Horticulture Innovation Australia

# Final Report

## In-transit ripening and prediction of outturn quality for mango

The Department of Agriculture and Fisheries (DAF)

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**MG12016**

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# Summary

Australian consumers prefer to purchase fruit and vegetables that are ready to eat. Climacteric fruit, such as banana, avocado, mango and tomato, are harvested mature but non-ripe, then ripened after harvest before placing on the retail shelf. These fruit are usually treated with ethylene gas for 1-3 days at the start of ripening. Ethylene is a natural plant compound that triggers the ripening process and results in more uniform, quicker ripening, and better quality fruit.

Traditionally, fruit are harvested and then cooled on-farm before transporting to the ripener at low temperatures to delay ripening until the fruit arrive at the ripener. During the ripening process the fruit use oxygen and produce carbon dioxide ($CO_2$) as a result of respiration. In most cases, the ripening rooms are regularly vented to maintain $CO_2$ concentrations below 2-4% because of the risk of fruit damage.

For mango, the above procedures require fruit to be cooled to about 13°C on the farm, then transported at this temperature. However, in-transit ripening will allow the fruit to be cooled and transported at 18°C, which would reduce cold room requirements at the pack house and energy costs during cooling and transport. In addition, less time should be required at the ripener to complete the ripening process. Therefore, in-transit ripening could save considerable costs for the whole supply chain, but good fruit temperature management and ethylene and $CO_2$ control are required.

This project aimed to:

- Test technologies to record fruit temperatures and $CO_2$ and ethylene concentrations during the 2-4 day refrigerated road trip of mango fruit from the Northern Territory to southern markets.
- Develop and test systems for releasing ethylene gas into the refrigerated container and for minimising $CO_2$ accumulation during transit.
- Develop decision aid tools that will estimate the behaviour of the fruit during transport based on the recorded temperature, ethylene and $CO_2$ concentrations and help the ripener to determine the additional days needed to ripen the fruit to the stage required by the retailers.
- Undertake semi-commercial testing of these technologies in selected mango supply chains and discuss the potential of these technologies with other relevant fruit industries.

Laboratory-based trials developed systems for slow release of ethylene from an Australian-developed ethylene releasing powder called Ripestuff™. Successful techniques were also developed for absorbing $CO_2$ using hydrated lime. Tests with commercial consignments using 12 m refrigerated containers from the Northern Territory to Brisbane confirmed that ethylene concentrations can be successfully maintained above the targeted 10 parts per million (ppm) for at least 48 hours and $CO_2$ concentrations can be maintained below about 5% throughout the 2-3 day journey. The quality of the ripe fruit was similar to those ripened under standard commercial conditions. The benefits in relation to quicker ripening depends on the transport duration and the transport temperatures.

Further research and commercial testing is required to reduce the application costs of ethylene and $CO_2$ management and to develop systems for maintaining oxygen concentrations above levels that will not impede ripening. More cost-effective means for monitoring ethylene concentrations are also required.

Detailed reports on the laboratory and commercial trials are available from the Department of Agriculture (Queensland), and from Horticulture Innovation Australia.

# Keywords

Ethylene, carbon dioxide, in-transit ripening, mango, temperature

# Introduction

Customers (agents and retailers) and consumers demand consistency of quality. For agents and retailers, this includes a specified stage of ripeness. For perishable products, predictable conditions (particularly temperature) from harvest to retail shelf, including during transport, are critical for quality consistency. Recent experience within mango project MG10008 ('Reducing skin damage and improving postharvest efficiency of Calypso$^{TM}$ mango') and with other commodities reveal frequent deviations from preferred or ideal conditions. It is unrealistic to expect "perfect every time" conditions. Recording transport conditions allows management decisions to be made as to how product should be efficiently handled on receival to minimise loss of quality. Understanding the response of fruit to variable postharvest temperature and other environmental conditions that affect storage and ripening will allow development of predictive tools to help product handlers to better manage the product.

For climacteric fruit like mango, a 4-7 day ripening period is required. This 'traditionally' involves transporting the fruit under relatively low temperatures to minimise ripening and then ripening them in-market. This practice wastes time, increases energy costs (e.g. extra cooling of fruit on farm and on the road) and requires additional cold room infrastructure in-market. Project MG10008 showed that 'B74' (Calypso$^{TM}$) mango can be partially ripened in-transit from the Northern Territory to Adelaide. However, the technologies piloted in MG10008 needed development to increase their efficacy, and requires evaluation under a broader range of commercial conditions. In addition, real time monitoring of transport conditions is needed to model predictable in-transit ripening outcomes.

This project was planned to:

- Confirm suitable technology to provide cost-effective solutions for real time monitoring of temperature, carbon dioxide ($CO_2$) and ethylene conditions during transport and handling of perishable 'B74' and 'Honey Gold' mango fruit.
- Compare several ethylene delivery technologies and optimise the most reliable one(s).
- Develop deterministic models for 'Honey Gold' mango using the measured data during transport to estimate product quality condition at outturn and its performance during further ripening and distribution to retailers.
- Reduce overall infrastructure costs and wastage of produce and resources (e.g. energy) in the supply chain from the farm to market by proof of concept implementation of reliable in-transit ripening with predictable ripeness stage at outturn.
- Undertake benefit cost analysis (BCA) of real-time monitoring systems and in-transit ripening.

