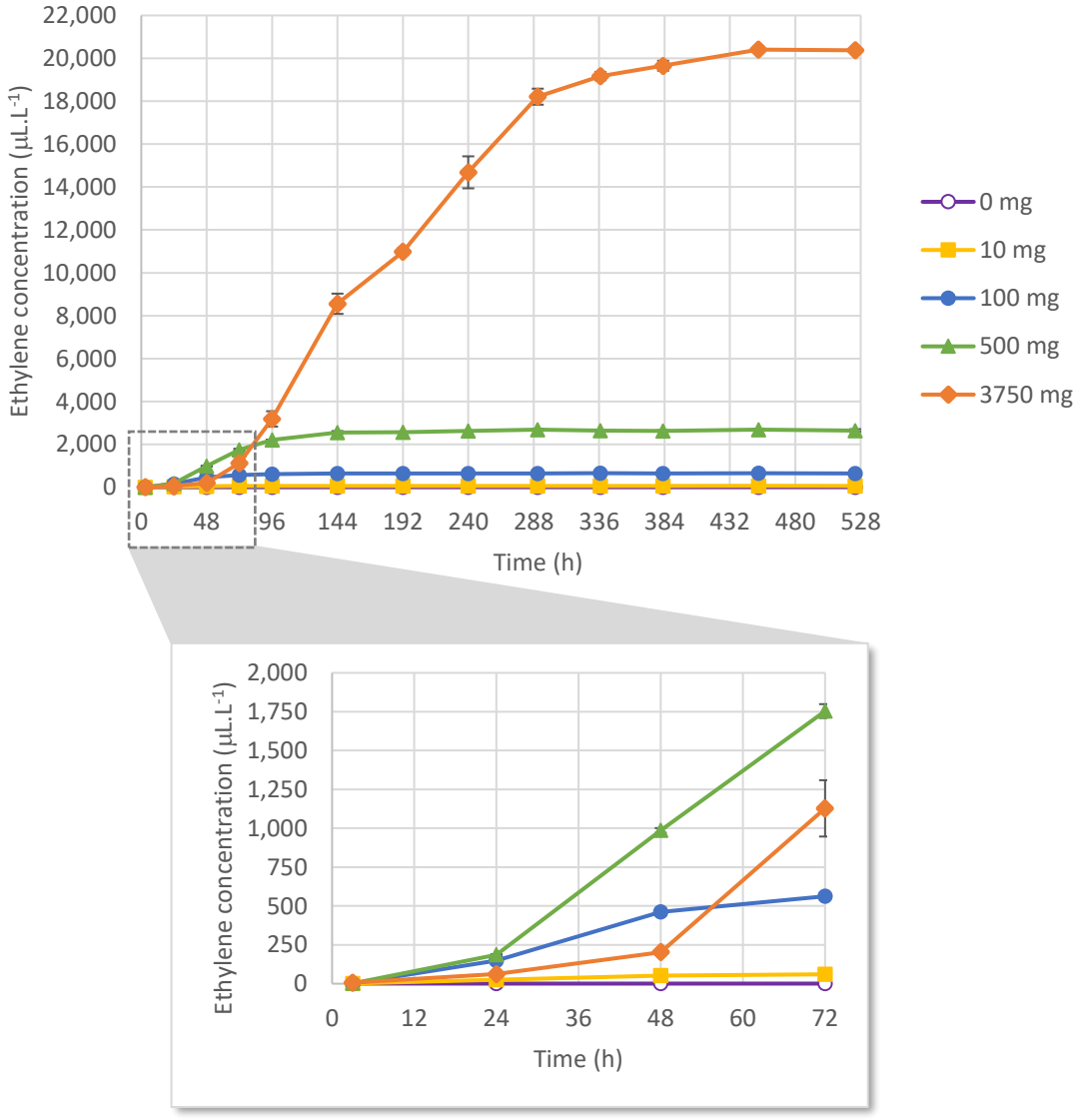

Figure 2. Headspace ethylene concentration in a 2 L chamber (sink) containing 0, 10, 100, 500 or 3750 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Error bars represent standard error of the mean (*n* = 2) and are too small to be visible in some instances.

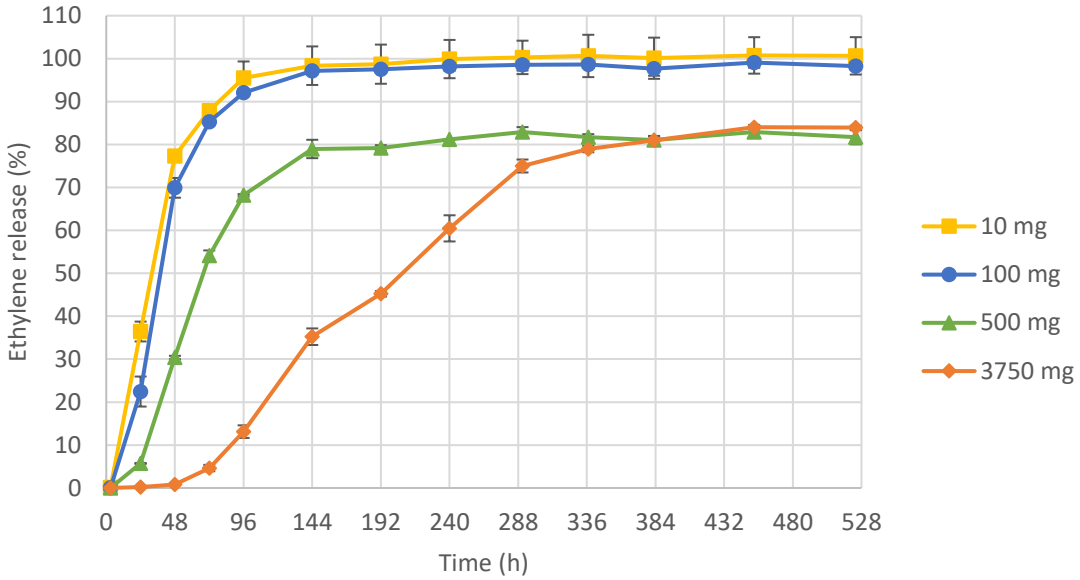

Figure 3. Ethylene release (expressed as a percentage of total ethylene in the system) in a 2 L chamber (sink) containing 10, 100, 500 or 3750 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid (source). Error bars represent standard error of the mean (n = 2) and are too small to be visible in some instances.

Table 1. Ethylene distribution between Ripestuff™ delivery system (source) containing 0, 10, 100, 500 or 3750 mg Ripestuff™ powder and 2 L chamber (sink) after 524 h. Means followed by the same letter do not significantly differ according to Fisher's LSD test (P > 0.01).

Treatment	Headspace ethylene concentration (µL.L^{-1})		Source to sink ratio
	Source	Sink	
0 mg	4	4	1.06 a
10 mg	69	69	1.00 a
100 mg	658	649	1.00 a
500 mg	2713	2648	1.01 a
3750 mg	21101	20378	1.03 a

3.2 Residual Ripestuff™ ethylene concentration and moisture content

All treatments assessed for residual ethylene exhibited concentrations corresponding to ~1% of the initial Ripestuff™ ethylene concentration (Table 2). When these values are considered in conjunction with ethylene release values (Figure 3), they account for all ethylene from the lower Ripestuff™ quantities, but not the 500 or 3750 mg treatments. Around 15-17% of ethylene from these larger quantities remained unaccounted for. Final moisture content of Ripestuff™ powder from these two treatments was similar to the initial level, whereas powder from the 100 mg treatment exhibited a 45% increase in moisture content (Table 2). Ripestuff™ quantities that fully

covered (3750 mg) or provided near full coverage (500 mg) of the Ripestuff™ container base (Figure 4) had formed a thin crusted surface layer by the end of the experiment.

Table 2. Ethylene concentration and moisture content of Ripestuff powder™ before (initial) and after (final) 524 h experimental period. Treatments were Ripestuff™ delivery systems comprising 100, 500 or 3750 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid. Means within a column and followed by the same letter do not significantly differ according to Fisher's LSD test (P > 0.01).

Treatment	Ripestuff™ ethylene concentration*		Moisture content (%)
	mol.mol^{-1}	% of initial	
Initial	0.543 a	100.0 a	6.18 b
Final			
100 mg	0.004 b	0.8 b	8.99 a
500 mg	0.005 b	1.0 b	7.39 b
3750 mg	0.007 b	1.2 b	6.73 b

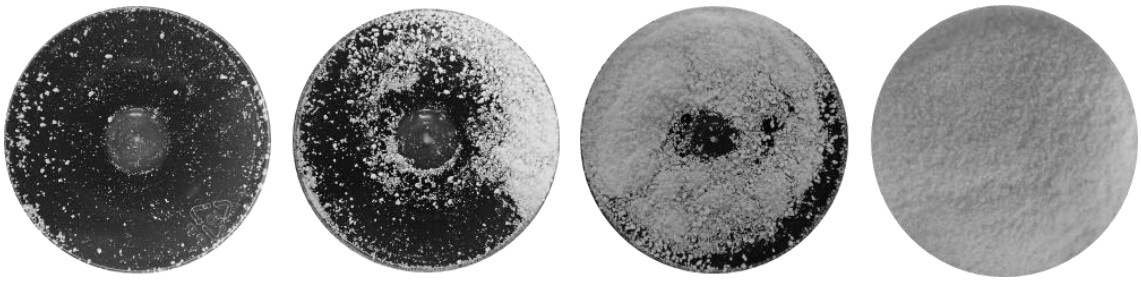

Figure 4. Images from left to right displaying the surface area coverage achieved by 10, 100, 500 and 3750 mg Ripestuff™ powder contained in a 70 mL specimen jar.

3.3 Headspace temperature and relative humidity

Sink headspace temperature during the experiment was 22.7 ± 1.0°C (mean ± SD) for all treatments. Similar profiles between treatments were also observed for sink RH, with all treatments exceeding 90% RH within 8 h (Figure 5b). Incomplete results for source RH were obtained due to equipment failure (i.e. loss of logger battery power) in the 100 mg and 3750 mg treatments. Source RH in the 500 mg treatment increased at a slower rate than in the 0 or 10 mg treatments, taking around twice as long (50 h as opposed to 26 h) to reach 90% RH (Figure 5a).

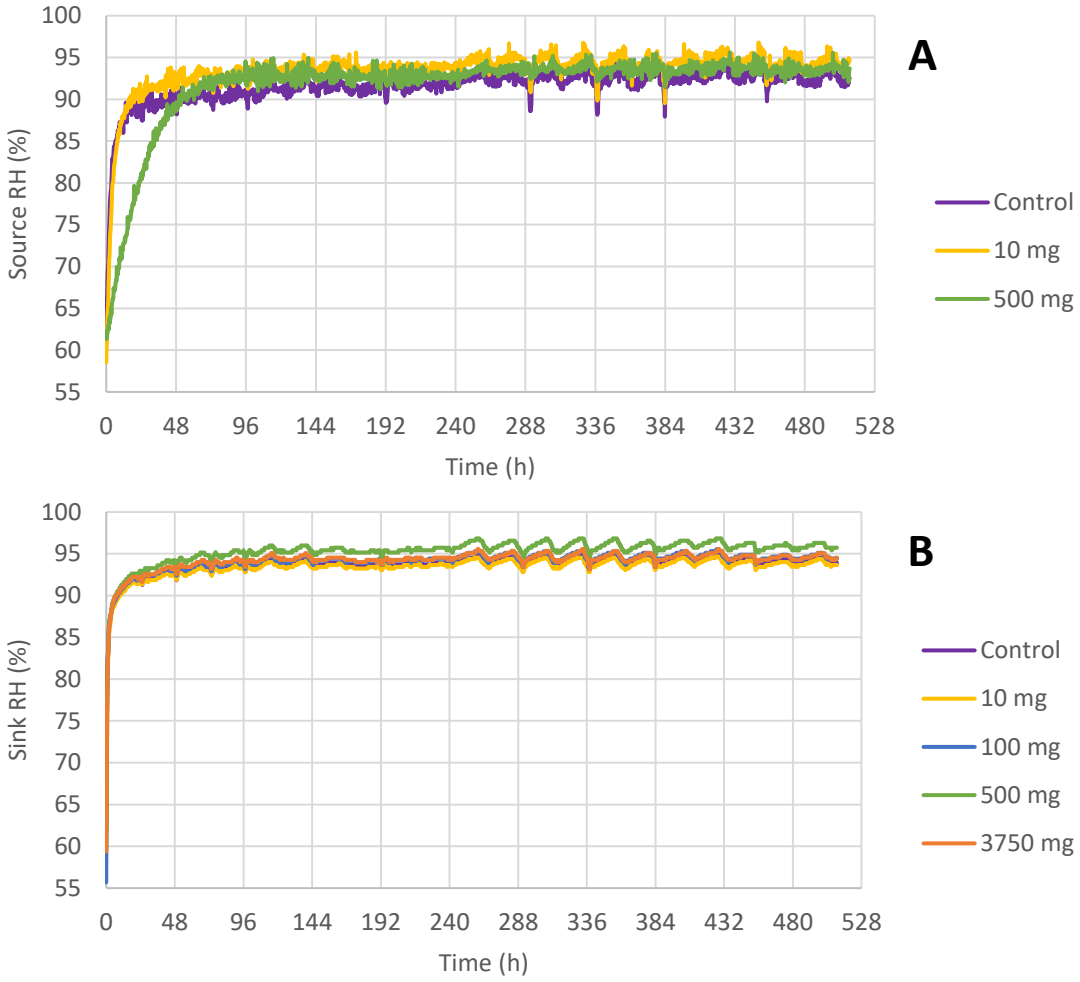

Figure 5. Headspace relative humidity inside (A) Ripestuff™ delivery system (source) and (B) 2 L chamber (sink) during 524 h experimental period. Treatments were Ripestuff™ delivery systems comprising 0, 10, 100, 500 or 3750 mg Ripestuff™ powder in a 70 mL specimen container with four holes of 0.5 mm diameter in the lid.

4 Discussion

Ethylene release profiles revealed the existence of an initial lag phase, the duration of which become longer as the amount of Ripestuff™ in the delivery system increased. As a result, the intermediate Ripestuff™ quantity of 500 mg was identified as the optimum amount to use with this delivery system in terms of achieving a high headspace ethylene concentration within 72 h (the typical period used for batch-ripening fruit). The finding supports the hypothesis that an inverse relationship exists between Ripestuff™ quantity and rate of ethylene release from the system. Hence, overloading of the system is the likely reason for its previous ineffectiveness in ripening mango fruit.

Ripestuff™ powder is comprised of an ethylene 'guest molecule' encapsulated within the hydrophobic cavity of an α-cyclodextrin (α-CD) 'host molecule' (Figure 6; Ho et al., 2011). This cavity can accommodate up to six water molecules when in a guest-free state (Angelova et al., 2017). In

order for ethylene release to occur, water molecules are required to displace the ethylene molecule from the α-CD cavity. It is not known how many water molecules must be bound to the α-CD molecule to facilitate ethylene release. However, a study on α-CD encapsulation of d-limonene showed that four water molecules needed to be displaced to accommodate the guest molecule (Yoshii et al., 1994). It seems likely that the reverse might also be true – binding of four water molecules to the α-CD molecule would facilitate release of the guest molecule.

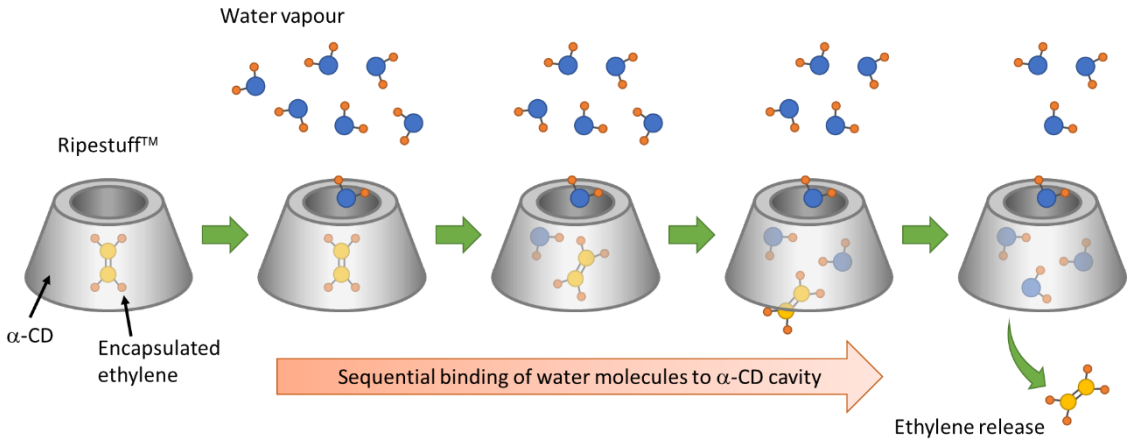

Figure 6. Proposed mechanism for ethylene release from Ripestuff™ powder based on the α-cyclodextrin (α-CD) hydration model developed by Angelova et al. (2017).

Hydration of the α-CD cavity is thought to occur sequentially (Figure 6), with the first water molecule becoming firmly bound to the narrow rim aperture of the cavity and acting as an 'anchor' for subsequent water binding (Angelova et al., 2017). The affinity of water for this initial binding site of the α-CD cavity is greater than its affinity for the second binding site of the cavity. Similarly, the affinity of water for the third binding site is higher than that for the fourth binding site. On this basis, it is plausible that water molecules would preferentially be distributed to all readily available 'initial' binding sites of α-CD molecules in the system before binding to the second site commences. Likewise, binding of water to all readily available third sites would take precedence over binding to the fourth site. Such a pattern of water binding would result in incomplete filling of α-CD cavities (and, therefore, no ethylene release) until sufficient water was present to fill all available α-CD cavities.

According to this theory, larger Ripestuff™ quantities have more α-CD cavities to fill and would need to absorb more water to trigger ethylene release. In the current delivery system, the amount of water available for binding is limited by the rate of moisture diffusion through the four holes in the lid. Hence, as Ripestuff™ quantity increases, more time would be needed for sufficient moisture to diffuse into the Ripestuff™ container, fill the α-CD cavities and trigger ethylene release. Ethylene release profiles observed in the current study support this theory.

Caking of the Ripestuff™ surface layer observed in the 500 and 3750 mg treatments at the end of the experiment may have created a barrier to moisture and ethylene diffusion between the powder and its headspace. The lack of difference between initial and final moisture content of Ripestuff™

powder from these treatments suggests that moisture diffusion was impeded. Furthermore, ethylene release from these treatments plateaued at ~80% despite almost no residual ethylene being detected in the Ripestuff™ powder. The 15-17% ethylene unaccounted for in these systems may have been released from the α-CD molecule but remained trapped within the void space between powder particles. Disruption of the surface layer caused by subsequent sampling is likely to have allowed any trapped gaseous ethylene to be lost to the atmosphere.

In conclusion, this study has highlighted the need for careful matching of Ripestuff™ quantity and delivery system to ensure ethylene release (and ultimately, fruit ripening) occurs in a timely fashion. It remains to be seen whether the optimum quantity of 500 mg Ripestuff™ powder identified here produces adequate levels of ethylene in a basket configuration to trigger fruit ripening. Further refinement of the prototype delivery system in terms of increasing the number of holes in the lid to accommodate faster moisture diffusion and, thereby, faster ethylene release from large Ripestuff™ quantities is recommended. Allowance may also need to be made for the potential (but as yet unproven) issue of ethylene becoming entrapped by the surface caking that occurs in Ripestuff™ powder when its quantity is large enough for particles to form a contiguous mass.

5 References

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Appendix G
DEVELOPMENT OF A PROTOTYPE RIPESTUFF™ DELIVERY SYSTEM CAPABLE OF SUSTAINED ETHYLENE RELEASE

UQ RESEARCH REPORT 5

prepared for
ACIAR Project HORT/2012/098

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Summary

Prototype ‘dry’ Ripestuff™ delivery systems (i.e. those that contain no added water) have generally been unable to trigger fruit ripening in a basket configuration. However, new knowledge suggests that the problem may have been caused by overloading of the system with Ripestuff™ powder, resulting in little ethylene release within the 72 h treatment period. Here we investigated ethylene release from ‘wet’ and ‘dry’ delivery systems comprising different combinations of Ripestuff™ quantity and number of holes in the lid of the Ripestuff™ container. Ethylene release from ‘dry’ delivery systems was strongly related to the ratio of these two factors. As Ripestuff™ quantity increased and/or hole number decreased (as indicated by a high ratio), the level of ethylene release at a given point in time decreased. No single Ripestuff™ delivery system was capable of sustained ethylene release for 72 h. However, it was suggested that combined use of multiple delivery systems with differing ratios of Ripestuff™ quantity to hole number could theoretically achieve the desired level of ethylene supply over this period. Two such combinations were proposed and recommended to be tested for batch ripening of mango fruit.

Contents

1 Introduction

Reliable batch ripening of fruit using a prototype Ripestuff™ delivery system (comprising a 70 mL specimen container with four 0.5 mm holes in the lid) requires the inclusion of water to facilitate ethylene release (Lacap and Bayogan, 2019, Experiments 8 and 9; Perkins and Joyce, 2019c). From a practical viewpoint, it would be preferable to eliminate this need for water addition to the system. Use of 'dry' delivery systems (i.e. systems with no added water) to batch ripen fruit has generally been unsuccessful (Lacap and Bayogan, 2019, Experiments 2.2 and 3). However, it has since been revealed that overloading with Ripestuff™ powder is likely to have hindered ethylene release from these systems (Perkins and Joyce, 2019d) and that the problem might be rectified by reducing the amount of Ripestuff™ powder and/or increasing the number of holes in the Ripestuff™ container lid.

Ripening of mango fruit by exposure to 10 $\mu L.L^{-1}$ ethylene at 20°C for 3 d is considered optimal in terms of achieving desirable fruit firmness and peel colour (Nguyen, 2003). Hence, the aim of the current study was to develop a new prototype 'dry' delivery system that is capable of achieving sustained ethylene release for a period of 72 h. Ethylene release from delivery systems employing different combinations of hole number and Ripestuff™ quantity was regularly monitored in a 2 L static chamber configuration. A limited number of 'wet' delivery systems were also included in the experiment for comparison purposes. Ethylene release data were modelled using Avrami's equation (Avrami, 1940), which has previously been used by Ho et al. (2011) to accurately describe ethylene release from α-cyclodextrin (α-CD). The fitted models enabled ethylene release rates for each delivery system to be predicted and, thus, their potential suitability for batch ripening of mango fruit to be determined.

2 Methodology

2.1 Ripestuff™ powder

The experiment used a batch of Ripestuff™ powder prepared in 2017 at The University of Queensland (St Lucia, Australia) by encapsulation of ethylene into amorphous α-CD. Moisture content was 7.48% (wet-weight basis) and ethylene concentration was 0.470 $mol.mol^{-1}$ α-CD when assessed at the time of the study. The powder was passed through a metal sieve (0.7 mm mesh size) to remove clumps that had formed during storage.

2.2 Ripestuff™ delivery system

Six delivery systems were employed in this experiment. Four were comprised of Ripestuff™ powder held within a 70 mL specimen container with either 4, 16, 32 or 64 holes of 0.5 mm ∅ in the lid. These were referred to as 'dry' delivery systems. A further two systems involved addition of 5 mL deionised water to Ripestuff™ powder held within a 70 mL specimen container with 1 or 4 holes in the lid. These were referred to as 'wet' delivery systems.

2.3 Chamber configuration

Chambers were 2 L preserving jars fitted with two headspace sampling ports, as described in UQ Research Report 1 (Perkins and Joyce, 2019a). A 10 mL aliquot of saturated KNO_3 solution in an unlidded 70 mL specimen container was added to each chamber to create a high humidity environment of ~94% at ambient temperature (Winston and Bates, 1960).

2.4 Treatments

The experiment was comprised of 18 treatments (Figure 1) which included three Ripestuff™ quantities of 0 (control), 500 or 1000 mg in combination with the six delivery systems described above. Treatments were named according to (hole number).(wet or dry).(Ripestuff™ quantity). For example, 16.D.1000 referred to a 'dry' delivery system containing 1000 mg Ripestuff™ and 16 holes in the lid.

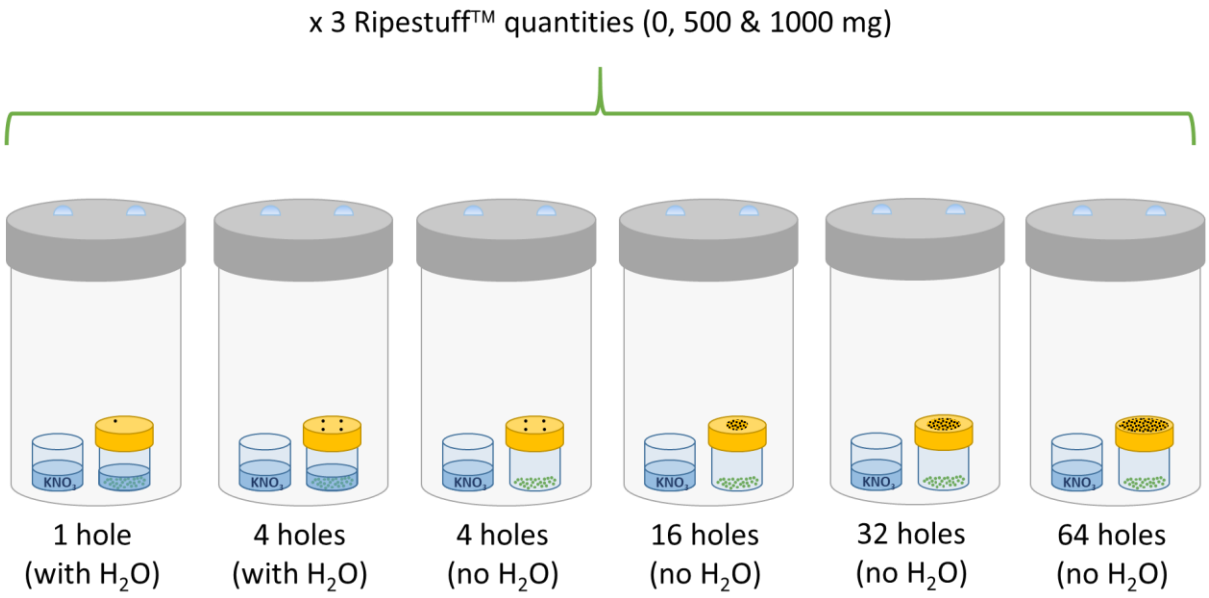

Figure 1. Treatments investigated for developing a sustained ethylene release Ripestuff™ delivery system in a high humidity (94% RH) environment. Variables included the number of holes in the lid of the Ripestuff™ container, the quantity of Ripestuff and the addition or no addition of 5 mL water to the container.

2.5 Data collection

2.5.1 Headspace ethylene concentration

A BD 1 mL disposable syringe with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm) was used to accurately sample 1 mL of headspace via a sampling port of each chamber at 3, 6, 9, 24, 32, 48 and 72 h. Treatments incorporating Ripestuff™ in solution (i.e. 'wet' treatments) were additionally sampled at 0.5 h, as ethylene release was expected to occur quickly in these treatments. During sampling, another syringe was used to introduce 1 mL of ethylene-free air into the chamber via the second sampling port to maintain pressure equilibrium. Headspace samples were immediately diluted by injection into a 40 mL headspace vial fitted with a PTFE/silicone septum screw cap (Macherey Nagel product code 702877, supplied by MicroAnalytix Pty Ltd, Taren Point, Australia). After ~3 min equilibration time, 2 mL diluted headspace was sampled using a BD 5 mL disposable syringe with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm) and immediately injected into a GC-FID, as described in UQ Research Report 1 (Perkins and Joyce, 2019a).

2.5.2 Residual Ripestuff™ ethylene concentration and moisture content

Upon opening the chambers at 72 h, Ripestuff™ sub-samples were immediately collected from the 'dry' treatments to determine residual moisture and ethylene concentrations. For residual ethylene determination, 5 mg Ripestuff™ was accurately weighed (to the nearest 0.1 mg) into 40 mL headspace vials to which 2.5 mL deionised water was then added and the vial immediately sealed. The vials were placed in a reciprocal shaking waterbath (Lab Companion™ BS-31) at 100 rpm for 30 min at 35°C then allowed to equilibrate with ambient laboratory conditions for 30 min. A headspace dilution step (as described in Section 2.5.1) was conducted prior to ethylene analysis by GC-FID.

For residual moisture determination, ~400 mg Ripestuff™ from each chamber was accurately weighed (to the nearest 0.1 mg) and dried to constant mass at 60°C to gravimetrically determine dry matter content (%) as:

$$\frac{final\ Ripestuff\ ™ mass}{initial\ Ripestuff\ ™ mass} \times 100$$

Dry matter was considered to represent the α-CD component of Ripestuff™ powder. The mass of residual moisture was obtained by subtracting the residual ethylene mass and the α-CD mass from the initial Ripestuff™ mass. Hence, moisture content (% wet-weight basis) was calculated as:

$$\frac{initial\ Ripestuff\ ™ mass\ - aCD\ mass - residual\ ethylene\ mass}{initial\ Ripestuff\ ™ mass} \times 100$$

2.5.3 Headspace temperature and relative humidity

Each Ripestuff™ container in Replicate 1 was fitted with Hygrochron HC data logger (DS1923; Thermochron Australia, Castle Hill, Australia) to record headspace temperature and relative humidity at 15 min intervals for the duration of the experiment. The same data were collected for the chamber headspace of five control (0 mg Ripestuff™) chambers in Replicate 1 using EasyLog data loggers (EL-USB-2; Lascar Electronics, Wiltshire, UK).

2.6 Ethylene release rate modelling

Modelling of ethylene release profiles for each delivery system was conducted using Avrami's equation (Avrami, 1940):

$$X = 1 - exp[-(kt^n)]$$

where *X* (-) is the release fraction of ethylene at time *t* (h), with a release rate constant of *k* (h^{-1}) and a release mechanism of *n* (-). Determination of the constants *n* and *k* was achieved by plotting ln(-ln(1-*X*)) against ln(*t*). This plot yields a straight line with gradient *n* and intercept ln(*k*). The values of *X* used to generate the linear plot were based on means calculated from ethylene release data obtained at each sampling time, which had been divided by 100 to convert the values from a percentage to a fraction.

Predicted *X* values generated by the model were converted to headspace ethylene concentrations by multiplication with the expected headspace concentration at full (100%) ethylene release. The derivative of these predicted headspace concentrations provided an estimate of the hourly ethylene

release rate ($\mu L.L^{-1}.h^{-1}$) at each point in time up to 72 h. The time at which the ethylene release rate was predicted to peak for each delivery system was reported as t_{max} (h).

2.7 Experimental design and data analysis

The experiment was a complete randomised design comprising three replicate chambers for each treatment. Staggered commencement times were used as in previous experiments, with a chamber being prepared every 3 min over a 2.7 h period to accommodate the time required for subsequent GC analyses.

Treatment effects on residual Ripestuff™ moisture content and ethylene concentration, ethylene release at 72 h and total measured ethylene at 72 h were determined by one-way analysis of variance using Minitab®, Version 17.3.1 (Minitab Pty Ltd, Sydney, Australia). Where significant differences were detected, means were compared using Fisher's LSD test at a significance level of 0.01. Linear regression analysis of the relationship between Ripestuff™ quantity to hole number ratio and ethylene release at 72 h was also conducted using Minitab®.

3 Results

3.1 Headspace ethylene concentration

Ethylene release into the chamber headspace occurred rapidly from treatments in which water had been added to the Ripestuff™ delivery system, more so when lids had four holes compared with one hole (Figure 2). In comparison to these 'wet' treatments, 'dry' treatments showed an initial lag phase of between 6 and 32 h before any substantial proportion of ethylene was released. Lag time was shorter with increasing hole number and decreasing Ripestuff™ quantity. Nearly identical ethylene release profiles were observed for treatments with the same ratio of Ripestuff™ quantity to hole number, these being 16.D.500 and 32.D.1000 (ratio = 31.25), and 32.D.500 and 64.D.1000 (ratio = 15.625).

Complete ethylene release within 72 h occurred only in the 4.W.500 treatment. For all other treatments, the proportion of ethylene released at 72 h ranged from 30% (for 4.D.1000) to 92% (for 64.D.500). A strong negative linear relationship ($R^2 = 0.98$, $P < 0.001$) was observed between the ratio of Ripestuff™ quantity to hole number and ethylene release at 72 h (Figure 3). The regression equation for this relationship was used to predict the maximum Ripestuff™ load capable of achieving 90% ethylene release at 72 h from delivery systems with different hole numbers in the lid (Table 1).

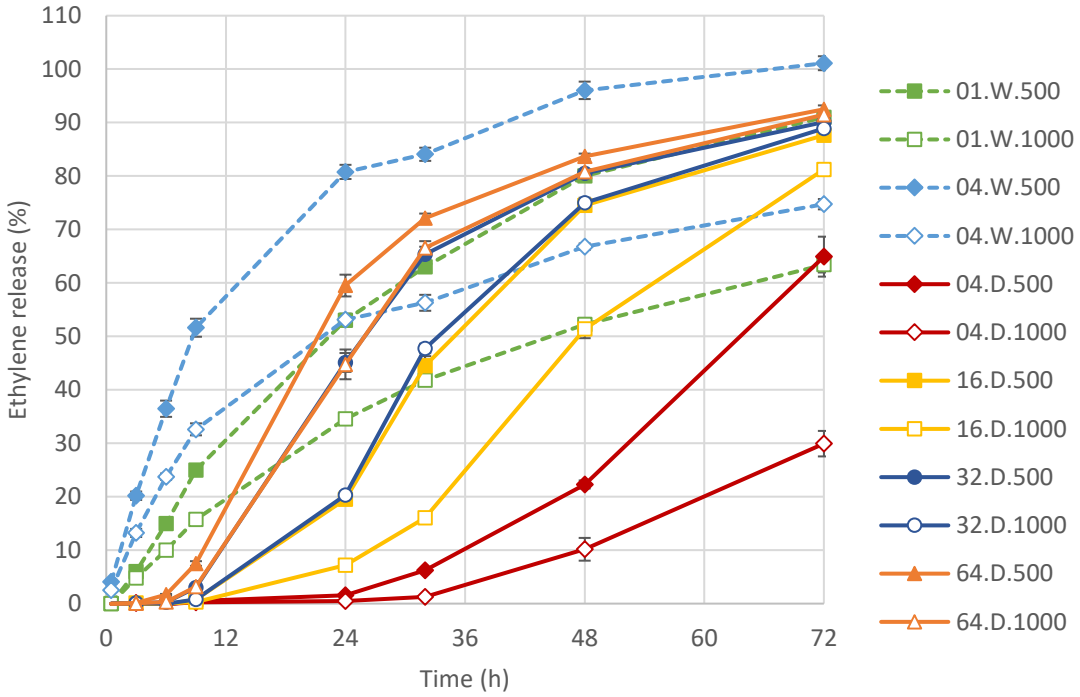

Figure 2. Ethylene release (expressed as a percentage of total ethylene in the system) from various Ripestuff™ delivery systems held at 23°C and 94% RH in a 2 L chamber. Error bars represent ± SEM (*n* = 3) and are too small to be visible in some instances.

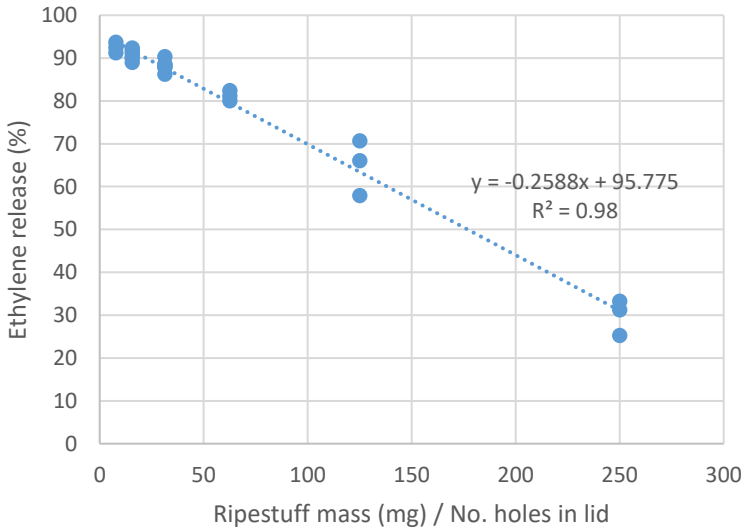

Figure 3. Relationship between Ripestuff™ quantity to hole number ratio and ethylene release at 72 h from prototype delivery systems in a 2 L static chamber at 23°C and 94% RH ($P < 0.001$).

Table 1. Predicted maximum loading limit for various 'dry' Ripestuff™ delivery systems to achieve 90% ethylene release at 72 h in a 2 L static chamber at 23°C and 94% RH.

No. holes in lid	Predicted maximum loading (mg Ripestuff™ powder)
1	22
4	89
16	357
32	714
64	1428

In terms of absolute ethylene headspace concentration in the chamber, the highest values recorded at 72 h were achieved using 64.D.1000 and 32.D.1000 (Figure 4). 'Dry' treatments with the same hole number initially released less ethylene from 1000 mg than from 500 mg Ripestuff™. This pattern of release is consistent with previous findings that larger Ripestuff™ quantities need to absorb more water before ethylene release will occur (Perkins and Joyce, 2019d). Conversely, 'wet' treatments with the same hole number released higher ethylene concentrations from 1000 mg as opposed to 500 mg Ripestuff quantity at all evaluation times (Figure 4).