As a result of this project, the need for on-farm cooling facilities and in-market ripening infrastructure should be significantly reduced because 'Honey Gold' mango fruit could be transported at 18°C and triggered to ripen during transit. This innovation would result in considerable cost savings and improved efficiency. The technologies refined with 'B74' mango were tested with 'Honey Gold' mango. This technology will also have relevance to other climacteric fruit, and to seafreight through triggering fruit to ripen to a predetermined stage before arrival.

# Methodology

## *Rationale*

The fundamental requirements for successful ripening of climacteric fruit are accurate and predictable temperature management, application of ethylene within the required concentration range, and maintaining $CO_2$ concentrations below about 2%, depending on cultivar. Project MG 10008 demonstrated the technical feasibility of maintaining fruit temperatures and managing ethylene and $CO_2$ concentrations in commercial refrigerated road container shipments from the Northern Territory (NT) to southern markets. Project MG 12016 was designed to refine and test these technologies during scale-up for regular commercial use.

The effects of temperature and ethylene and to a lesser extent $CO_2$ on mango ripening are well-established and so required no further investigation. For in transit ripening, 16-18°C is preferred to reduce the risk of fruit temperature build-up from the greater heat generated by the warmer fruit. About 10 ppm ethylene for at least 2 days is required to enhance the rate and consistency of mango ripening. Concentrations of 100-150 ppm are not likely to negatively affect mango ripening based on work with 'Kensington Pride', and these higher container concentrations at the start of the journey may ensure that all fruit in the container are quickly exposed to the minimum 10 ppm. $CO_2$ concentrations of more than about 3% can be hazardous and need to be managed.

Evaluating the potential for commercial in-transit ripening required monitoring of fruit temperatures and $CO_2$ and ethylene concentrations in non-treated and treated 20 pallet refrigerated road containers and then assessing the effect of these conditions on fruit ripening and quality. However, this is complicated by the significant variation within commercial consignments, such as:

- Product temperatures, both across the load in the container and between containers. This is largely the result of variable fruit temperatures at loading, the stowage pattern in the container, and airflow characteristics of the container. Higher temperatures significantly increase fruit respiration, heat generated by the fruit, and $CO_2$ production, and shorten fruit ripening times.
- The air-tightness (leakage) of the container. The concentrations of ethylene (from the ethylene release systems) and $CO_2$ are determined both by fruit production and ethylene powder release rates and leakage from the container.
- Batch-to-batch variation in fruit ripening characteristics because of, for example, fruit maturity at harvest and production conditions.

The initial approach to commercial testing with 'B74' mango was to apply in-transit ripening treatments to as many commercial consignments as possible to understand the variability through increased data. This required semi-commercial use of the technology. Lower 'Honey Gold' mango production volumes necessitated testing on fewer containers, but with more detailed monitoring. It also allowed sampling fruit at arrival at the ripener and assessment of ripening characteristics under controlled laboratory conditions.

The project consisted of the following four programs:

## *Environmental logging technologies*

The most appropriate logging technology is a balance between equipment cost, the cost of retrieving the logger and retrieving/analysing the data, and the risk of logger loss before data retrieval. Available

temperature logger technologies were reviewed at the start of the project. A wireless system was selected that appeared to have the best balance of the above requirements.

Wireless platforms for ethylene and $CO_2$ are not yet available. $CO_2$ loggers manufactured and used extensively by Mitsubishi Australia (MAXtend® division) Australia were used. Reliable, portable ethylene measurement is difficult and relatively costly. Most loggers with the required sensitivity are based on electro-chemical (EC) cell or photo-ionisation detector (PID) systems, each with their own advantages and disadvantages. Electrochemical ethylene loggers specifically designed for the project to provide at least 5 days battery life were supplied by GasAlarm (Sydney). To test the potential to provide a lower cost multi-sensor logger, a prototype logger containing ethylene, $CO_2$ and temperature sensors was designed and constructed in-house and tested in commercial consignments. The logger was based on the common Arduino microcomputer platform.

## *Ethylene application and $CO_2$ control systems*

Two ethylene release technologies were studied:

1. Common plastics are permeable to ethylene, allowing slow-release from plastic bags based on the surface area to volume ratio, compositional characteristics and thickness of the plastic, and the ethylene concentration inside the bag. Project MG 10008 tested 30-70 µm thick PVC bags filled with approximately 4% ethylene in carbon dioxide (Ripegas™). Ten bags, each 1 x 0.3 m, were fastened to each of 10 pallets in the container. To simplify commercial use, the current project tested non-explosive 4% ethylene in nitrogen and the use of two 5 x 0.3 m bags either attached to the pallets or the walls of the container.
2. Ripestuff™ is an alpha-cyclodextrin encapsulated ethylene product developed by The University of Queensland. Ethylene is released from the powder on exposure to high humidity or water. The first formulation of Ripestuff™ was tested in MG 10008. A less expensive novel formulation was developed at the start of this project. The basic release mechanisms and kinetics of the new formulation were studied to provide a sustained release system for testing in road containers. The final technique used a large number (150-200) of small (70 cc) plastic screw top containers with 4 x 0.5 mm holes through the lid of each container and each container holding 12 g Ripestuff™. Some testing of Ripestuff™ on banana was undertaken to allow out-of-season testing between mango seasons.