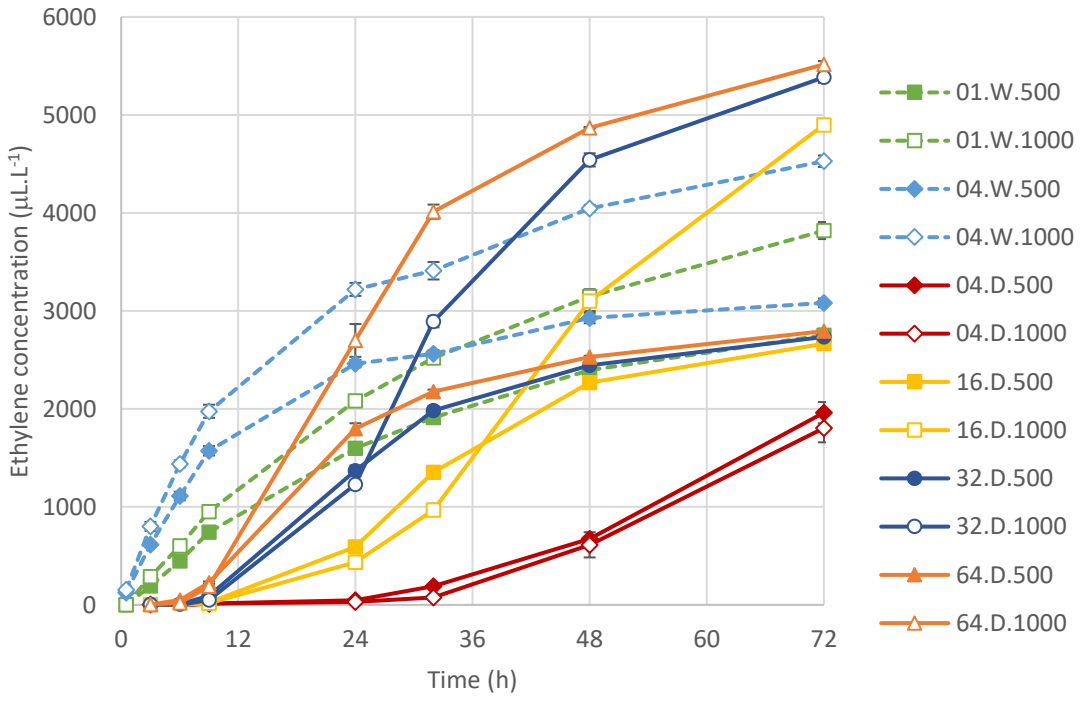

Figure 4. Headspace ethylene concentration in a 2 L chamber containing various Ripestuff™ delivery systems and held at 23°C and 94% RH. Error bars represent ± SEM (*n* = 3) and are too small to be visible in some instances.

Modelling of ethylene release curves using the Avrami equation enabled prediction of ethylene release rates over the course of 72 h for each treatment (Figure 5). Peak ethylene release rate occurred almost immediately for most 'wet' treatments, whereas peak times for 'dry' treatments ranged from 20 to more than 72 h (Table 2). No single treatment achieved sustained ethylene release for 72 h (Figure 6). However, addition of predicted ethylene release rates from multiple treatments revealed that a combination of delivery systems may provide a constant supply of ethylene during this period. Combined use of five delivery systems (04.W.500, 04.D.500, 04.D.1000, 16.D.500 and 64.D.500) was estimated to produce a stable ethylene release rate of ~175 $\mu L.L^{-1}.h^{-1}$. A second combination comprising seven delivery systems (04.W.1000, 04.D.500, 2 x 4.D.1000, 32.D.1000 and 2 x 64.D.500) was estimated to produce a higher rate of ~300 $\mu L.L^{-1}.h^{-1}$ across the 72 h period (Figure 6).

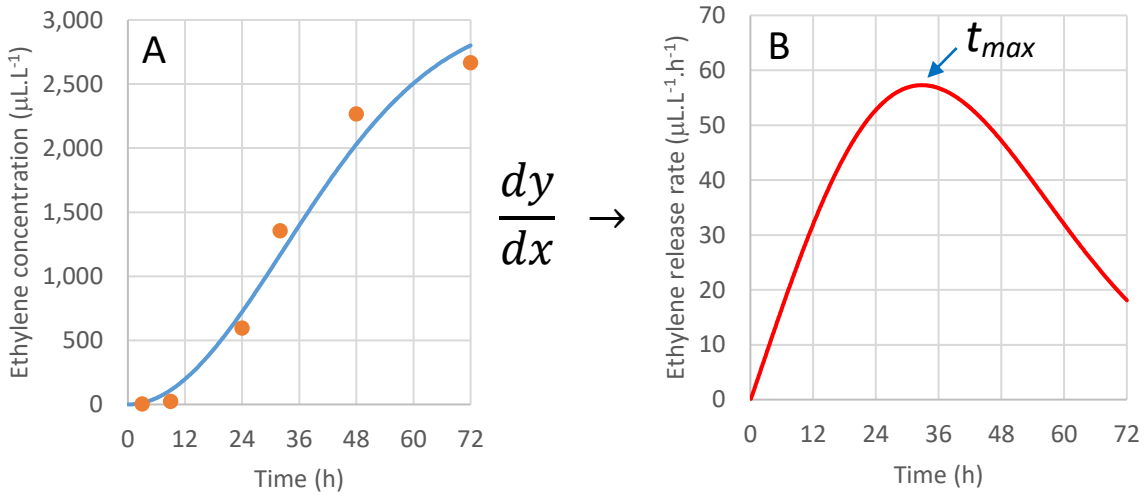

Figure 5. Time of peak ethylene release rate (t_{max}) was predicted for each treatment (16.D.500 shown here as an example) by: (A) using the Avrami equation to fit an ethylene release curve (blue line) to the experimental data (orange markers), and (B) plotting the derivative of the fitted curve (red line) to estimate ethylene release rate.

Table 2. Time of peak ethylene release rate (t_{max}), release rate constant (k), release parameter (n), and coefficient of determination (R^2) for ethylene release from various Ripestuff™ delivery systems based on Avrami's equation at 94% RH and 23°C.

Treatment	t_{max} (h)	k (h^{-1})	n	R^2
01.W.500	4.6	-3.8899	1.1255	0.9962
01.W.1000	< 0.5	-3.9590	0.9481	0.9942
04.W.500	< 0.5	-2.5030	0.9314	0.9970
04.W.1000	< 0.5	-2.5939	0.6962	0.9875
04.D.500	64.5	-15.931	3.7492	0.9934
04.D.1000	> 72.0	-18.176	4.0391	0.9873
16.D.500	32.8	-7.7109	2.0131	0.9432
16.D.1000	48.0	-13.002	3.2643	0.9997
32.D.500	28.5	-7.7017	2.1082	0.9277
32.D.1000	33.3	-7.6753	1.9904	0.9393
64.D.500	19.6	-5.9061	1.6821	0.9271
64.D.1000	28.1	-7.6744	2.1085	0.9341

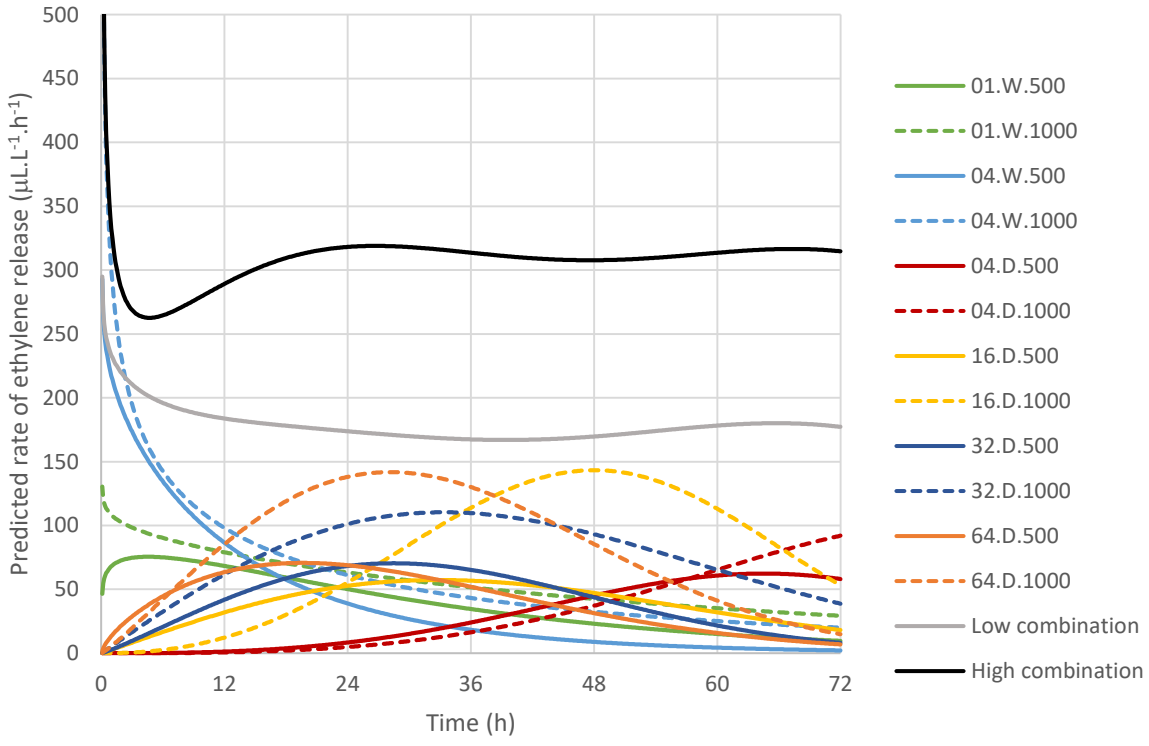

Figure 6. Predicted ethylene release rates from various single or combined Ripestuff™ delivery systems, based on Avrami's equation at 94% RH and 23°C. Low combination was calculated by adding the predicted release rates of 04.W.500, 04.D.500, 04.D.1000, 16.D.500 and 64.D.500. High combination was calculated by adding the predicted release rates of 04.W.1000, 04.D.500, 4.D.1000 (x2), 32.D.1000 and 64.D.500 (x2).

3.2 Residual Ripestuff™ ethylene concentration and moisture content

Evaluation of residual ethylene content in Ripestuff from 'dry' treatments at 72 h revealed that most treatments retained less than one tenth of their ethylene (Table 3). However, treatments with a Ripestuff™ quantity to hole ratio of 62.5 or higher retained significantly ($P < 0.01$) more ethylene. Reasonably good agreement existed between Ripestuff™ residual ethylene content and ethylene release measurements (Table 3). For example, Ripestuff™ from 64.D.500 had retained 5% of its original ethylene content at 72 h and chamber headspace ethylene concentrations suggested 92% had been released by this time. Hence, 97% of total ethylene in the system had been accounted for. Similar values were also obtained for other treatments, with the exception of 91% for 04.D.500. No difference in Ripestuff™ moisture content was observed between treatments at 72 h or between initial (0 h) and final (72 h) values for any treatment (Table 3).

Table 3. Ethylene concentration and moisture content of Ripestuff™ powder from 'dry' treatments before (initial) and after (final) 72 h treatment period. Relative quantities of ethylene retained in the powder and released into the chamber headspace at 72 h were combined to give a total quantity of measured ethylene (expressed as a percentage of total ethylene in the system). Means within a column and followed by the same letter do not significantly differ according to Fisher's LSD test ($P > 0.01$).

Treatment	Moisture content (%)	Ripestuff™ ethylene concentration		Ethylene release (%)*	Total measured ethylene (%)
		$mol.mol^{-1}$	% of initial		
Initial	7.48 a	0.470 a	100 a	-	-
Final					
04.D.500	7.48 a	0.125 c	27 c	65 c	91 b
04.D.1000	8.26 a	0.313 b	67 b	30 d	97 a
16.D.500	7.65 a	0.036 de	8 e	88 ab	95 ab
16.D.1000	8.10 a	0.068 d	14 d	81 b	96 ab
32.D.500	8.67 a	0.031 e	8 e	90 a	97 a
32.D.1000	7.92 a	0.038 de	8 e	89 a	97 a
64.D.500	7.65 a	0.023 e	5 e	92 a	97 a
64.D.1000	8.41 a	0.027 e	6 e	91 a	97 a

**Values from Figure 2.*

3.3 Headspace temperature and relative humidity

Chamber temperature throughout the experiment was 23.1 ± 1.0°C (mean ±SD). Chamber RH was similar for all five control treatments measured, reaching 90% RH by 12 h (data not shown). Temperature of the Ripestuff™ delivery system during the experiment was consistent between treatments and ranged from 21.2 to 24.7°C, with a mean of 22.9°C (Figure 7). RH of the delivery system increased faster as the number of holes in the lid increased and the Ripestuff™ quantity decreased (Figure 8A-D). All treatments attained RH equilibrium between the chamber and delivery system headspaces by 72 h, with the exception of 04.D.1000 which failed to reach a delivery system RH of 90%. Almost identical RH profiles were exhibited by delivery systems with the same ratio of

Ripestuff™ quantity to hole number (Figure 8 E-F), these being 16.D.500 and 32.D.1000 (ratio = 31.25), and 32.D.500 and 64.D.1000 (ratio = 15.625).

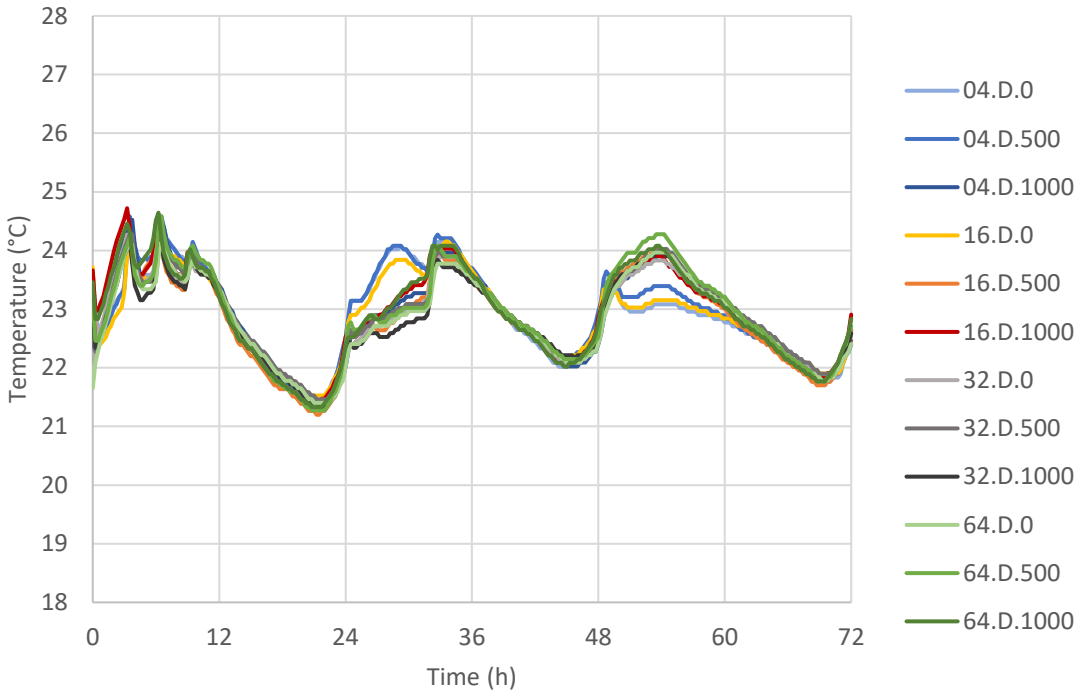

Figure 7. Headspace temperature inside various Ripestuff™ delivery systems held at 23°C and 94% RH in a 2 L static chamber.

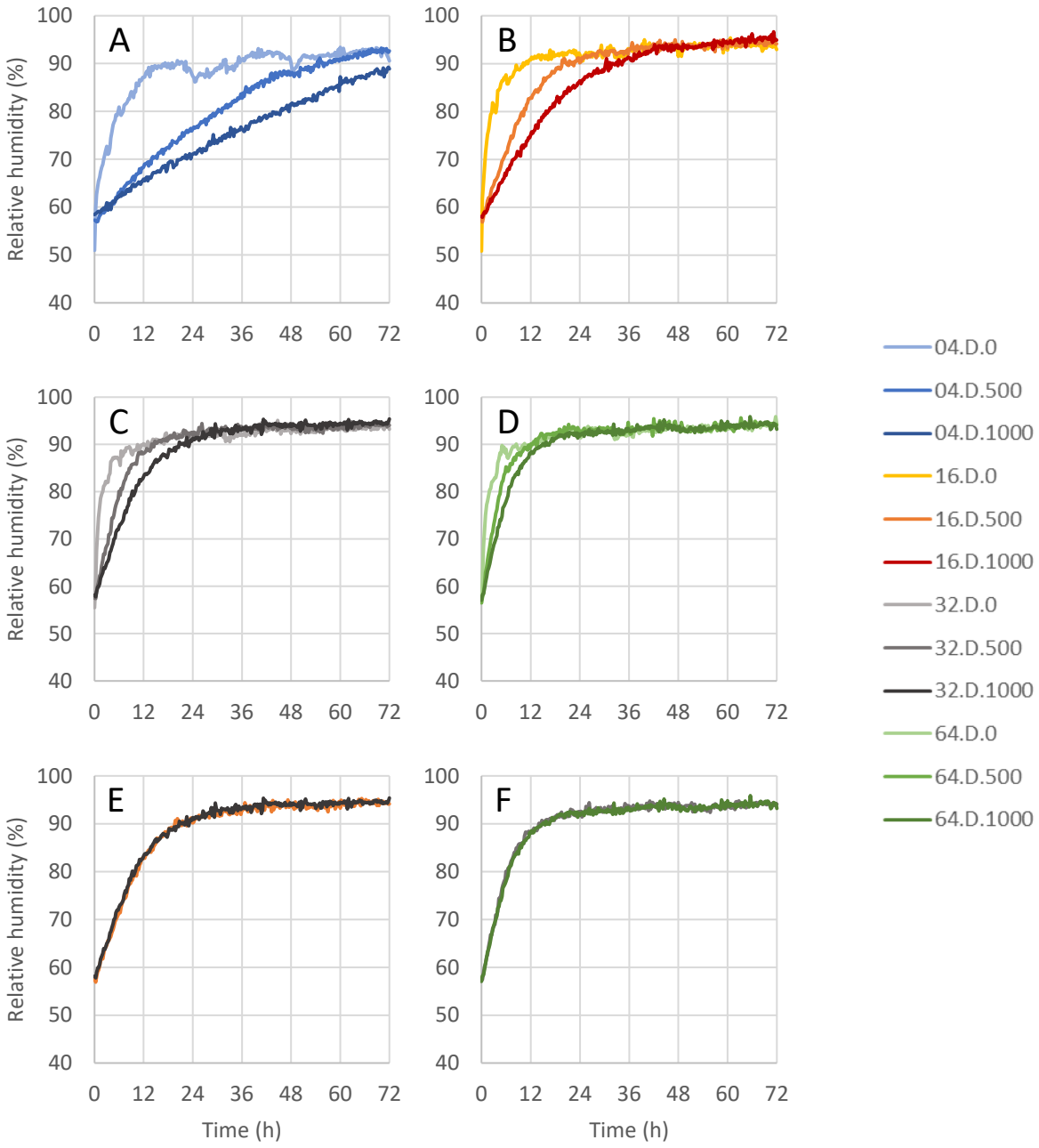

Figure 8. Headspace relative humidity (RH) inside Ripestuff™ delivery systems with (A) 4 holes, (B) 16 holes, (C) 32 holes or (D) 64 holes in the lid, and held at 23°C and 94% RH in a 2 L static chamber. Delivery systems with a Ripestuff™ quantity to hole number ratio of (E) 31.25 or (F) 15.625 were plotted together to highlight the similarities in their RH profiles.

4 Discussion

This study showed that ethylene release from 'dry' Ripestuff™ delivery systems can be manipulated by altering the amount of Ripestuff™ powder and the number of holes in the lid. In particular, the ratio of Ripestuff™ quantity to hole number was found to be a strong predictor of the level of ethylene release at a given point in time. The influence of hole number can be explained by Fick's Law which states that rate of diffusion is directly proportional to the surface area available for

diffusion (Berk, 2018). In the case of 'dry' Ripestuff™ delivery systems, the limiting process appears to be the rate of moisture diffusion in, not ethylene diffusion out (Perkins and Joyce, 2019a,b). As hole number increases, so too does the surface area available for moisture to diffuse into the delivery system from the high humidity external environment (i.e. the chamber). Larger Ripestuff™ quantities need to absorb more moisture before ethylene release will commence, as our earlier work has shown (Perkins and Joyce, 2019d). Hence, as Ripestuff™ quantity increases and/or hole number decreases (as indicated by a high ratio of these two factors), the level of ethylene release at a given point in time decreases.

Inconsistent ripening responses previously observed in mango fruit exposed for 72 h to a 'dry' Ripestuff™ delivery system (Lacap and Bayogan, 2019, Experiments 2.1, 2.2 and 3) were considered to be a result of overloading the system with Ripestuff™ powder (Perkins and Joyce, 2019d). In those situations, Ripestuff™ quantities of 1250 to 5000 mg were placed into 70 mL specimen containers with 4 holes in the lid. It has now been revealed that the amount of Ripestuff™ used in that system should not exceed 89 mg if 90% ethylene release is to be achieved within 72 h at 23°C. It should be noted that this load limit was determined using a 2 L static chamber and is likely to differ slightly when applied to the basket configuration used to ripen fruit. Even so, the finding points to a staggering degree of overloading in the abovementioned fruit ripening experiments.

This study aimed to develop a Ripestuff™ delivery system capable of sustained ethylene release over a 72 h period. No single treatment was found to provide this constant supply of ethylene. However, by combining multiple delivery systems it was considered theoretically possible to achieve the desired level of sustained ethylene release. A combination of delivery systems capable of generating ~300 $\mu L.L^{-1}.h^{-1}$ ethylene was proposed because this was the initial release rate observed in 04.W.500 – a delivery system recently shown to produce reasonable ripening of 'Carbao' mango fruit (Lacap and Bayogan, 2019, Experiment 8). In that experiment, 04.W.500 produced a short-lived initial spike of 26 $\mu L.L^{-1}$ ethylene in the basket headspace. Exposure of mango fruit to 10 $\mu L.L^{-1}$ ethylene at 20°C for 3 d is considered optimal for achieving desirable fruit firmness and peel colour (Nguyen, 2003). Higher ripening temperatures and ethylene concentrations promote undesirable skin blotchiness (Nguyen, 2003), as was observed in the abovementioned UPMin experiment. Hence, a combination of delivery systems capable of generating a lower ethylene dose of ~175 $\mu L.L^{-1}.h^{-1}$ have also been proposed here. This 'low' combination may offer uniform ripening without causing skin blotchiness.

For most treatments, the combined measurements of headspace ethylene in the chamber and residual ethylene in Ripestuff™ at 72 h accounted for ≥ 95% of all ethylene in the system. One exception was the 04.D.500 treatment, for which only 91% of all ethylene was measured. Ethylene release from this delivery system was entering a stage of rapid increase at 72 h and it is likely that substantial build-up of ethylene gas in the Ripestuff™ container headspace was occurring at the time. The gas would have been lost to the atmosphere when the container was opened at 72 h to sample the Ripestuff™ powder for residual ethylene analysis. A similar delivery system used to batch-ripen bananas produced a headspace ethylene concentration that was 733 times greater in the Ripestuff™ container than in the basket at 72 h (Perkins and Joyce, 2019c). The occurrence of a similarly high concentration in the current study could account for the remaining 9% of ethylene in the system.

In conclusion, no single Ripestuff™ delivery system tested here was capable of sustained ethylene release for 72 h. However, this goal may be achieved by the combined use of multiple delivery systems with differing ratios of Ripestuff™ quantity to hole number. The next step is to demonstrate that the suggested delivery system combinations are capable of achieving uniform fruit ripening in a

'real world' situation. Batch ripening of fruit in the Philippines is likely to be conducted at higher temperatures than the 23°C used in this experiment and therefore result in larger, sharper and earlier ethylene release rate peaks. Heat generated from ripening fruit may also influence temperature. Production of endogenous ethylene, carbon dioxide and water vapour by fruit are additional variables to consider. Furthermore, newspaper-lined baskets are not a static system and loss of ethylene to the external environment introduces an unknown variable.

Both the 'high' and 'low' combinations proposed here use a large number of Ripestuff™ containers (seven and five, respectively). Use of this many containers serves the immediate research purpose of proving that sustained ethylene release is achievable, but it does not provide a practical solution for industry. If one of the combinations proves successful for mango ripening, further work will be needed to incorporate its essential elements into a delivery system that is commercially viable.

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Appendix H

AIR PRESSURE OSCILLATION EFFECTS ON RIPESTUFF™ ETHYLENE RELEASE FROM A PROTOTYPE DELIVERY SYSTEM

UQ RESEARCH REPORT 6

prepared for
ACIAR Project HORT/2012/098

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Summary

Ethylene release from a 'dry' prototype Ripestuff™ delivery system (i.e. one without added water) tends to occur very slowly when used to ripen mango fruit in newspaper-lined baskets but very rapidly when used to ripen palletised cartons of mango fruit during road shipment. This study aimed to determine whether air pressure oscillations generated during transit could be responsible for the increased rate of ethylene release observed in this situation. Repeated air pressure oscillations of 0, 250, 500, 1000 or 2000 Pa were applied over a period of 48 h to a prototype 'dry' delivery system held within a 2 L humidified chamber. Headspace RH of the delivery system showed a slight but significant ($P < 0.05$) increase in response to pressure change. However, no effect on ethylene release was observed between treatments. Ethylene release commenced at 6 h and had reached completion by 48 h in all treatments. A comparison with heavy vehicle cabin pressure values obtained from scientific literature suggest that the pressure treatments investigated in this study may have greatly over-estimated the magnitude and greatly under-estimated the frequency of pressure oscillations encountered during road transportation. It was speculated that actual pressure oscillations during shipment could induce a rapid increase in delivery system headspace RH which would in turn promote ethylene release. Recommendations were made to characterise the magnitude and frequency of in-transit pressure oscillations encountered within a fruit consignment and evaluate their effects on Ripestuff™ ethylene release from a 'dry' delivery system under controlled laboratory conditions.

Contents

1 Introduction

Our earlier studies have shown that ethylene release from a prototype 'dry' Ripestuff™ delivery system (i.e. 70 mL specimen container with four 0.5 mm ∅ holes in the lid) is insufficient to trigger uniform ripening of fruit in newspaper-lined baskets when relatively large Ripestuff™ quantities (> 1.25 g) are employed (Lacap and Bayogan, 2019, Experiments 2.2 and 3; Perkins and Joyce, 2019c). However, Duong et al. (2017) showed that 12 g Ripestuff™ powder incorporated in this delivery system initiated ripening of 'Honey Gold' mangoes when exposed to the fruit for 3 to 4 d in-transit (i.e. in refrigerated road containers maintained at 14-23°C). Ethylene concentrations in these road containers reached a peak of ~170 $\mu L.L^{-1}$ after 6 h when 20 Ripestuff™ delivery systems were included in the shipment.

The rapid ethylene release observed from this very large amount of Ripestuff™ powder might be explained by fluctuations in air pressure caused by flexing of the road container as it travels. It is hypothesised that such pressure fluctuations would force moisture laden air from around the fruit into the delivery system (under periods of high pressure) and back out again (under periods of low pressure). This continual exchange of air would likely lead to a rapid rise in relative humidity (RH) of the delivery system which would, in turn, promote ethylene release from the Ripestuff™ powder.

To test this theory, a prototype 'dry' delivery system was subjected to oscillating air pressures of differing magnitude in 2 L humidified static chambers. The effects of these treatments on headspace ethylene concentration, temperature and RH of both the chamber and delivery system were quantified over a 48 h period.

2 Methodology

2.1 Ripestuff™ powder

The experiment used a batch of Ripestuff™ powder prepared in 2017 at The University of Queensland (St Lucia, Australia) by encapsulation of ethylene into amorphous α-CD. Moisture content was 6.16% (wet-weight basis) and ethylene concentration was 0.541 $mol.mol^{-1}$ α-CD when assessed at the time of the study. The powder was passed through a metal sieve (0.7 mm mesh size) to remove clumps that had formed during storage.

2.2 Ripestuff™ delivery system

The experiment employed a delivery system comprising 0 (control) or 10 mg Ripestuff™ powder in a 70 mL specimen container with four 0.5 mm ∅ holes in the lid.

2.3 Chamber configuration

Chambers were 2 L preserving jars fitted with four headspace sampling ports, as described in UQ Research Report 1 (Perkins and Joyce, 2019a). A sheet of chromatography paper (Whatman™ 3MM CHR grade, 11 x 14 cm; catalogue no. 3030-6185) was placed against the inner wall of each chamber and wetted with 5 mL deionised water.

2.4 Treatments

The experiment consisted of six treatments (Figure 1), whereby chambers containing delivery systems with 10 mg Ripestuff™ powder were subjected to pressure oscillations of 0, 250, 500, 1000 or 2000 Pa. Chambers containing delivery systems with no Ripestuff™ powder and subjected to no

pressure change served as the control. Pressure oscillations of 250, 500, 1000 and 2000 Pa were achieved by removal and subsequent re-injection of 4.6, 9, 19 and 37 mL of chamber headspace, respectively, using an appropriately sized BD disposable graduated syringe fitted with a 25 gauge BD PrecisionGlide™ needle (0.5 mm x 25 mm). These headspace volumes represented 0.25, 0.5, 1 and 2% of the total chamber headspace volume, respectively. One 'pumping event' consisted of three pressure oscillations applied over a 3 min period (Figure 2). Chambers were subjected to hourly pumping events for 6 h each day. Hence, the total number of oscillations applied to each chamber during the experiment was 54. The first pumping event commenced immediately upon enclosure of the delivery system within the chamber (i.e. 0 h).

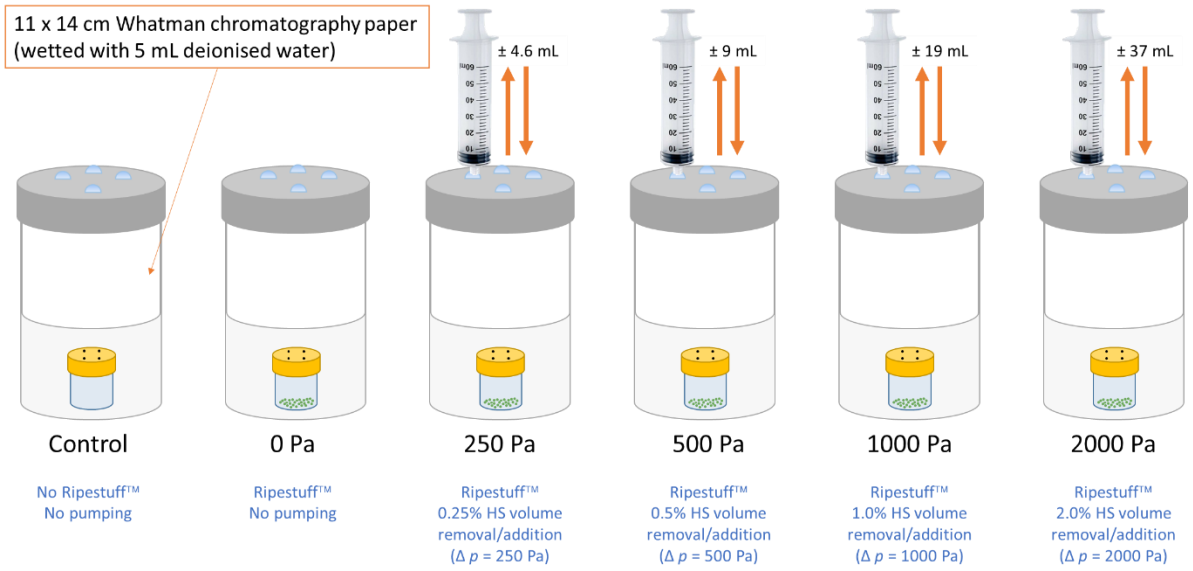

Figure 1. Treatments investigated for air pressure oscillation effects on ethylene release from a Ripestuff™ delivery system in a high humidity 2 L static chamber. A total of 54 oscillations of varying magnitude (equivalent to a change in chamber pressure of 0, 250, 500, 1000 or 2000 Pa) were applied to each chamber during a 48 h period by removal and subsequent re-injection of an appropriate headspace (HS) volume.

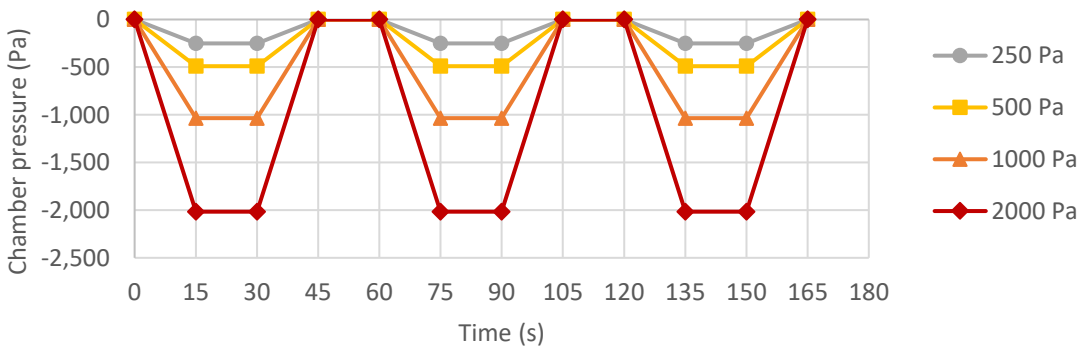

Figure 2. Chamber pressure profile during one pumping event (conducted hourly for 6 h each day).

2.5 Data collection

2.5.1 Headspace ethylene concentration

Sampling and analysis of chamber headspace ethylene concentration was conducted using the procedure described in UQ Research Report 1 (Perkins and Joyce, 2019a). Sampling from each chamber was conducted at 3, 6, 9, 24, 30 and 48 h. At the end of the experiment (i.e. 48 h), a single headspace sample of 2 mL was immediately taken from each Ripestuff™ container (via one of the four holes in the lid) to determine whether ethylene partitioning between the headspace inside and outside the container had reached equilibrium.

2.5.2 Headspace temperature and relative humidity

Each chamber in Replicate 1 (excluding the control) was fitted with an EasyLog data logger (EL-USB-2; Lascar Electronics, Wiltshire, UK) affixed to the inner wall with self-adhesive Velcro. Ripestuff™ containers in these same chambers were fitted with a Hygrochron HC data logger (DS1923; Thermochron Australia, Castle Hill, Australia) affixed to the inner surface of the lid with self-adhesive Velcro. Subsequent to the main experiment, headspace temperature and RH was monitored at 2 min intervals in a separate set of humidified chambers containing a 10 mg Ripestuff™ delivery system and subjected to either the 0 or 2% pressure oscillation treatments for 5 h (resulting in a total of 15 oscillations being applied to each chamber). No Velcro was used to affix the loggers in this instance – EasyLog data loggers were placed loosely in the chamber base and Hygrochron HC data loggers were placed sensor-side up in the base of each Ripestuff™ container.

2.6 Experimental design and data analysis

The main experiment was a complete randomised design comprising three replicate chambers for each treatment. Staggered commencement times were used as in previous experiments, with a chamber being prepared every 3 min over a 54 min period to accommodate the time required for subsequent GC analyses. The follow-up experiment was a complete randomised design comprising two replicate chambers for each treatment.

Ethylene release data from the main experiment and headspace RH data from the follow-up experiment were subjected to a two-factor (treatment x sampling time) analysis of variance using Minitab®, Version 17.3.1 (Minitab Pty Ltd, Sydney, Australia). Means were compared using Fisher's LSD test at a significance level of 0.05. Ethylene release data from Replicate 1 were excluded from the analysis for reasons discussed in Section 3.1.

3 Results

3.1 Headspace ethylene concentration

No significant difference ($P > 0.05$) in ethylene release was observed between treatments at any sampling time (Figure 3). For all treatments, ethylene release commenced at 6 h and had reached completion by 48 h. Full ethylene release corresponded to a headspace ethylene concentration of ~75 $\mu L.L^{-1}$. The data presented in Figure 3 are based on values obtained from Replicates 2 and 3 only, since Replicate 1 exhibited a vastly different ethylene release profile (Figure 4). It was believed that the presence of Velcro-affixed loggers in Replicate 1 chambers and delivery systems hindered ethylene release and that exclusion of Replicate 1 data from the statistical analysis was justifiable on this basis.

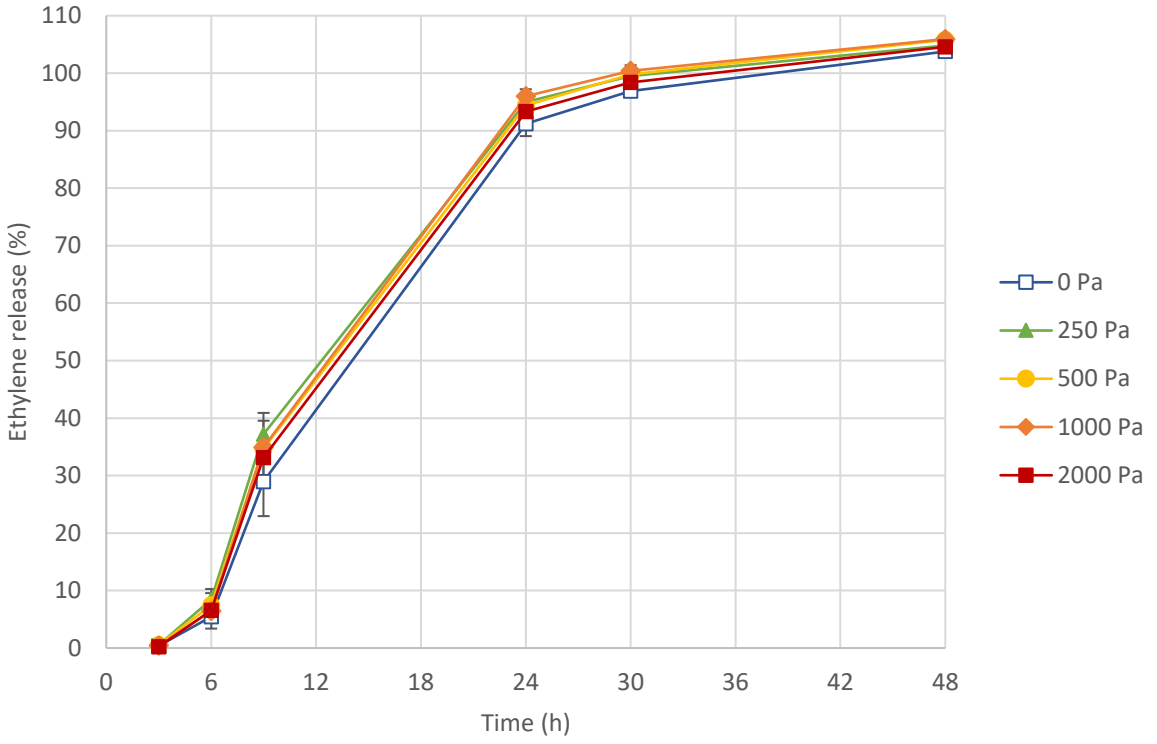

Figure 3. Ethylene release (expressed as a percentage of total ethylene in the system) from 10 mg Ripestuff™ powder in a prototype delivery system exposed to air pressure oscillations of 0, 250, 500, 1000 or 2000 Pa in a humidified 2 L chamber. Error bars represent standard error of the mean (*n* = 2) and are too small to be visible in some instances.