Discussions with experts in other disciplines indicated alternative mechanisms for ethylene storage (for example, metal organic frameworks), but changes in project structure and funding did not allow this to be pursued.

Hydrated lime is the most common and cost-effective method for absorbing excess $CO_2$ in static controlled atmosphere cold rooms. MAXtend® utilises hydrated lime in paper bags placed on top of pallets within the seafreight container. A similar system was trialled for in-transit ripening. Later laboratory trials refined the MAXtend® recommendations to address the high $CO_2$ production rates of ripening mangoes.

These gas control systems were tested each year on three to 10 commercial mango consignments from the Northern Territory and North Queensland to either Adelaide or Brisbane markets.

## *Predictive models for mango ripening*

Understanding how mango responds to conditions during in transit ripening could allow prediction of

remaining ripening time and shelf life. Hence, the ripening responses of 'Honey Gold' mango to 18-26°C, 0-80 ppm ethylene and 1-8% $CO_2$ during the first 4 days of ripening were studied. The trials were repeated over two seasons with fruit from the NT, North Queensland and Southeast Queensland.

The ethylene application and predictive model sections were to be part of a PhD program. However, after difficulty finding a suitable candidate, the candidate appointed resigned for personal reasons after about 6 months. However, a second candidate (Ms Khamla Mott) was found and appointed with a consequent delay to the commencement of research. Nonetheless, two seasons' laboratory data on fruit responses and progress in evaluating the potential to develop predictive models based on these data were realised. A significant reduction in funding associated with the withdrawal of the initial co-investor also lessened the detail of investigation undertaken within each of the Ethylene Application and Predictive Models programs.

The commercial trials determined significant reductions in $O_2$ concentrations during in-transit ripening. This aspect had not been included in the above modelling trials because of the known generally more significant adverse effects of high $CO_2$ on mango ripening as compared with low $O_2$. Nevertheless, in a separate trial the effects of typical combinations of 5-20% $O_2$ and $CO_2$ on 'Honey Gold' ripening were studied.

## *Benefit cost analysis*

A benefit cost analysis (BCA) was undertaken at the start of the project based on approximately 50% adoption with 'B74' mangoes after about 4 years and approximately 5% adoption to other mango cultivars. The BCA was refined toward the end of the project based on 'Honey Gold' engagement.

## *Other mango cultivars*

In-transit ripening was evaluated with 'B74' and 'Honey Gold' mango. Evaluation on other cultivars did not eventuate because of budget reductions.

## *Technology transfer and evaluation*

The key initial targets for adoption were the co-investing mango chains. The concepts were then to be demonstrated with chains involved with other mango cultivars such as 'Kensington Pride' and 'R2E2', with the technology and its benefits discussed with other chains involved with climacteric fruit such as banana and avocado.

Evaluating commercial feasibility required working closely with the co-investing chains and especially with the key chain members who were intimately involved in treatment application in the pack house and evaluation at the ripener. In addition, at least two pre-season and one post-season meetings were held with key members of the co-investing chains. Presentations were given at each of the Australian mango conferences (see Outputs) and articles were submitted to the mango industry magazine "Mango Matters".

# Outputs

## *Environmental logging technologies*

### Literature review of temperature monitoring systems

This review at the start of the project documented the broad classifications of available temperature monitoring systems from traditional recorders through electronic platforms requiring retrieval of loggers to manually download the data to emerging wireless technology that automatically downloads logger data to a web-based portal. The later allows stakeholder access to the summarized data via username/password. Of the wireless systems available at the start of the project, the Xsense/BT9 system was determined to be most appropriate for testing and evaluation in commercial consignments.

### The DAF ethylene, $CO_2$ and temperature logger

Battery operated loggers containing only an ethylene sensor and another version containing ethylene, $CO_2$ and temperature sensors were developed in-house using an Arduino platform. These were also tested in the laboratory and during commercial in-transit trials.

## *Ethylene application and $CO_2$ control systems*

### Report on release of ethylene from Ripestuff™

These reports provide an initial understanding of factors affecting ethylene release from novel Ripestuff™ formulations. They provided the basis for the release technologies used in practice in the trial commercial consignments as described in the Methodology section. The rate and duration of ethylene release from the new Ripestuff™ formulation was manipulated by varying numbers of holes in the lids of the 70 cc containers.

### Report on a bagged hydrated lime system for $CO_2$ absorption

This report compares the lime activity of various sources of hydrated lime. The lime activity is the capacity of the hydrated lime to absorb $CO_2$, and at least 85-90% lime activity is required for effective $CO_2$ absorption. The report also documents the permeability of the hydrated lime bag used by MAXtend® in their sea freight systems and the permeability of off-the-shelf large paper bags as alternatives to the MAXtend® bag. The results explained the poor $CO_2$ control results of the 2015/16 season and informed a successful $CO_2$ absorption strategy for the commercial trials in 2016/17. The respiration of 'Honey Gold' mango during ripening, with and without ethylene treatment, was also studied to enable calculations on lime requirements.