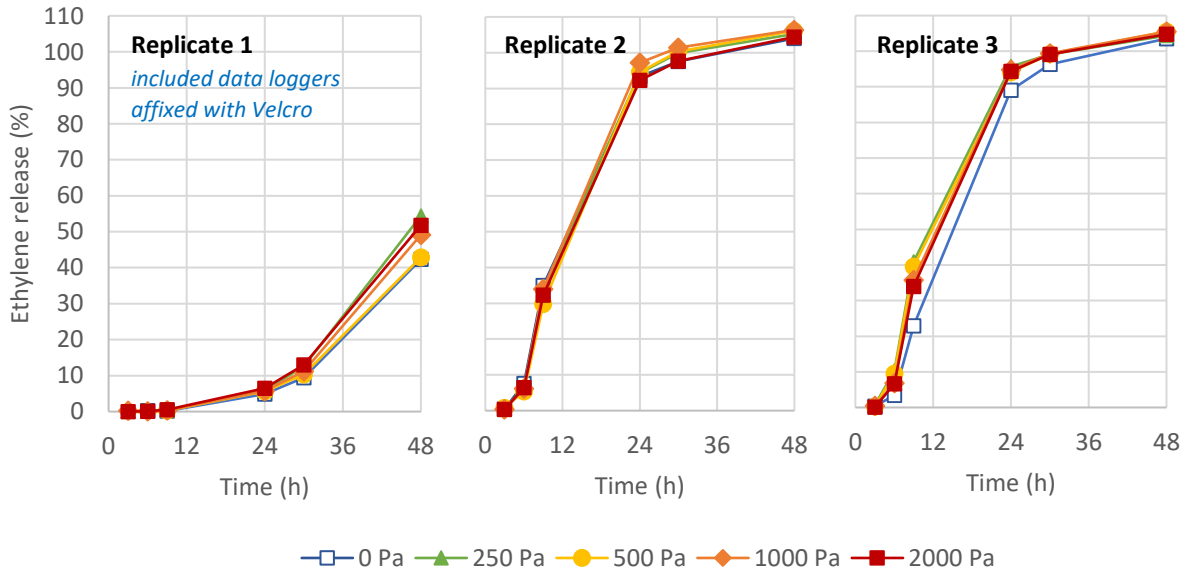

Figure 4. Ethylene release profiles for individual replicates of 10 mg Ripestuff™ powder in a prototype delivery system exposed to air pressure oscillations of 0, 250, 500, 1000 or 2000 Pa in a humidified 2 L chamber.

A comparison of delivery system (source) and chamber (sink) headspace ethylene concentrations at 48 h revealed that all treatments from Replicates 2 and 3 had a source to sink ratio of one, which is indicative of a system in equilibrium (**Error! Not a valid bookmark self-reference.**). However, all Ripestuff™-containing treatments from Replicate 1 appeared to be in a state of flux, exhibiting source to sink ratios of between four and six.

Table 1. Ethylene distribution between headspace of Ripestuff™ delivery system (source) and 2 L chamber (sink) after 48 h. Treatments were air pressure oscillations of 0, 250, 500, 1000 and 2000 Pa applied to the chamber at regular intervals. A delivery system containing no Ripestuff™ powder and subjected to no pressure change served as the control. Data for individual replicates are presented to highlight the substantial variation between replicates.

Treatment	Headspace ethylene concentration ($\mu L.L^{-1}$)		Source to sink ratio
	Source	Sink	
	— REPLICATE 1 —		
Control	1	1	1
0 Pa	161	30	5
250 Pa	180	40	4
500 Pa	176	32	6
1000 Pa	175	35	5
2000 Pa	171	37	5
	— REPLICATE 2 —		
Control	1	1	1
0 Pa	74	73	1
250 Pa	74	74	1
500 Pa	80	78	1
1000 Pa	78	78	1
2000 Pa	77	76	1
	— REPLICATE 3 —		
Control	1	1	1
0 Pa	76	74	1
250 Pa	77	74	1
500 Pa	74	72	1
1000 Pa	76	73	1
2000 Pa	78	77	1

3.2 Headspace temperature and relative humidity

Headspace temperature of the delivery system (source) during the experiment was 24.5 ± 0.4°C (mean ± SD) for all treatments. Similar RH profiles between treatments were observed for the source and chamber (sink), with all treatments exceeding 90% RH within 30 h and 0.5 h, respectively (Figure 5).

Variation in ethylene release profiles between Replicate 1 and the other two replicates (Figure 4) suggested that the presence of Velcro-affixed loggers in the former may suppress RH increase in the source. A comparison of source RH profiles with those subsequently obtained using loggers without Velcro showed this to be the case (Figure 6A). Source RH increased rapidly when Velcro was excluded, reaching 86-90% RH by the end of the 5 h monitoring period. By comparison, the inclusion of Velcro in the main experiment resulted in source RH reaching only 75-77% at this time.

A slight but significant ($P < 0.05$) difference in source RH was detected from 10 min onwards between the 0 Pa and 2000 Pa treatments when Velcro was excluded. Source RH of the 2000 Pa treatment was generally 2 to 3 percentage points higher than that of the 0 Pa treatment. In terms of sink RH, similar profiles were obtained irrespective of whether Velcro was present or not (Figure 6B). No difference in sink RH was detected between the 0 Pa and 2000 Pa treatments at any time when Velcro was excluded.

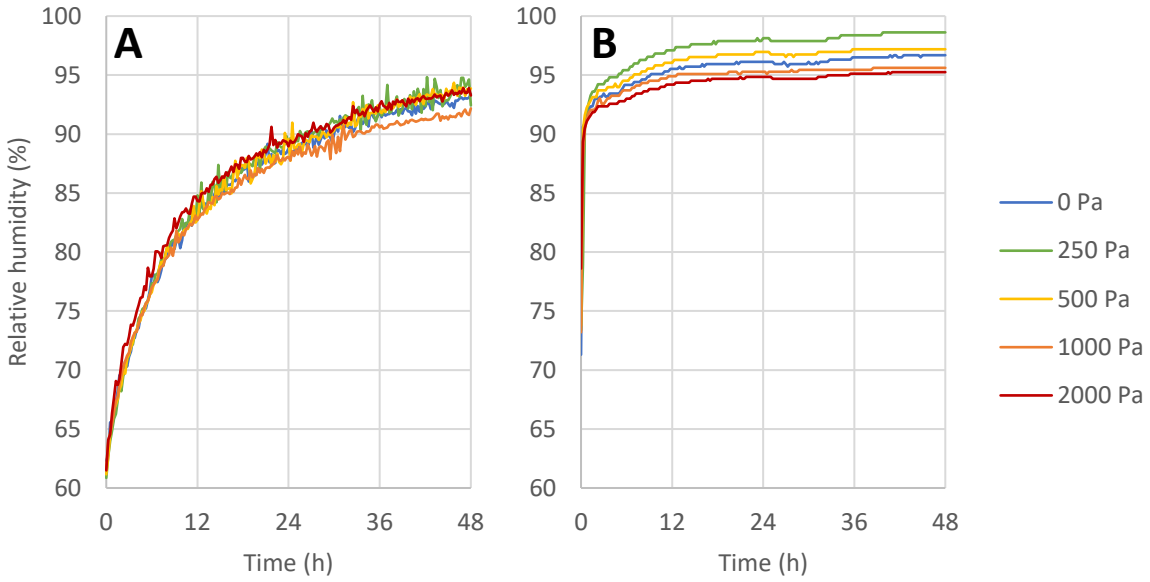

Figure 5. Headspace relative humidity inside (A) Ripestuff™ delivery system (source) and (B) 2 L humidified chamber (sink) during 48 h treatment period. Treatments were air pressure oscillations of 0, 250, 500, 1000 or 2000 Pa applied to the chamber at regular intervals.

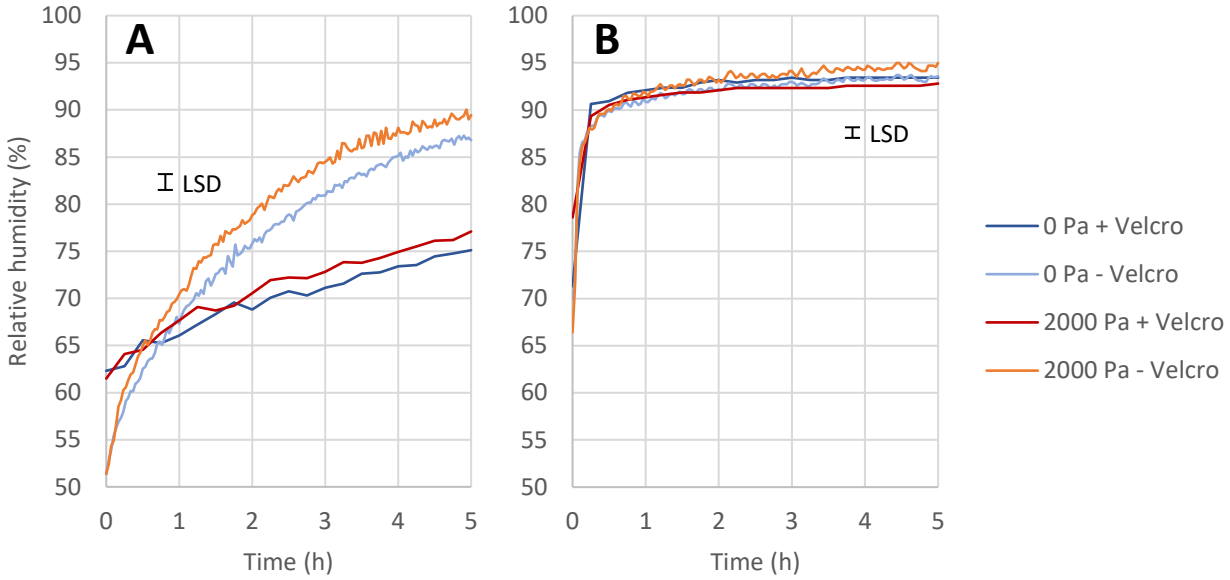

Figure 6. Effect of Velcro on headspace relative humidity inside (A) Ripestuff™ delivery system (source) and (B) 2 L humidified chamber (sink) during 5 h treatment period. Treatments were three air pressure oscillations of 0 or 2000 Pa applied to the chamber at hourly intervals commencing at 0 h. Data for treatments with Velcro were obtained from the main experiment (Figure 5; *n* = 1). Data for treatments without Velcro were obtained from a separate follow-up experiment (*n* = 2). LSD values presented on graphs relate only to treatments without Velcro.

4 Discussion

The air pressure oscillation treatments investigated in this study did not enhance ethylene release from a 'dry' delivery system held in a high humidity environment. However, this result does not dispel the theory that pressure changes during transit influence Ripestuff™ ethylene release. Given that road containers are in continual motion for several hours each day, the number of air pressure oscillations experienced in-transit is likely to far exceed the 54 oscillations applied here. For example, vehicles generate infrasound at frequencies between 1 and 20 Hz (i.e. cycles per second). Infrasound in vehicles is considered to arise from: (1) turbulence from the moving vehicle or other traffic, infusing through the vents; (2) flexing of the body causing volume changes; (3) acceleration of the vehicle, causing an inertial reaction from the enclosed and external air; and (4) pressure variations due to altitude changes (Vanderkooy, 2014). Infrasound levels of 100 dB (equivalent to 2 Pa) have been reported within the cabin of heavy vehicles travelling between 70 and 90 $km.h^{-1}$ (Broner, 1978; Nowacki et al., 2008; Williams and Tempest, 1975). This pressure level is far lower than the pressure changes investigated in the current study. However, such frequent small pressure oscillations could have a greater effect on air volume exchange between the delivery system and chamber headspaces than 54 pressure oscillations of higher magnitude. When viewed in terms of the volume of air exchange, the highest magnitude treatment in the current study resulted in the delivery system headspace being replaced 1.1 times in 48 h. In comparison, exposure to infrasound levels of 100 dB at 10 Hz for 48 h would theoretically result in the headspace being replaced 68

times (i.e. once every 42 min). This calculation assumes that the road container is subjected to the same infrasound levels as the cabin.

Delivery system RH was shown to respond to a fractional turnover of headspace volume. This response was evident in the follow-up experiment conducted without Velcro, which showed three pressure oscillations of 2000 Pa (equivalent to an exchange of 6% of total headspace volume) were sufficient to generate a small but significant increase in delivery system RH within 10 min. The much higher in-transit headspace volume exchange rate proposed here suggests that Ripestuff™ delivery systems in a road container would undergo a rapid rise in RH which would in turn trigger ethylene release.

Self-adhesive Velcro in the delivery system appeared to ab/adsorb moisture to such an extent that it restricted ethylene release. This effect was not observed in an earlier study which incorporated Velcro-affixed loggers in some delivery systems (Perkins and Joyce, 2019b). In that study, wet or dry chromatography paper was included in the delivery system and would have likely overridden any effect that Velcro might have had on headspace RH and, thus, ethylene release.

In conclusion, ethylene release from a 'dry' Ripestuff™ delivery system was unaffected by repeated air pressure oscillations up to a magnitude of 2000 Pa. However, these treatments appear to have greatly over-estimated the magnitude and greatly under-estimated the frequency of pressure oscillations encountered during road transportation in heavy vehicles. Calculations based on values obtained from scientific literature suggest that in-transit pressure changes could account for the rapid Ripestuff™ ethylene release reported by Duong et al. (2017). It is recommended that infrasound levels within a consignment of fruit be monitored during road transportation. Once characterised, these levels should be simulated in the laboratory to determine their effects on Ripestuff™ ethylene release from a 'dry' delivery system in a static chamber configuration under controlled temperature and humidity conditions.

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Appendix I

Preliminary investigation of ethylene release from silicone tubing in flowing and static systems

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1.1 Introduction

Mango (*Mangifera indica* L.) fruit grown in northern, tropical areas of Australia are currently harvested mature green and transported 2,000 – 4,000 km at 12 - 16°C to southern ripening centres. They are exposed to either 'shot' or 'trickle' release systems of ethylene (C_2H_4) gas for 2 - 3 days inside controlled temperature ripening rooms operated at 18 - 20°C and >85% relative humidity (RH) (Better Mangoes 2002; Ledger et al. 2010). Fruit ripening, which includes skin colour changes from green to yellow and fruit softening, is initiated before they are sent to market (Ledger et al. 2010). Typically ripening room C_2H_4 concentrations range from 1 - 100 µL L^{-1}. C_2H_4 treatment helps to coordinate more uniform and predictable fruit ripening and facilitates orderly marketing.

There is potential to engage fruit time in transport to initiate ripening in consignments of mangoes by alternative C_2H_4 delivery means (Mott et al. 2017), and similarly to employ alternative C_2H_4 release mechanisms in other situations, such as when current methods are unsafe. In the Philippines, calcium carbide (CaC_2) at a rate of 5 - 6 g kg^{-1} fruit is used to ripen mangoes (Australian Centre for International Agricultural Research (ACIAR) 2017; Mango Production n.d.). CaC_2 is produced by heating calcium oxide with charcoal under reducing conditions. Once hydrolysed, CaC_2 releases acetylene (C_2H_2) along with trace amounts of C_2H_4 and so can be used in ripening fruit (Reid 2002). Thompson and Seymour (1982) exposed bananas (*Musa* AAA group) to C_2H_2 or C_2H_4 at 10, 100 and 1000 µL L^{-1} for 24 h at 18°C and high humidity. All concentrations of C_2H_4 initiated ripening while all concentrations of C_2H_2, except 10 µL L^{-1}, induced a similar rise in climacteric respiration and ripening of skin colour and soluble solids content, as compared to C_2H_4. Medlicott et al. (1987) compared the ripening

of cv. 'Tommy Atkins' mango fruit exposed to C_2H_4 or C_2H_2 concentrations for 24 h at 25°C and high humidity. Fruit exposed to 1000 µL L^{-1} C_2H_2 achieved similar ripening and consequent softening and peel colour development as compared to fruit exposed to 1000 µL L^{-1} C_2H_4. The availability and low cost of CaC_2 means that it is commonly used in India for ripening of mango, banana and papaya (Chandel et al. 2018). However, due to its toxicity as a carcinogen and human health concerns resulting in neurological disorders, ulcers, hypoxia and other health issues (ScienceLab.com 2013; Ramachandra et al. 2016) it has been banned in India and most western countries (Ho & Bhandari 2016; Ramachandra et al. 2016).

Alternative C_2H_4 release methods to initiate ripening of cv. 'Anjou' pears in transit have been investigated (Sharrock et al. 2010; Sharrock & Henzell 2010). Thimble sized capsules (Sharrock & Henzell 2010) and specially produced Ethylene Release Canister's™ (Sharrock et al. 2010) were used to ripen clamshells and pallets of 'Anjou' pears. More recently, Xu (2017) investigated an in-transit pear ripening technology consisting of sachets of C_2H_4 encapsulated in α-cyclodextrin. C_2H_4 from the encapsulated complex was released by water vapour transmission from the sachet materials (PVA film and Tyvek®) under 90% RH at 4°C.

Hofman et al. (2013) evaluated two slow release C_2H_4 systems applied in commercial consignments of cv. 'Calypso™' mangoes. The first system employed C_2H_4 encapsulated in an α-cyclodextrin (α-CD) inclusion complex (IC) powder (Ho 2013) known commercially known as Ripestuff™ (UniQuest 2014). Plastic containers with holes in the lid with IC powder were installed on top of pallets inside a refrigerated truck trailer. The second system employed large plastic bags (10 of 50 cm x 1.1 m dimensions) filled with 3.8% C_2H_4 in nitrogen gas attached to the top of pallets inside a refrigerated trailer. While both systems released C_2H_4 over time, the authors suggested that improvements to enhance the ease of use and efficacy of each system were needed.

Poole and Joyce (1993) adapted s method of Saltveit (1978) to design a simple and reliable C_2H_4 permeation system for plant studies. Eight (8) mm internal diameter polyvinyl chloride (PVC) tubing at various lengths was pressurised with 50 kPa of pure C_2H_4. Lengths of 1.5, 15, 150 and 1500 cm gave concentrations of 0.12, 0.99, 7.8 and 100 µL L^{-1} C_2H_4 inside 64 L gassing chambers, respectively.

Macnish et al. (2004) developed a simple PVC sustained release device containing 1-methylcyclopropene (1-MCP) in deionised water to protect flowers against abscission from exposure to C_2H_4 during export. 1-MCP gas inhibits C_2H_4 action in plants by binding to C_2H_4 receptors. The 14 cm lengths of tubing with an internal diameter of 6 mm contained 2.8 ml of 1-MCP solution and were sealed at each end with solid glass rods. 1-MCP was released from

the tubing and sustained concentrations at ≥0.03 μL L^{-1} for 132 h (5.5 days) providing longer term protection against C_2H_4 to export flowers.

The current study was part of the 'Improved postharvest management of fruit and vegetables in the Southern Philippines and Australia' project conducted by ACIAR. One of the many objectives of the project was to improve fruit quality through developing effective postharvest strategies. Potentially safer ripening agents alternative to CaC_2 were investigated.

In the current preliminary study, initial laboratory experiments examined the potential of an alternative slow release ripening system. Hollow silicone tubing of different diameters was filled with C_2H_4 and C_2H_2 gas and monitored for release. Gas concentrations were measured from replicated flow-through and static chamber systems and a bamboo basket system. This preliminary work was towards a larger commercial release system for use in developing countries, such as the Philippines.

1.2 Materials and Methods

1.2.1 Materials

The following preliminary experiments were conducted in laboratories at Maroochy Research Facility (MRF), Nambour (26.6237° S, 152.9588° E), University of Philippines Mindanao (UP Min), Davao (7.0857° N, 125.4841° E) and The University of Queensland, Gatton campus (UQG), Gatton (27.5554° S, 152.3372° E from August 2015 - February 2017.

Hollow tubing

Silicone rubber tubing (Gecko Optical Scientific Equipment, Perth, Western Australia, Australia) of various different internal and external diameters and tube thicknesses and lengths were employed (Table 1). Each length of tubing was blocked-off at either end with a stainless steel or aluminium stopper or else folded and clamped tight with cable ties (CABAC, 200 x 4.8 mm, Agricultural Requirements, Gatton, Queensland, Australia).

Table 1: Details of length, internal diameter (ID), outer diameter (OD) and wall thickness of hollow silicone rubber tubing used in the preliminary experiments.

		Silicone tubing details			
Experiment No.	Treatment No	Length (cm)	ID (mm)	OD (mm)	Wall thickness (mm)
1	1	15	8	14	3
	2	15	10	12	2.5
	3	15	9	12	1.5
2		120	7.9	NA	NA
3	1	15	6	12	3
	2	15	9	13	2
	3	15	7	10	1.5
4	1	15	12	18	3
	2	15	10	16	3
	3	15	8	14	3
	4	15	6	12	3

Gas sources

Various lengths of silicone tubing were filled with 3.8% C_2H_4 in nitrogen (N_2) as Ripegas™ (BOC Gas, Australia), 97 ± 5 µL L^{-1} C_2H_4 in N_2 (BOC Gas, Australia) or C_2H_2 gas (local boilermaker 2016 pers. comm. 3 August, Mindanao, Philippines). To fill 15 cm lengths of tubing, a small PVC hose was attached to the regulator on the C_2H_4 cylinder and a 25 gauge (0.5 x 25 mm) hypodermic needle (Terumo Neolus®, Livingstone International, Rosebery, New South Wales, Australia) was attached at the other end (Figure 1). The needle end of the hose was inserted into the end of the silicone tubing. A second needle was inserted into the far end of the silicone tubing. This arrangement allowed flow of C_2H_4 gas through the needle into the full length of stoppered silicone tubing and was flushed through for ~60 s (Figure 1). Stainless steel or aluminium rods were then inserted to block off the small holes left by the needles. This technique was refined slightly by placing the end of the tubing into water to show C_2H_4 gas bubbling out through the end (Figure 1). Similarly for 1.2 m lengths of tubing, a small hose was attached to the regulator on the C_2H_2 cylinder and a small nozzle attached at the other end. The nozzle end of the hose was inserted into the silicone tubing and the other end of the silicone tubing was placed in water (Figure 1). C_2H_2 gas was flowed through the length of silicone tubing for ~2mins to bubble out slowly at the far end. The silicone tube end in the water was pinched closed, folded two times upon itself and clamped closed with a cable tie. The silicone

tube attached to the nozzle was pinched closed and folded two times and closed off with a cable tie.

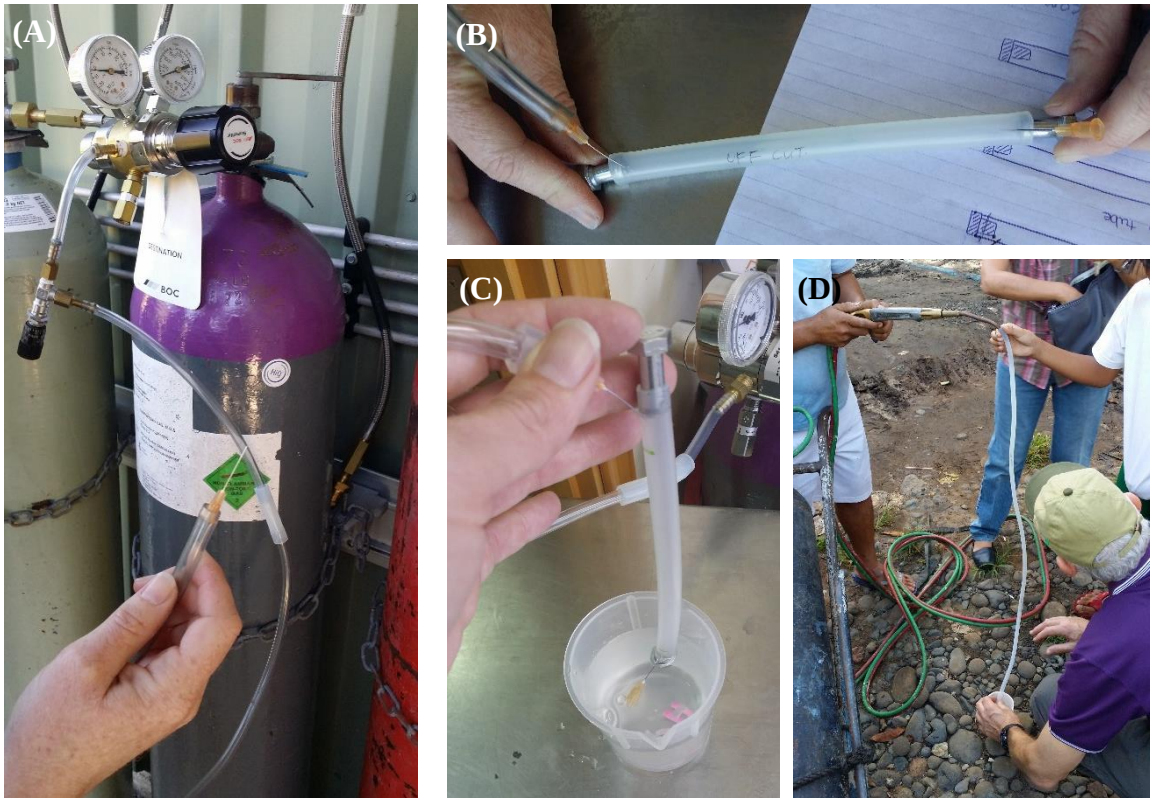

Figure 1: Ethylene (C_2H_4) cylinder with regulators, PVC tubing and needle attached (A) to fill stoppered length of silicone tubing (B). Filling method was adapted by placing tube end in water (C). Filling length of silicone tubing with acetylene (C_2H_2) from a gas cylinder using a similar technique where the tube end is placed in water (D).

Chamber systems

Modified flow-through and static chamber systems were used in the following experiments. The flow-through chamber system consisted of 525 ml glass jars with screw top lids (viz., recycled preserving jars) with two ~50 cm lengths of 6 mm ID PVC tubing attached to the lid creating 'in-flow' and 'out-flow' channels (Figure 2). An external Champion Dominator Ci55 air compressor (Sullair®, Dandenong South, Victoria, Australia), supplied air that was regulated with a needle valve. The air flowed to a manifold with six individual ports with the flow rate from each port being controlled by individual hollow glass tubing inserted in the tubing. The glass capillaries were calibrated to provide 100 ml min^{-1} or 10 ml min^{-1} per port. The port outlets were attached to the 'in-flow' tube for each chamber. The 'out-flow' tube air

stream from each chamber was passed through a container of potassium permanganate ($KMnO_4$) to absorb excess C_2H_4 concentrations.

Static chambers as used in the Philippines were 42 L woven bamboo baskets lined and covered with ~5 layers of newspaper (Figure 2).

Static chambers used in the remaining experiments were 525 ml (as above), 1 L and 2 L Ball® glass preserving jars (BallMason Australia Pty. Ltd., Kempsey, New South Wales) with screw top lids. Lids on the 525 ml glass jars had two lengths of 6 mm ID ~20 cm of PVC tube attached to assist in gas sampling (Figure 2). The lids on the 1 L (930 ml volume) glass jars had 2 Suba-seal® serrated bungs (Suba-seal 9, Sigma-Aldrich, Castle Hill, New South Wales, Australia) installed for gas sampling (Figure 3). The lids of the 2 L (2000 ml volume) glass jars were modified by inserting two ~20 cm lengths of 4 mm ID UV stabilised Flexi Tube (Neta, Agricultural Requirements, Gatton, Queensland, Australia) into the lid. Each tube extending from the lid had a 4 mm Inline micro tap (Pope® barbed valve, Agricultural Requirements, Gatton, Queensland, Australia) attached for gas sampling (Figure 3).

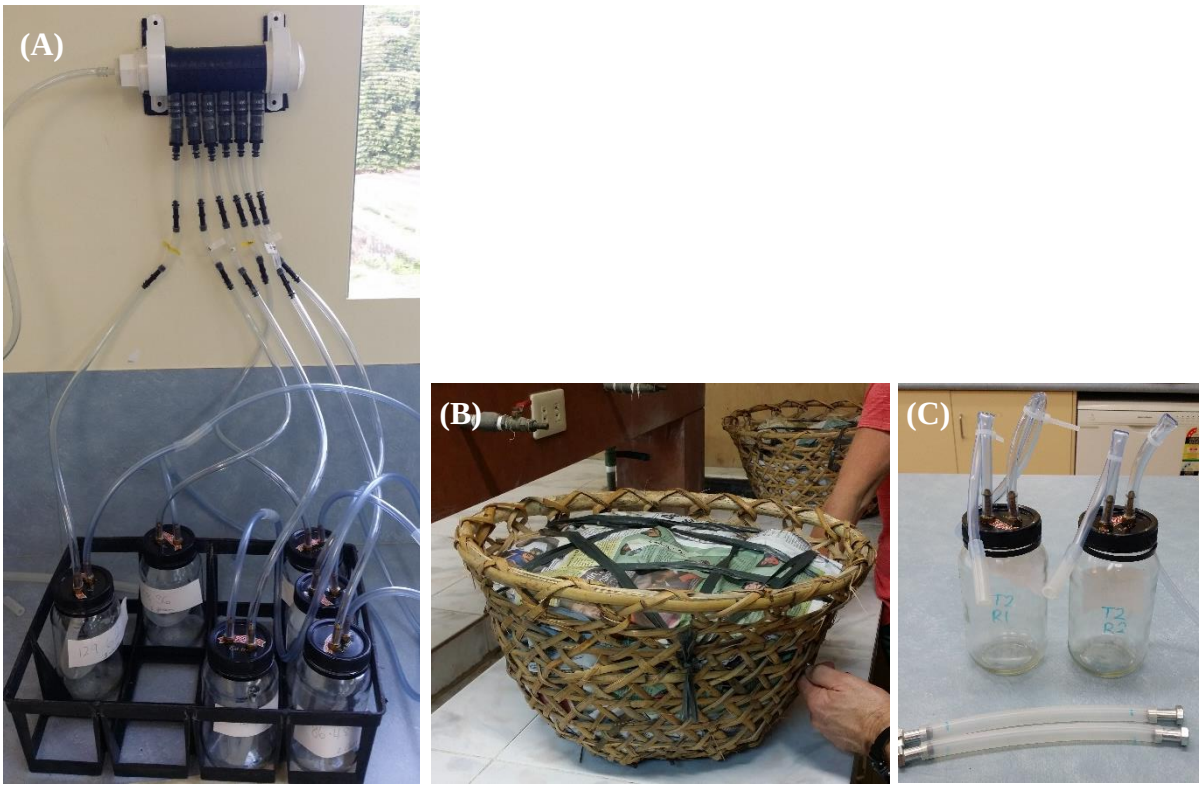

Figure 2: Flow through 525 ml chamber system (A), woven 42 L bamboo basket system lined and covered with newspaper (B) and static 525 ml chamber system with in- and out-flow tubes for gas sampling (C).

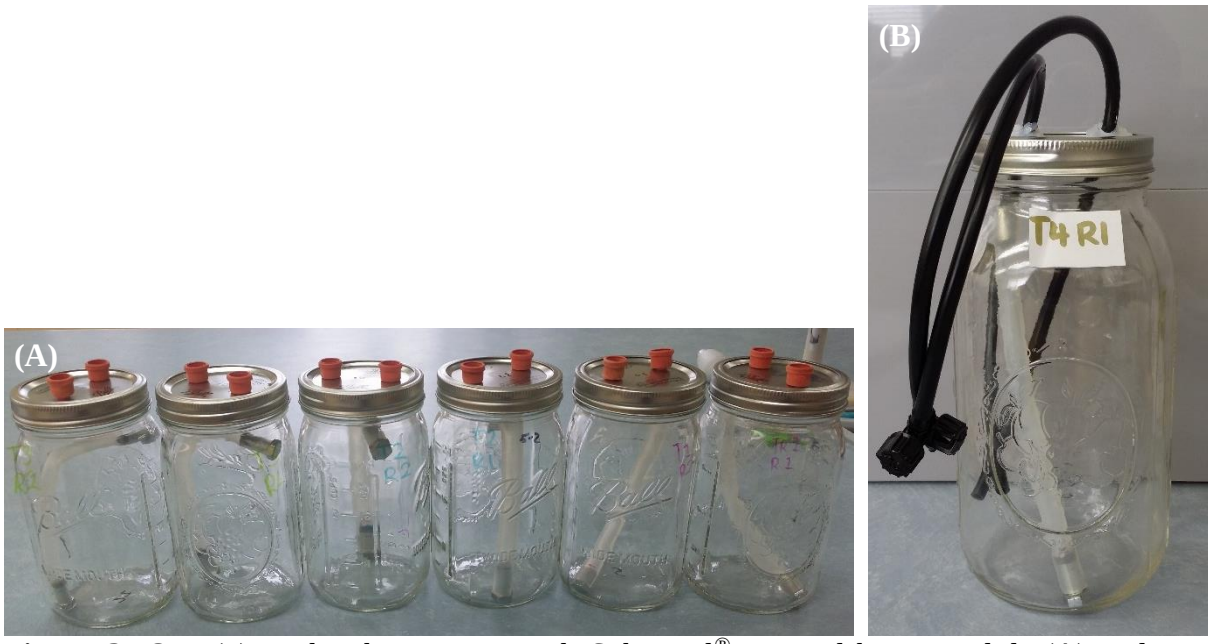

Figure 3: One (1) L chamber system with Suba-seal® serrated bungs in lids (A) and 2 L chamber system with in- and out-flow tubes with micro taps attached to permit gas sampling (B).

Plant material

Fresh mango (*Mangifera indica* L.) fruit cv. 'Carabao' were obtained from local markets that were sourced from two farms in the Philippines and transported to the University of Philippines Mindanao campus. Fruit were graded for similar size and quality, with 20 individual fruit per basket being weighed upon arrival and prior to treatment. Approximately 20 kg of 'Carabao' mangoes were used per basket (~42 L volume) as filler fruit.

Gas measuring devices

C_2H_4 concentrations in experiment 1 were determined using a Shimadzu (GC-2010 Plus) gas chromatograph (GC) fitted with a 30 m long Rtx-BAC Plus 2 (Restek) column (0.32 mm internal diameter, 0.60 µm film thickness) and a flame ionization detector (FID), Nitrogen (15 ml min^{-1}) was the carrier gas. The column temperature was maintained at 200°C, the injector temperature was 260°C, and the detector temperature was 240°C. The lowest detection limit for the GC was ~1 - 2 µL L^{-1}.

In experiment 2, a Kitagawa® AP-20 aspirator pump fitted with either C_2H_4 or C_2H_2 gas sampling tubes (Kanagawa, Japan) was used to test for C_2H_4 and C_2H_2 over ranges between 50 - 1000 µL L^{-1}. A Felix F-950 (Felix Instruments, FreshView, Eight Mile Plains, Australia) three

gas analyser was also used in experiment 2 to monitor C_2H_4, carbon dioxide (CO_2) and oxygen (O_2) concentrations. The Felix F-950 measured C_2H_4 between 0 - 200 µL L^{-1} and CO_2 and O_2 between 0 - 100%.

An ICA-56 ethylene sensor (ProFesh Systems Pty. Ltd, Rocklea, Australia) with a measuring range of 0 - 99 µL L^{-1} was used to measure C_2H_4 concentrations in experiments 3 and 4.

1.2.2 Methods

Experiment 1 - Ripegas™ in flow through chambers

Experiment 1 was repeated (replicated) two times using silicone tubes with 15 cm length and three different ID and OD sizes using six flow-through 525 ml chambers in total (Table 1). After filling a silicone tube with Ripegas™ and sealing its ends, it was placed inside a glass chamber and the lid then sealed tightly. Prior to closure, a small amount of petroleum jelly was smeared on the inside of the lids rubberised seal to further minimise any potential leakage of air or gas. The target airflow through each chamber was 100 ml min^{-1}. Actual air flow measured through each chamber ranged between 86 - 144 ml min^{-1}. A 5 ml disposable Terumo® syringe (Livingstone International, Rosebery, New South Wales, Australia) was used to collect air samples from the outlet flow of each chamber. Each sample was analysed using a Shimadzu GC. One sample per day was taken from each chamber for 3 days. At the end of the 3 days extra samples were taken to investigate the possibility of contaminated air throughout the flow system. Samples were also taken directly from the silicone tubes on the final day to measure if C_2H_4 was present.

To measure release under static conditions, a 15 cm length of stoppered silicone tubing (10 mm ID, 12 mm OD) filled with Ripegas™ was placed in a 1024 ml glass chamber with a screw top lid. Air samples were taken from the chamber every 30 min from time zero, when the lid was secured to the chamber. All samples were analysed using the GC. In total, 13 samples were taken throughout the day. The final sample was taken 24 hours after the first sample was taken. The initial flow-through experiment using Ripegas™ with three treatments and two replications was repeated with an adjusted air flow of 10 ml min^{-1}.

The flow-through experiment then exchanged Ripegas™ which was not available at the time for 97 ± 5 µL L^{-1} C_2H_4 gas with 10 ml min^{-1} flow rate and was repeated. Sampling was conducted hourly, with seven samples being taken throughout the day and the final sample taken 24 hours after the first sample.