### Annual reports on testing in-transit ripening in commercial mango consignments

These reports summarise results of commercial trials in 20-pallet refrigerated containers, mostly from the Northern Territory to Adelaide or Brisbane. The trials were undertaken with 'B74' mango in the first two seasons and with 'Honey Gold' mango in the last two seasons. Ethylene release and $CO_2$ control systems were included and fruit temperature, and ethylene, $CO_2$ and $O_2$ concentrations were monitored. The first two seasons' results revealed inconsistent temperature management, which is critical to successful in-transit ripening. 'Honey Gold' mango temperature management was more consistent and the 2016/17 results indicated satisfactory ethylene application and good $CO_2$ control.

Out-of-season evaluation of temperature management in banana consignments was also undertaken. In addition, the leakage rates of several refrigerated road containers was tested in both stationary and on-the-road situations.

## *Predictive models for mango ripening*

### Temperature, ethylene and $CO_2$

The report for the 2015/16 and 2016/17 seasons describes the effects of typical in-transit conditions of temperature, ethylene and $CO_2$ on the ripening responses of 'Honey Gold' mango. Preliminary predictive models have been developed from these data. Their detailed development will be undertaken by the PhD student in the subsequent related project AM 15002 ("Serviced Supply Chains: Monitoring and modelling to improve the quality of Australian fresh produce into Asian markets").

### Low $O_2$ concentrations

The commercial trials confirmed significant reductions in $O_2$ during in-transit ripening due to fruit respiration. This report summarises the effects on 'Honey Gold' ripening of several combinations of 5-20% $O_2$ and $CO_2$, as recorded in commercial consignments.

## *Benefit cost analysis*

The BCA developed at the start of the project was adjusted to reflect the fact that 'Honey Gold' businesses were the commercial partners in the last two years of the project. Slight cost adjustments were made for e.g. hydrated lime, altered cost structure for the XSense/BT9 temperature monitoring system, the higher cost of $CO_2$ and ethylene monitoring (disposable loggers are not yet available), a delayed adoption rate, and reduced project costs because of the reduced voluntary contributions. It did not include potential benefits to other climacteric fruit industries such as avocado, banana and tomato.

This report is confidential and is not included in the appendix.

## *Technology transfer*

In addition to the above reports, the following activities contributed toward technology transfer.

*Articles published in Mango Matters, or submitted for publication:*

Hofman, P., Macnish, A., Ho, B., Marques, R., Bhandari, B., and Joyce, D. (2013). Ripening mangoes during transport. Winter 2013: 28-33.

Hofman, P. (2014). Reporting transit conditions for mango quality. Mango Matters Autumn 2014: 27.

Hofman, P., Joyce, D., Mott, K. and Macnish, A. Can we predict the quality of our mango fruit on arrival at the consumer? Submitted to Mango Matters December 2016.

Hofman, P., Macnish, A. and Joyce, D., Transporting your mangoes to market –do you know how they are being treated? Submitted to Mango Matters December 2016.

*Presentations at industry or international conferences:*

Hofman, P.J., Ledger, S., Macnish, A., Marques, J., Ho, B., Bhandari, B. and Joyce, D. (2013). Critical success factors in transporting mango fruit. 9th Australian Mango Conference. Cairns, Australia, 14-17 May 2013.

Hofman, P. Ledger, S. (2015) Critical success factors in transporting mango fruit. 10th Australian mango conference, Darwin (25-28 May 2015).
Mott, K., Hofman, P., Joyce, D., Macnish, A., Bhandari, B. (2015). In-transit ripening of mango fruit: concept and considerations. XI International Mango Symposium in Darwin (28 Sept-2 Oct 2015).
Hofman, P., Mott, K., Joyce, D., Macnish, A., Bhandari, B. (2017). In-transit ripening - increasing ripening efficiency and reducing costs. 11th Australian mango conference, Bowen (2-5 May 2017).

*Meetings with co-investing chains:*

About three meetings were held before the start of each mango season to determine R&D priorities for that season, and to plan the details for each trial. At least one meeting was held after the end of each season to report on the R&D results.

# Outcomes

## *Environmental logging technologies*

The Xsense/BT9 wireless temperature monitoring system provided a good balance between cost and efficiency. The system, however, required installation of a communications unit (CU) at the ripener to download the data from the temperature tags to the Internet. In this regard, the hard-wired CU model did not integrate well with the 'Calypso' business strategy because third-party ripening facilities were used and it could be problematic to install these loggers in time. Accordingly, a mobile CU based on individual SIM cards is potentially more convenient, even if more expensive.

The Xsense/BT9 was effective in retrieving and graphing the data. The website was relatively slow, but this has since been improved by BT9.

Real-time monitoring of temperatures with the Xsense system was also tested. This required CU installation inside the refrigerated container with the GPS and mobile aerials placed outside the container through the doors. However, this impost created extra delays with container loading, which was problematic during periods of high harvest and dispatch volumes.

The MaxTend® $CO_2/O_2$ loggers proved to be very effective, with compact design and adequate battery life. These loggers are not commercially available.

Ethylene logging proved problematic. The commercially-supplied loggers containing an EC sensor had ongoing issues with inadequate battery life and were relatively costly at about $2500 each. The DAF in-house built loggers contained both EC and PID sensors. Their calibration was only possible through firmware upload, which required training. Combining several sensors onto the one platform provided a compact all-inclusive unit, but it will require further technological development to optimise reliability and performance.

The two ethylene sensing technologies operating on different principles were compared. Both are influenced to varying degrees by other organic volatiles; for example, acetylene and internal combustion engine exhaust fumes. Consequently, ethylene concentrations recorded across the commercial trials were not always similar and were sometimes difficult to interpret.

## *Ethylene application and $CO_2$ control systems*

### Ripestuff™ laboratory trials

Laboratory trials indicated rapid release of ethylene from Ripestuff™ upon exposure to high humidity air. Slower release was obtained by enclosing Ripestuff in 70 cc containers with one to four holes of approximately 0.5 mL diameter in their lids.

Release rates were also influenced by the weight of Ripestuff™ powder per container and the relative humidity within the container. Relative humidity (RH) above about 75% significantly increased the release rate from the container. The RH in refrigerated containers with mangoes can vary between 80-95%, so further research is required to determine if these RH conditions affect release of ethylene from Ripestuff™. The effect of Ripestuff™ particle size on release rates also needs to be evaluated. Using 70 cc containers worked well in commercial trials (see below). However, simpler application methods that provide the required release rates remain to be developed for use in commerce.

### Carbon dioxide absorption

Hydrated lime sources purchased from local suppliers had lime activity over 90%, which is the recommended minimum for effective $CO_2$ absorption. The MAXtend® bag had significantly lower permeability than did the off-the-shelf bags and, therefore, were less suitable for the higher $CO_2$ production conditions within containers of ripening mango. Bags should have maximum permeability, but retain the hydrated lime to prevent contamination of fruit. Discussions with paper bag suppliers indicated little understanding of the characteristics of the paper used in the bags, so permeability tests on new batches is advised.

Based on these findings and the $CO_2$ production rate as determined for 'Honey Gold' mango, the following revised hydrated lime protocol was tested in commercial consignments:

- 192 kg of hydrated lime evenly distributed within Detpac (Brompton, South Australia) checkout bags providing a surface area of 24 $m^2$ and lime depth of 1.5 cm, and,
- 128 of these bags placed evenly in cartons on the top of the 18-20 pallets within a standard 12 m refrigerated road container.

### Commercial trails

The first two seasons' commercial trials with 'B74' mangoes revealed inadequate fruit consignment temperature management. The main contributing factors were poor pre-cooling at the packhouse before loading and stowage patterns that allowed the cold delivery air to short-circuit to the return vent. 'Honey Gold' mango temperature management was more consistent, with fruit temperatures generally maintained between 16-22°C. This allowed better comparison of the gas control treatments applied to different containers, and of fruit responses.

The use of thin-film PVC bags under commercial conditions proved problematic. Installation of the bags in the container, either by attaching to the pallets or to the walls of the container, required about 30 minutes during container loading, which often created difficulties with loading staff and drivers. This approach was abandoned after the second season.

Using about 200 containers with a total of about 2 kg of Ripestuff™ resulted in concentrations in excess of 170 ppm within about 12 h, and at least 2 days above the minimum required concentration of 10 ppm. The high initial concentration would have helped ensure rapid exposure to ethylene through the

whole load. However, concentrations of about 100 ppm would be more cost-effective.

MAXtend® recommendations for hydrated lime as applied in 2015/16 resulted in $CO_2$ concentrations in excess of 20% after 2.5 days. Using off-the-shelf paper bags with considerably higher permeability and a greater volume and exposed surface area of hydrated lime maintained $CO_2$ concentrations below 5% in all treated containers in 2016/17. The $O_2$ used during fruit respiration resulted in $O_2$ concentrations decreasing from 21 to below 3% in several consignments, which would adversely affect fruit ripening.

## *Predictive models for mango ripening*

Preliminary model analysis suggests that 'Honey Gold' fruit firmness after 4 days in the treatment chambers was significantly affected by growing region, temperature and ethylene concentration across both seasons, with inconsistent effects from $CO_2$. Skin colour (yellow / green balance, based on Hue) was also significantly affected by temperature, ethylene and $CO_2$. However, at the ripe stage (eating soft), temperature and ethylene treatment affected yellow / green skin colour, with no effect of $CO_2$. Ethylene treatment reduced the days to ripe, but there was little effect of increasing concentration from 20-80 ppm.

The results overall indicate that temperature and ethylene were the main factors influencing ripening times. An early stage predictive model using temperature and $CO_2$ was developed in Excel™. This model will be refined within project AM15002 and compared to other platforms (e.g. dedicated software programs) that may provide more accurate estimates and / or a more user-friendly interface.

An allied study indicated that more than 8% $CO_2$ and less than 5% $O_2$ delayed the loss of green colour and firmness during ripening compared to ethylene treatment alone, and required an additional 1-2 days to reach the eating soft stage. These results indicate the need to address the low $O_2$ concentrations recorded in the commercial trails.