Experiment 2 – C_2H_2 in bamboo baskets

Three replicate baskets of green 'Carabao' mangoes were treated with peroxide cured silicone tubing (Masterflex® 96400-18, John Morris Scientific Pty Ltd) filled with C_2H_2 gas. C_2H_4 gas was not used in this experiment as it was too expensive to source in the Philippines and also difficult to access. A short time (~15 mins) after the 1.2 m lengths of silicone tube were filled with C_2H_2 gas, they appeared shrunken and flat, perhaps creating a vacuum. Each bamboo basket was lined with newspaper and a layer of 'Carabao' mangoes then placed in the bottom. The silicone tubing filled with C_2H_2 was placed inside the basket on top of the layer of mangoes (Figure 4). Then 20 selected and pre-weighed mangoes were placed around the tubing and the remainder of the basket void was filled with filler fruit. The fruit were covered with ~5 layers of newspaper and secured tightly with string and monitored for C_2H_4 and C_2H_2 release using a Kitagawa® aspirator pump and ethylene and acetylene tubes, respectively. A Felix F-950 3 gas analyser was used to monitor C_2H_4, carbon dioxide (CO_2) and oxygen (O_2) concentrations.

Figure 4: Image showing a 1.2 m length of silicone tube filled with acetylene (C_2H_2) gas placed into a woven, bamboo basket lined with newspaper and layers of cv. 'Carabao' mangoes.

Experiment 3 –C_2H_4 in static chambers

Silicone tubing of three different IDs and wall thicknesses (Table 1) was filled with 97 ± 5 µL L^{-1} C_2H_4 with stoppered ends. Tubes were placed into individual 525 ml glass jar chambers with PVC tubing attached to the lid for C_2H_4 sampling. C_2H_4 concentrations were measured using an ICA-56 ethylene sensor device (ProFesh Systems Pty. Ltd, Rocklea, Australia). Silicone tubing (6 mm ID, ~5 cm L) was attached to the 'in' and 'out' flow of the device, creating a tight seal for the PVC tubing from each chamber.

To enhance the gas sampling technique and minimise potential C_2H_4 leakage from the chamber space via the PVC sampling tubes (Figure 2), the experiment was repeated using 930 ml glass jar chambers with two Suba-seal® bungs in the lid for C_2H_4 sampling. C_2H_4 concentrations were measured using the ICA-56 ethylene sensor device. PVC tubing of 6 mm ID and ~30 cm length was attached to the in- and out-flow of the device. A 25 gauge (0.5 x 25 mm) hypodermic needle (Terumo Neolus®, Livingstone International, Rosebery, New South Wales, Australia) which was inserted into the bungs on the lid of the chamber was attached at the opposite end of the PVC tubing (Figure 5).

Figure 5: Image showing the ICA-56 ethylene sensor device measuring concentrations (µL L^{-1}) from silicone tubing inside the 1 L glass chamber via Suba-seal® bungs and PVC tubing.

Experiment 4 - C_2H_4 in static 2 L chambers with modified lids

Silicone tubing of four different internal and external diameters and the same wall thickness (Table 1) were chosen to compare the effect of surface area in this experiment replicated three times. Tubing was cut to 15 cm internal lengths, with each end being sealed with aluminium bolts. The tubes were filled with 97 ± 5 µL L^{-1} of C_2H_4 gas, sealed and then placed inside separate 2 L static glass chambers with screw-top lids. C_2H_4 concentrations inside the chambers were measured at 1, 2, 3, 4 and 5 hours and then daily (every 24 hours) for 22 days using the

ICA-56 device until equilibrium was reached. Each chamber contained an iButton® Thermocron temperature (TC DS1922L-F50, iButton Link, Whitewater, WI, USA) and/or relative humidity logger. The logger was attached by self-adhesive dots (Velcro®, Gatton Plaza News, Gatton, Queensland, Australia) to the inside, top section of the glass chamber.

1.3 Results and discussion

Experiment 1 - Ripegas™ in flow through chambers

During 3 days of measurement, no (i.e., 0 µL L^{-1}) C_2H_4 was recorded for each chamber with ~100 ml min^{-1} flow rate. On the final day samples were taken directly from the silicone tubing and 0.547 µL L^{-1} C_2H_4 was measured from TR 1 replication 1. Due to the lack of C_2H_4 detection in the flow-through system, various sections of the system were sampled to test for leakage or contamination. Samples were taken from near the manifold, pre-needle valve, post-needle valve and from an empty chamber (i.e., glass jar) was also sampled. All samples registered 0 µL L^{-1} C_2H_4.

C_2H_4 was detected in a single static chamber at concentrations <1.0 µL L^{-1}, this being the lower end of the available GC's nominal detection range. The marginally detectable low concentrations trended to increase over time. The highest reading of 0.086 µL L^{-1} was reached at 300 min after the lid was closed. The sample taken 24 hours after the lid was closed was 0.082 µL L^{-1} (Figure 6).

The air flow for the flow-through system was reduced to 10 ml min^{-1} and the experiment was repeated. Sampling was conducted every 1 - 2 hours, with C_2H_4 concentrations registering initially but then disappearing. No C_2H_4 was detected for samples measured at 24 h after treatment.

In the experiment 1 using 97 ± 5 µL L^{-1} C_2H_4 gas with a 10 ml min^{-1} flow rate, C_2H_4 concentrations were also <1.0 µL L^{-1}. For treatment 3 silicone tubing (wall thickness of 1.5 mm), C_2H_4 concentrations for the two replications peaked at the initial sampling time, i.e., when the lid was closed with 0.755 µL L^{-1} and 0.914 µL L^{-1}, respectively, and decreased over time (Figure 7). For treatments 1 and 2 with wall thicknesses of 3 and 2.5 mm respectively, C_2H_4 peaked at the second sampling time of 1 hour after lids were closed. C_2H_4 continued to decrease over time (Figure 7). After 24 h when samples were taken directly from the silicone treatment tubes, C_2H_4 concentrations were all <1.0 µL L^{-1} C_2H_4.

Poole and Joyce (1993) compared the release of C_2H_4 from pressurised PVC and silicone tubing in flow through systems with a flow rate of 2 L min^{-1}. Their results indicated that silicone tube C_2H_4 efflux was 75 times greater than that from PVC tubing. Relatively fast permeation

from silicone tubing may be why C_2H_4 was problematic to measure (detect) in the present flow-through systems viz., being at very low concentrations even when the flow rate was reduced from 100 to 10 ml min^{-1}.

Based on the afore-mentioned findings, it was concluded that a flow-through system with un-pressurised tubing released its C_2H_4 rapidly, before samples were collected for measurement, and that a static chamber system may be a more suitable approach in the context of these preliminary investigations.

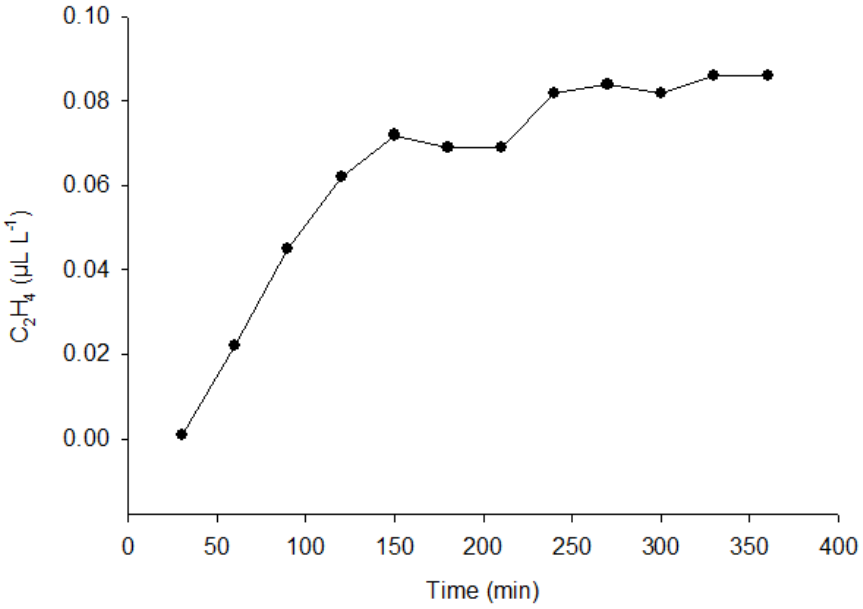

Figure 6: Ethylene (C_2H_4) concentrations (µL L^{-1}) released over time (min) from 15 cm length of hollow silicone tubing of 10 mm internal diameter (ID), 12 mm outer diameter (OD) filled with 97 ± 5 µL L^{-1} C_2H_4 in a static 1 L glass chamber.

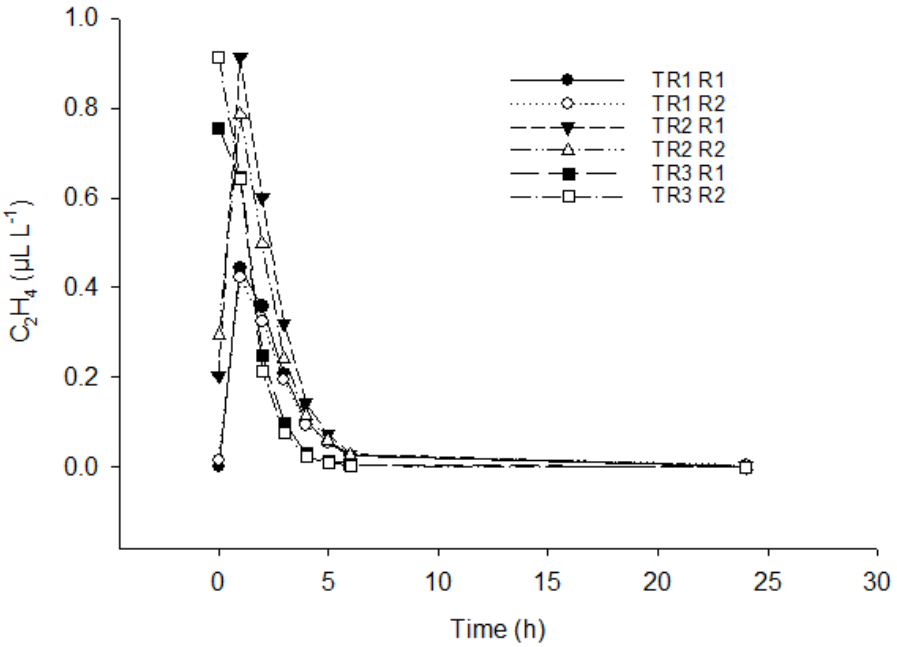

Figure 7: Ethylene (C_2H_4) concentrations (µL L^{-1}) released over time (h) from 10 ml min^{-1} flow-through 525 ml chambers with three silicone tube treatments (TR 1 – 3), replicated twice (R1-2). TR 1: 8 mm internal diameter (ID), 14 mm outer diameter (OD) and 3 mm wall thickness; TR 2: 10 mm ID, 12 mm OD and 2.5 mm wall thickness and TR 3: 9 mm ID, 12 mm OD and 1.5 mm wall thickness.

Experiment 2 - C_2H_2 in bamboo baskets

Shortly (~15 min) after 1.2m lengths of silicone tube were filled with C_2H_2 gas, they became shrunken and flattened. It was surmised that the C_2H_2 partitioned rapidly in the silicone matrix thereby creating a vacuum in the tubing void space. Nonetheless, the tubes were still used in the initiated experiment as it was considered C_2H_2 in the silicone tube membrane could still release into the basket and ripen the fruit. Concentrations of 10 µL L^{-1} C_2H_2 at 25°C for 24 h significantly ($P = 0.05$) hastened softening of 'Tommy Atkins' mango while concentrations at 1000 µL L^{-1} were considered suitable for initiating ripening under commercial ripening situations (Medlicott et al. 1987). In the present study it was observed upon opening the baskets after 3 days, that the silicone tubing had resumed their original shape. However, over the 3 days of treatment, no C_2H_2 or C_2H_4 was detected with the Kitagawa® tubes.

Poole and Joyce (1993) surmised C_2H_4 diffusion out of plastic tubing to occur simultaneously with inward diffusion of air (viz., mainly N_2 and O_2) in systems at atmospheric pressure. They

considered this exchange to lead to a gradual decline over time in the amount of C_2H_4 diffusing out of the tube through dilution of C_2H_4 remaining in the tubing (Poole & Joyce 1993). However, diffusion and, indeed, partitioning between phases (i.e., gas vs. matrix), would not be the same for different gases (Barrer & Chio 1965).

Experiment 3 –C_2H_4 in static chambers

C_2H_4 was measured hourly using the ICA-56 detector for silicone tubes of 15 cm length filled with 97 ± 5 µL L^{-1} C_2H_4 gas and placed into 525 ml glass chambers. After 24 h, C_2H_4 concentrations inside the 525 ml chambers peaked at ~4 µL L^{-1}.

With a view to increase the effectiveness of the C_2H_4 sampling, the experiment was repeated using 1 L glass chambers with Suba-seal® bungs in the lids and needles attached to PVC tubing coming from the ICA-56 C_2H_4 sensing device. After 24 h, C_2H_4 concentrations inside the 930 ml chamber peaked at 2.1 µL L^{-1}. The small pump inside the ICA-56 device was emitting a strange sound and having difficulty in circulating air from the chamber past the sensor and back into the chamber. It was surmised that the needles in the PVC tubing used for sampling were too restrictive for the ICA-56 sensor, as this was not an issue when using the 4 mm PVC and silicone tubing attached to the device.

Experiment 4 –C_2H_4 in 2 L static chambers with modified lids

C_2H_4 concentrations for all treatments generally increased over a 22 d period (Figure 8). The highest concentration of C_2H_4 (15.3 µL L^{-1}) was recorded for TR 1, replication 2. This level was higher than those for the two other replications that recorded 4.2 and 5.5 µL L^{-1} C_2H_4 at the same time.

Macnish et al. (2004) found that concentrations of 1-MCP released from PVC tubes in individual sealed 2.2 L glass jars were low after 12 h. Thereafter, concentrations of 1-MCP increased over time and became constant at ~140 µL L^{-1} for between 108 and 156 hours.

The C_2H_4 release from silicone tubing in the present experiment evidently followed a similar trend.

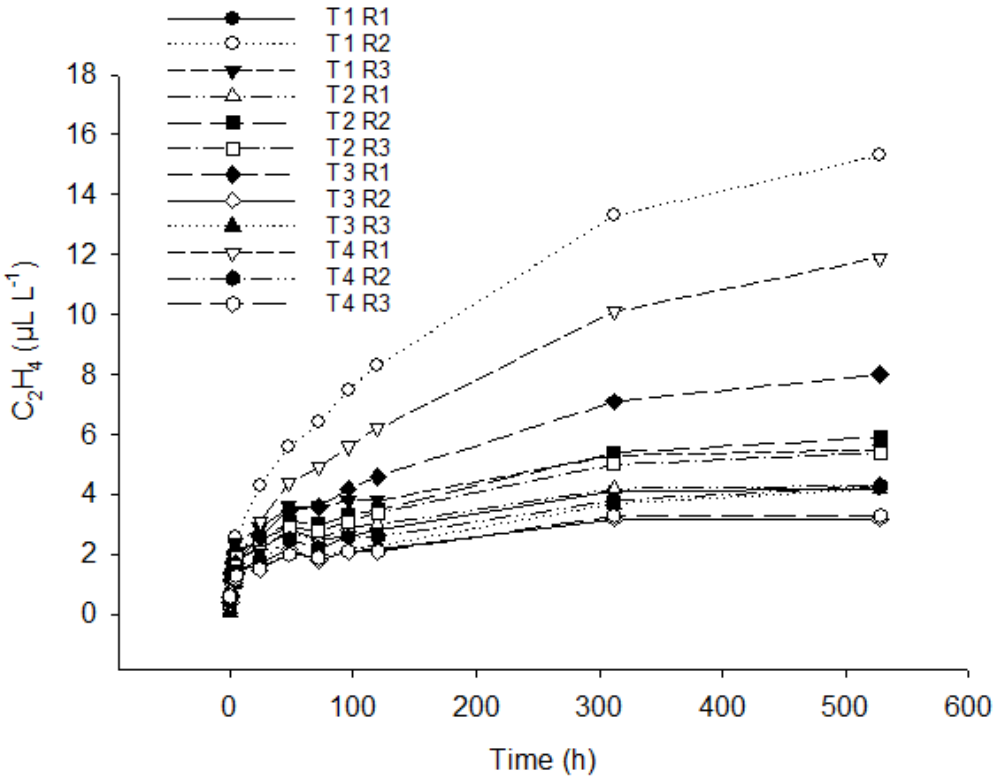

Figure 8: Ethylene (C_2H_4) concentrations (µL L^{-1}) released over time (h) from 2 L static glass chambers containing four silicone tube treatments (T 1 - 3), replicated three times (R 1 - 3). T 1: 10 mm internal diameter (ID), 16 mm outer diameter (OD) and 3 mm wall thickness; T 2: 10 mm ID, 16 mm OD and 3 mm wall thickness; T 3: 8 mm ID, 14 mm OD and 3 mm wall thickness and T 4: 6 mm ID, 12 OD and 3 mm wall thickness.

1.4 Conclusion

The present study demonstrated that a simple system of sealed silicone tubes filled with C_2H_4 gas can sustain the release of C_2H_4 at ≤15.3 µL L^{-1} concentrations into static chamber systems for up to 22 d. The simple sustained C_2H_4 release delivery system could be used to release C_2H_4 concentrations in fruit ripening situations, such as in-transit ripening. It would be relatively safe and easy to use in-transit without requiring specialised training compared to ripening fruit with CaC_2. The system itself is easy to construct (Poole & Joyce 1993) using materials sourced from online stores, commercial agricultural stores and gas suppliers. Challenges arose with chamber design and also local capacity to accurately measure C_2H_4 and C_2H_2 gas concentrations. In particular, it was difficult to capture low C_2H_4 concentrations from the 100 ml min^{-1} flow-through chamber systems. Additionally, it was difficult to capture C_2H_4 and/or C_2H_2 from the 42 L woven bamboo baskets in the Philippines. In the developing countries

context of this chapter, the techniques investigated would be problematic to implement in places such as the Philippines. There, it is very expensive and difficult to access compressed cylinders of C_2H_4 gas for horticultural purposes (EV Bayogan 2016 pers. comm., 29 July). Also silicone tubing is very expensive and so, it is recommended to optimise with other plastic tubing. PVC tubing be could be investigated in this regard. It is also vital to have access to relatively sophisticated equipment and calibration gases, such as a GC and C_2H_4 standards, respectively. Further investigation into the use of plastic tubing filled with C_2H_4 gas on a small scale is needed to optimise simple systems for in-transit and also local produce market ripening. This work should include investigating C_2H_4 release from tubing under transit temperatures and environmental conditions. Such a simple system could provide a safe alternative to current systems using banned CaC_2. Further research on a smaller scale is needed before the system can be upgraded to commercial fruit ripening situations.

1.5 Acknowledgements

The authors would like to thank the Department of Agriculture & Fisheries technical staff, in particular Liz Singh, for their assistance in conducting experimental work at the Maroochy Research Facility, Nambour, Australia. The authors would also like to thank Jenny Ekman and Adam Goldwater from Applied Horticulture Research (AHR) and the Australian Centre for International Agricultural Research (ACIAR) for their assistance and collaboration in experimental work conducted in the Philippines.