## *Were all intended outcomes achieved*

Very significant progress was made in this project. Nonetheless, external factors impacted on project progress and outcomes:

- The initial mango chain co-investor withdrew from the project after the second season. The reasons for this withdrawal are obscured by commercial considerations, but appear to include:
    - Very good fruit temperature management is essential for effective in-transit ripening, and reliable pre-cooling of fruit before container loading is one of the key steps. This was often difficult for this commercial partner during the peak Northern Territory harvest because of insufficient pack house cooling capacity. Consequently, some consignments arrived at the ripener with fruit temperatures in excess of 30°C. This could not be rectified in the short term and so ongoing research on in-transit ripening became impractical for their supply chain system.
    - Management and staff changes within the company from the start of the project. The mango component of the business was sold after the second season.
- A second mango chain was eager to co-invest in years three and four, but at a markedly lower level because of existing commitments. As a result trials on in-transit ripening under simulated seafreight conditions were not conducted, and the depth of activities in the other components reduced.
- Delays in appointing and then re-appointing a suitable PhD student, which impacted progress on ethylene release systems and the development of predictive models.

### *Outcomes likely to be achieved in the longer term*

The potential financial benefits of in-transit ripening of climacteric fruit remain as evidenced, for example, by hard data to date and BCAs. However, they remain to be validated and realised in practice, including because of the delays outlined above prevented the development of commercially robust ethylene and $CO_2$ control systems. Further investments in laboratory development of the Ripestuff™ system and in alternative ethylene release technologies along with additional commercial trials to optimise the ethylene and $CO_2$ systems are warranted.

The research to develop predictive tools for mango ripening will continue. A major focus of project AM 15002 (project leader: Dr Peter Hofman) is to develop models to predict the quality of horticulture products on arrival in Asian markets. John Lopresti (Agriculture Victoria) is a project member and an expert in this area. The modelling component of the aforementioned PhD program will be completed in this project with John Lopresti's support.

# Evaluation and Discussion

### *Appropriateness of the methodology*

Temperature logging technologies have advanced rapidly over the life of the project. The Xsense wireless system was the most appropriate at the start of the project and is still a suitable system for integrated chains with ripening facilities. More flexible real-time temperature logging options are becoming available that do not require CU installation at receival points. These provide greater flexibility although at a higher per unit cost per logger.

Accurate ethylene measurement during transport is still problematic. Recent developments using, for example, nanotube technology may soon provide cheaper and more accurate monitoring. Several novel near-market devices will be tested within AM 15002 as and when prototypes become available.

Commercial testing provided the greatest methodology challenge because of the inherent variability in fruit characteristics, temperature management and variable refrigerated container performance. The initial approach of testing with larger numbers of commercial consignments and less detailed monitoring was problematic, largely because of a shift in the initial co-investor's priorities. Fewer commercial trials more carefully monitored provided a better approach, although with a greater workload for the project team.

Hydrated lime was the most appropriate method to reduce $CO_2$ accumulation. Good control was achieved under reasonable fruit temperature management. Attention needs now to focus on preventing the significant reduction in $O_2$ concentrations. This may require a 'bleed' valve that allows passive entry of outside air due to the potentially lower pressures inside the container upon the absorption of $CO_2$.

Ripestuff™ will be an effective ethylene application system given further development of its slow release systems and more consistent product characteristics, such as Ripestuff™ particle size.

### *Impact of the project*

The project identified significant deviations from recommended transport temperatures and practices. A strong focus on the importance of temperature management in the reports and at mango industry conferences may have resulted in practice improvements, although this was not quantified. Observations over the duration of the project suggested improvement in the use of refrigerated containers through the

adoption of ceiling air delivery chutes to help distribute cold delivery area throughout the container. However, this practice change may be restricted to one or two transport companies. Practice change with the initial co-investor was not realised because of their sale of the 'Calypso' mango business.

The project demonstrated the technical feasibility for in-transit ripening. Difficulties encountered in the project (viz. delays in PhD student appointment / reappointment, first co-investor withdrawal) delayed project progress. In-transit ripening has not been adopted as standard commercial practice by the second co-investor chain or more broadly as yet. This is primarily because robust commercial systems are still to be fully developed. Accordingly, further development and commercial testing is warranted and can be justified.

## *Efficiency of the delivery mechanism/s*

Output delivery was primarily via end-of-season progress reports, post-season meetings with the co-investing chains, and presentations at industry conferences.

The commercial trials required working closely with the investing chains and their members directly involved in pack house and in-market receival facilities. This hands=on gave them first-hand experience with the technology.

## *The effectiveness of project activities in delivering project outputs and outcomes*

Commercial testing was challenging because key variables often cannot be controlled. These include fruit maturity, numbers and timing of containers being dispatched to a ripener close to the research laboratory, fruit temperatures at loading, and refrigerated container performance as associated with container age and air circulation characteristics.

The first co-investor was encouraged to implement in-transit ripening more extensively from year two to allow more consignments to be treated to address this variability. However, this proved not to be practical / successful because:

- The XSense CU system did not suit their operations model, despite their initial choice of the CU units.
- Applying the $CO_2$ and ethylene control systems to each container required about 30 minutes, which was problematic during peak workload periods. However, technology development and refinement is an iterative process, where semi-commercial testing importantly helps identify the needs / areas for further improvement.
- The co-investor was moving towards the sale of their mango business, which eventuated the following year.

Perhaps more detailed semi-commercial trials to specifically reduce application times, and a more detailed assessment of pack house and logistics practices during peak periods may have resulted in smoother transition to up-scaled commercial implementation.

## *Learnings from the project and overall relevance to industry*

A key learning was the challenges associated with scaling-up and commercial testing of new ideas and novel technologies in fresh produce supply chains with commercial partners subject to business pressures, including those of other supply chain players. Within the context of this project, adherence to basic temperature management practices was an essential starting point, but this was not observed with the initial commercial partner during the first 2 years of the project. In association, an

understanding of operating constraints during the pressures of busy harvesting seasons was gained by the project players. Collectively, these and other operating environment constraints suggest that more extensive simulation testing may have increased application efficiency; e.g., in relation to installing ethylene bags. Out of season engagement with a banana supply chain did allow some out-of-season testing of temperature monitoring technologies, container leakage rates and ethylene release technologies. However, this could not be sustained because of significant budget cuts. Experimental design for commercial testing needs to be factored into such considerations. In-field testing often necessitates less stringent statistics as compared with controlled laboratory experiments and simulation trials.

The learnings from the Predictive Models for Mango Ripening program in this project are being adopted by the Predictive Tools program of AM 15002.

# Recommendations

- Continue evaluation of emerging temperature and $CO_2$ monitoring platforms, particularly those based on web portal reporting systems to reduce the need for intervention, increase reliability of reporting, and provide real-time capability. This will continue within HIA project AM 15002.

- Monitor emerging ethylene sensing technologies given the significance of ethylene for climacteric fruit. A start-up company in the US was approached in relation to their nanotube technology for ethylene monitoring. This too will be followed up within HIA project AM 15002.

- Engage with manufacturers and users of refrigerated road containers to outline project results on temperature monitoring and suggest improvements, particularly around managing airflow.

- Continue to develop economical Ripestuff™ application systems for generating the required ethylene concentrations in refrigerated containers. This would include additional laboratory work to understand the release kinetics of new Ripestuff™ formulations, refinement of manufacturing procedures to determine the right balance between manufacturing cost and commercial use, and ongoing semi-commercial trials.

- Continue to explore options for alternative ethylene release systems, including:
    - Low-cost systems based on semipermeable membranes,
    - Other technologies for containing ethylene until required release,
    - More controlled systems such as continual monitoring of ethylene concentrations, and dosing of e.g. Ripestuff to maintain the required concentrations. Several manufacturing companies have expressed interest.

- Conduct further commercial trials to refine lime requirements and application systems to minimise $CO_2$ accumulation. The procedures tested in 2016/17 provided good results, but further development and testing is required to reduce labour costs.

- Investigate the feasibility of alternative $CO_2$ absorption systems given the application and disposal costs of hydrated lime. $CO_2$ absorption/release systems are being investigated for environment control in greenhouses, and these may have application to in-transit ripening.

- Undertake laboratory and commercial trials to determine the conditions under which in transit ripening will reliably reduce ripening times. These conditions include:

- Fruit maturity (more mature fruit ripen more quickly).
- The temperature difference between containers without and within transit ripening. Temperature is a major determinant of ripening time, so non-treated fruit transported at 18°C will ripen considerably faster than fruit transported at 13°C. However, 'Honey Gold' mangoes from the NT cannot be transported below 16°C because of the risk of under skin browning (see the final report for MG 13016). Therefore, the benefits of in transit ripening are less. Also, the benefits would be greater with longer transport durations.

# Scientific Refereed Publications

Journal article

Ho, Binh T., Hofman, Peter J., Joyce, Daryl C. and Bhandari, Bhesh R. (2016) Uses of an innovative ethylene-α-cyclodextrin inclusion complex powder for ripening of mango fruit. Postharvest Biology and Technology, 113 77-86.

Chapter in a book or paper in conference proceedings

Mott, K., Hofman, P., Joyce, D., Macnish, A., Bhandari, B. (2015) In-transit ripening of mango fruit: concept and considerations. Acta Horticulturae (in press).

# Intellectual Property/Commercialisation

No commercial IP generated.

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# Appendices

1. Supply chain monitoring of product and environmental conditions of horticultural products
2. Characteristics of Ethylene Release from Ripestuff (α-CD encapsulated ethylene)
3. Developing effective system for $CO_2$ management under in-transit ripening condition
4. In-transit ripening report 2013-14
5. In-transit ripening report 2014-15
6. In-transit ripening report 2015-16
7. In-transit ripening report 2016-17
8. Effect of simulated in-transit gas conditions on ripe fruit quality of 'Honey Gold' mango
9. Modelling the responses of 'Honey Gold' mango fruit to in-transit ripening conditions – preliminary results
10. Paper in press: In-transit ripening of mango fruit: Concepts and considerations.
11. Published paper: Uses of an innovative ethylene-a-cyclodextrin inclusion complex powder for ripening of mango fruit

# Supply chain monitoring of product and environmental conditions of horticultural products

2013/14

In-transit ripening and prediction of outturn quality for mango
HIA project MG12016

Roberto Marques, Azmat Bajwah, Peter Hofman

This publication has been compiled by Peter Hofman of Horticulture and Forestry Sciences, the Department of Agriculture and Fisheries.