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Appendix J. Ripestuff™ research activities during the HORT/2012/098 project variation

No.	Activity	Output/milestones	Date	People involved
1	Introduction and planning of experiments (Skype meeting)	Draft preschedule	31 May 2018	Sohail Mazhar Angelyn Lacap
2	Discussion of preschedule (Phone discussion)	Comments on preschedule received	8 Jun 2018	Sohail Mazhar Angelyn Lacap
3	Finalization of experimental plan (Skype meeting)	Updated experimental plan for UQ and UP Min	31 Jul 2018	Daryl Joyce Emma Ruth Bayogan Sohail Mazhar Angelyn Lacap
4	Receipt of portable ethylene analyzer from Australia to the Philippines	Ethylene analyzer used for monitoring ethylene from Ripestuff™	01 Aug 2018	Jenny Ekman Angelyn Lacap
5	Melinda Perkins replaced Sohail Mazhar as Research Coordinator for UQ		08 Oct 2018	Sohail Mazhar Melinda Perkins
6	Collaborative research in UQ Gatton	Conducted experiments on 1) Ripestuff™ release kinetics and 2) determination of ethylene concentration in old batches of Ripestuff™	10-14 Dec 2018	Angelyn Lacap Melinda Perkins
7	Research update (UQ Gatton)	Reviewed experiments conducted in UP Min and designed future experiments based on results from experiments in UQ Brought Ripestuff™ powder with known amount of ethylene from Australia to the Philippines	13 Dec 2018	Daryl Joyce Melinda Perkins Angelyn Lacap
8	Research update (Skype meeting)	Shared results of experiments in UQ (Experiments 1-4) and UP Min (Experiments 4-5), and discussed future Ripestuff™ experiments	13 Mar 2019	Daryl Joyce Emma Ruth Bayogan Melinda Perkins Angelyn Lacap
9	Research update (Skype meeting)	Shared results of experiments in UQ (Experiments 5-6) and UP Min (Experiments 6-7)	07 May 2019	Daryl Joyce Emma Ruth Bayogan Melinda Perkins Angelyn Lacap
10	Research update and wrap-up (Skype meeting)	Discussed results of experiments conducted in UP Min (Experiments 8-10)	2 Jul 2019	Daryl Joyce Melinda Perkins Angelyn Lacap

Appendix K
‘Carabao’ Mango

- Premium variety
- Also known as ‘Manila Super Mango’ or ‘Philippine Mango’
- Reputed internationally due to its sweetness and exotic taste
- Ranks as the third most important fruit crop in the Philippines next to pineapple and banana
- Considered as one of the priority and high value crops of the country

Sensory systems

Color vision
Smell
Taste

Food detection and selection

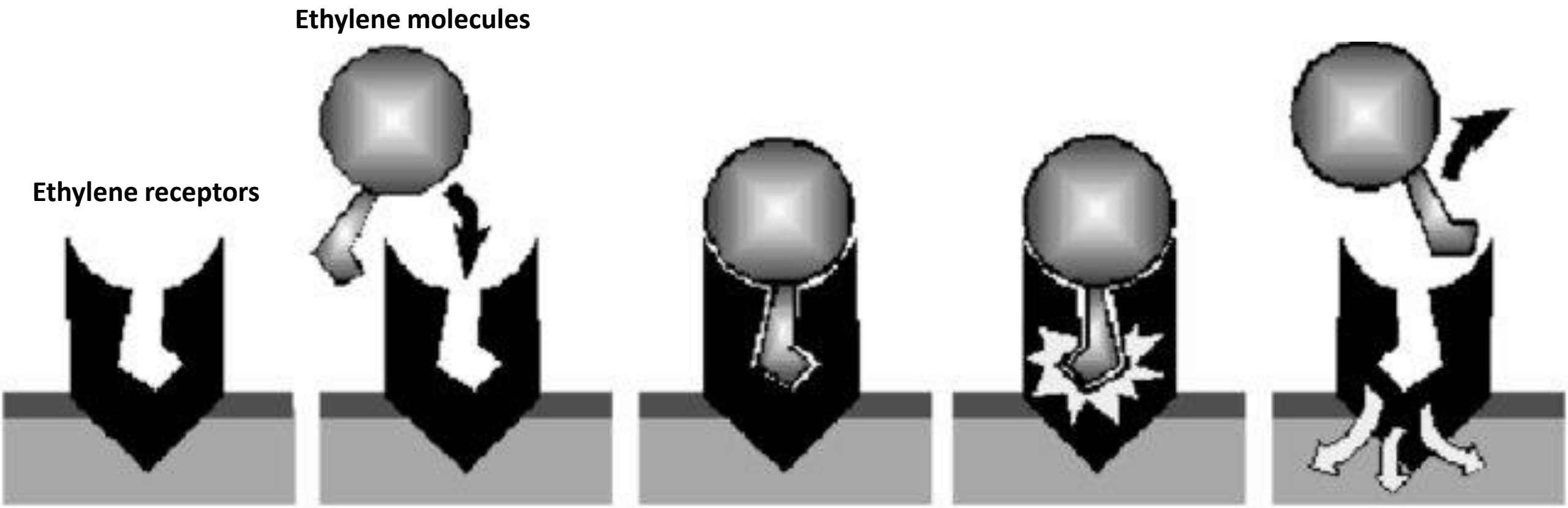
Fruit ripening

Color development
Firmness
Sweetness
Odor

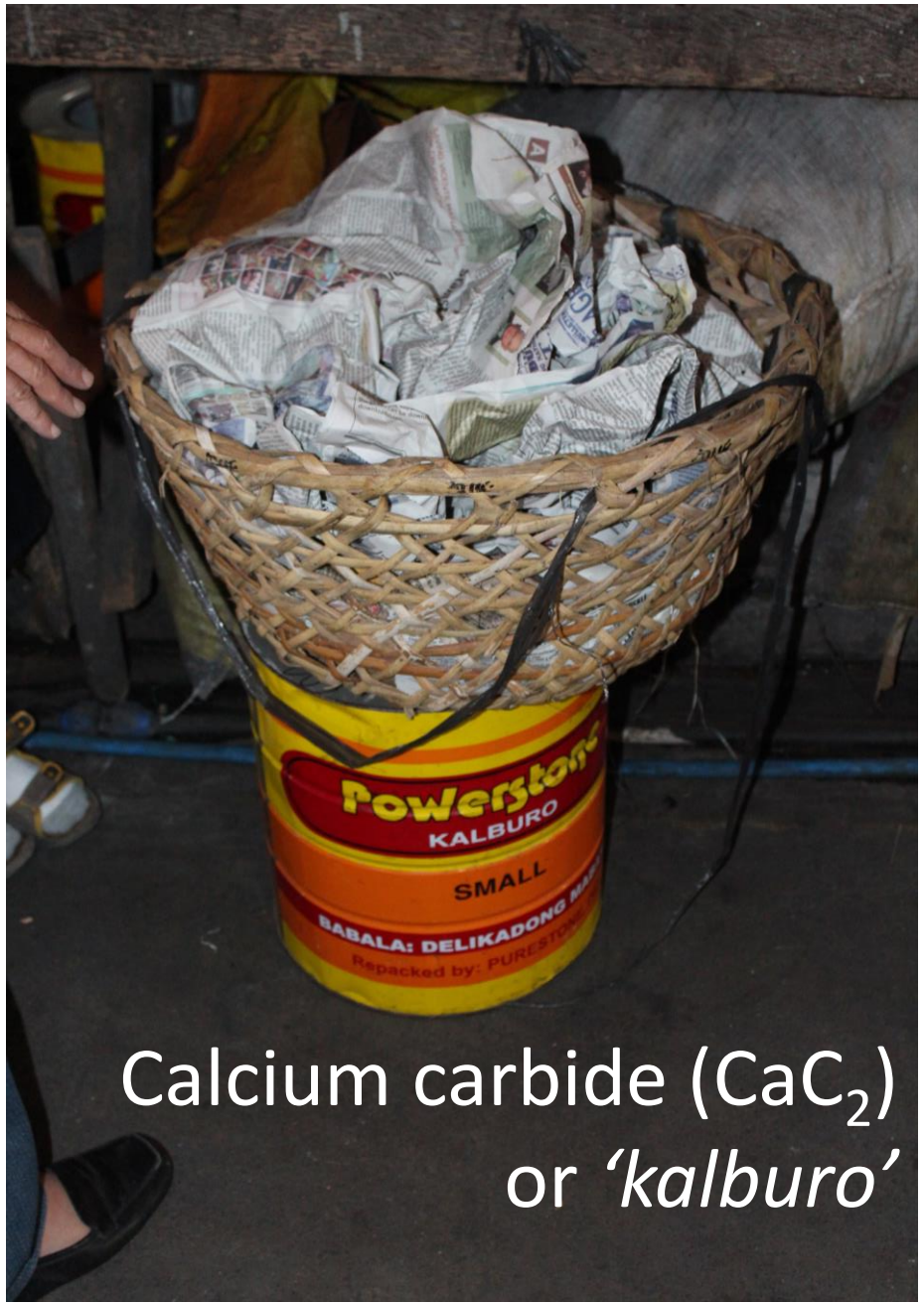
Increase fruit detectability

Ripening

Ethylene (C_2H_4) gas



Ethylene receptors are embedded in the cell.	Ethylene molecules in the air bind onto the receptors.		The ethylene molecule acts like a key, "unlocking" the receptor.	A chemical signal is sent to the cell, and the ethylene molecule releases.



Calcium carbide (CaC_2) or *'kalburo'*

200-250 g CaC_2 per 25 kg mangoes (1 basket)

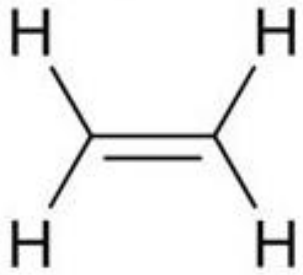
$$CaC_2 + 2H_2O \rightarrow Ca(OH)_2 + C_2H_2$$

Acetylene

C_2H_2

Structural analog

Ethylene



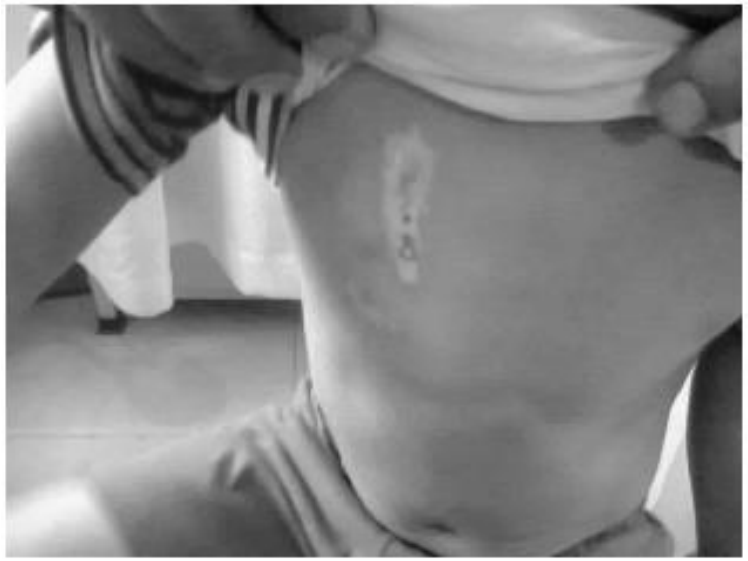
C_2H_4

Dangers of calcium carbide

Flammable

Eye/skin irritant

Carcinogenic



: Mucosal and Cutaneous contact burns due to calcium carbide.

Arsenic

and

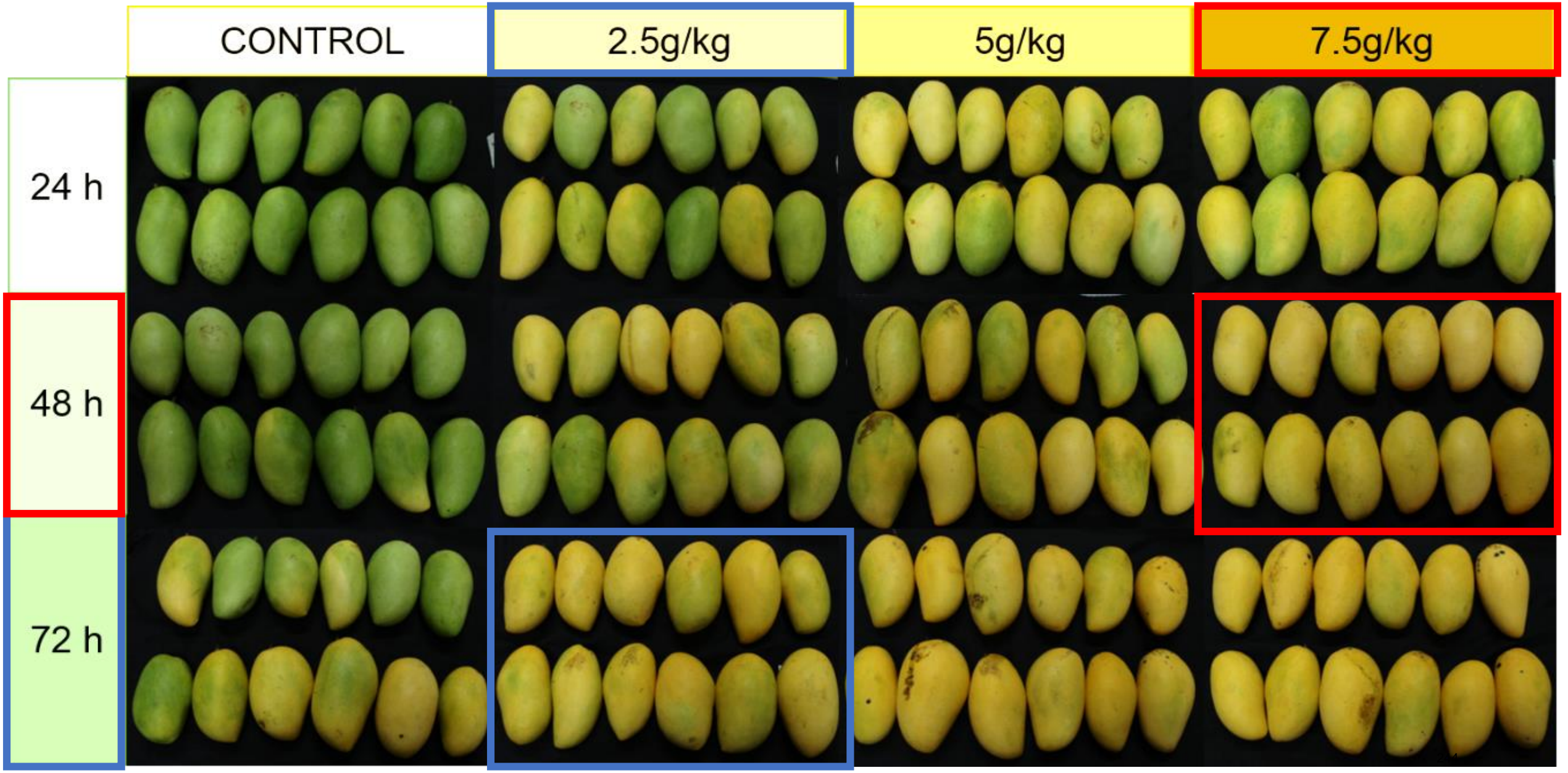
phosphorus

residues

Arsine gas (AsH_3) and phosphine (PH_3)

How to reduce the risks of calcium carbide?

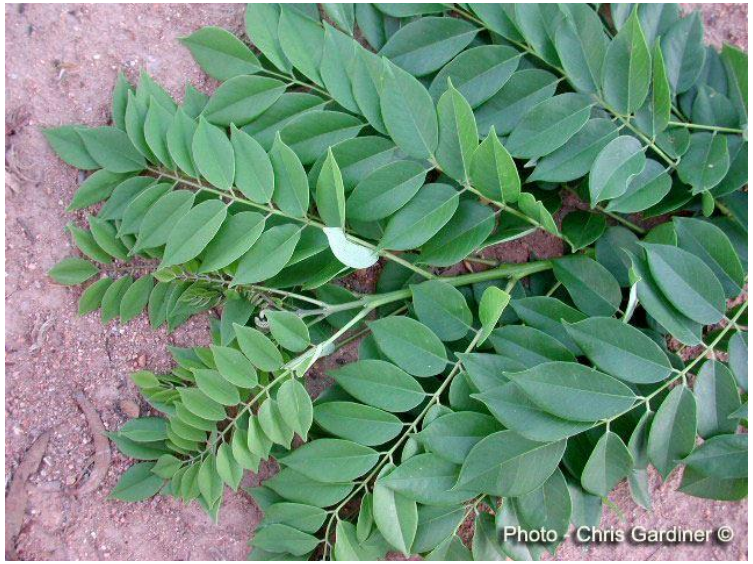
Calcium carbide concentration vs. treatment duration



Alternative ripening agents?

Ethephon

Bioethylene sources



Kakawate leaves
(*Gliciridia sepium* leaves)

More yellow than green
'Saba' banana

Calcium carbide vs. alternative ripening agents





I wish list for remake

desmark
I wish list for remake

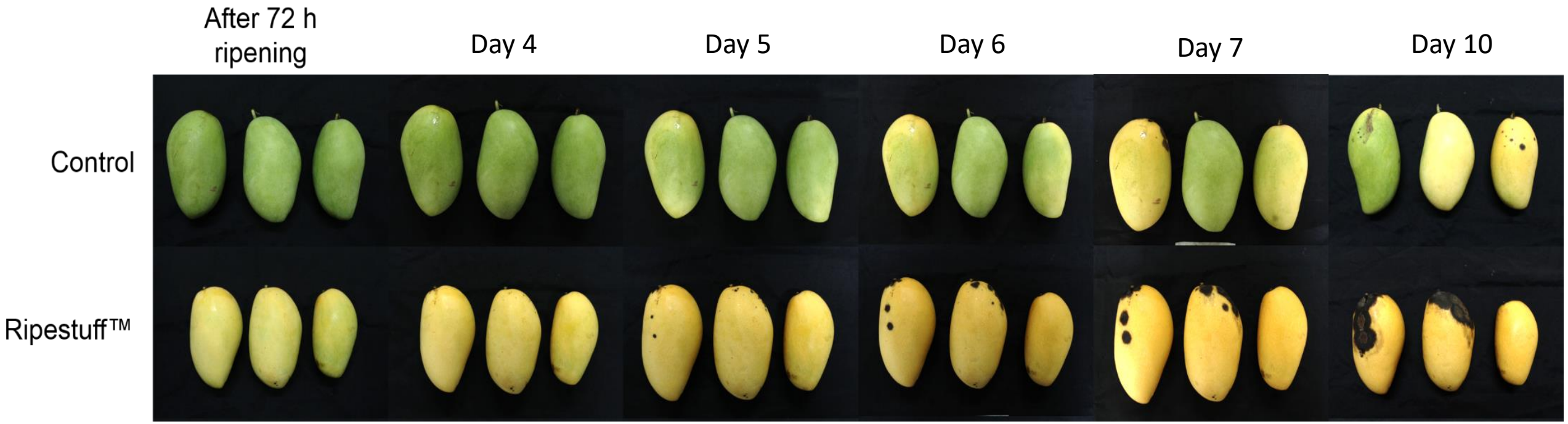

desmark

FORD RANGER

Ripestuff™

Ethylene-α-cyclodextrin inclusion complex powder

Control vs. Ripestuff™



“The fruit industry is ripe enough to advance into a safer ripening technology, and leave the harmful calcium carbide behind.”

Australian Government

Australian Centre for International Agricultural Research

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