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# Summary

Temperature manipulation of perishable products is a critical tool in managing quality after harvest. Therefore, recording product temperature is essential for quality control and continual improvement. Traditional temperature monitoring systems have been based on retrieving data loggers to download the recorded temperature data, which, along with the cost of loggers and the risk of loss, has at least partly resulted in slow adoption of regular temperature monitoring programs in Australian horticulture supply chains.

Radio frequency identification systems (RFIDs) consist of information stored in the RFID tag being wirelessly transmitted to an RFID reader. They were traditionally used to track product flow and movement as an alternative to barcodes, but recently their value has been recognised as a platform for transmitting sensor (e.g. temperature) data from the tags to provide a wireless sensor system. These wireless sensors do not have to be retrieved to download the sensor data. Recent enhancements of this technology, including improved reliability and accuracy, lower cost, integration with internet-based data reporting systems, greater reading distances between tag end RFID reader, and the ability of tags to communicate with each other (called wireless sensor networks; WSN), has provided an exciting platform for cost-effective and continual recording of product temperatures, including in real time. It is possible the same platform can be used for recording gas concentrations (e.g. ethylene and carbon dioxide) around a product to further enhance the predictability of product outturn after transport and ripening.

This mini review summarises the current options for temperature monitoring and the various systems available in Australia, and recommends a monitoring system for commercial testing in mango supply chains.

# Introduction

The demand from consumers for quality and food safety has driven customers (e.g. retailers) to implement traceability systems in the supply chain (Trautman et al, 2008). The emergence of traceable agricultural supply chains is the outgrowth of a long line of developments in improving food quality and safety management. Food traceability is described as the part of a logistics management system which is able to provide all information regarding the food product, starting from its origin to delivery to the consumer, and including the various intermediate steps involved in its processing (Dabbene et al, 2013; Bosona and Gebresenbet, 2013). In order to ensure food safety and quality, it has now become essential for food based companies in most developed countries to maintain and implement correct food traceability systems in their standard operating procedures (Costa et al, 2013; Figure 1).

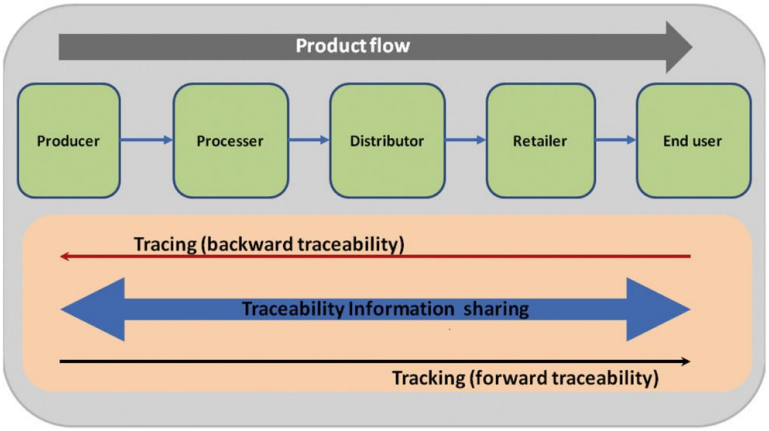


Figure 1: Conceptual representation of product and traceability information flows relevant to the food supply chain (Bosona and Gebresenbet, 2013).

Traceability is an important tool supply chain members can use to ensure specific processes and safety measures are correctly implemented. The real value of a traceability system within an agri-food supply chain is illustrated when there is a food safety breach causing potential health implications. An effective traceability system can identify the cause of the breach and indicate improvements (Costa et al, 2013). In addition, good traceability systems will identify breaches in protocols that may result in product loss (e.g. inappropriate temperature conditions during transport/storage) and allow corrective actions to be taken.

# 1. The importance of temperature

Temperature is critical in maintaining the integrity of perishable horticulture products. The product continues to respire after harvest, with increasing respiration rates usually associated with more rapid senescence, shorter storage and shelf life and increased wastage. Maintaining storage temperatures that balance the need to minimise respiration rate without causing chilling damage, are essential for optimising storage life and outturn quality after long transport. Also, ripening of climacteric fruit requires specific temperatures to ensure maximum ripe fruit quality.

Because of the above, most horticulture products are transported with some degree of temperature control. Accurate monitoring of fruit temperatures during this phase is an important traceability requirement to allow corrective actions if transport temperatures breach set limits, and to allow adjustment in post-arrival treatments to minimise the impact of these breaches. It is also an essential element of continual improvement in transport and post-arrival systems.

Significant advances in electronics and miniaturisation has facilitated rapid development in effective temperature management and recording. This brief review outlines the basic groups of technologies available, and the advantages and disadvantages of the systems to horticulture product temperature monitoring from farm to retail.

# 2. Product and environmental monitoring systems

Effective monitoring systems have the following requirements:

- sensing the variable of interest, e.g. temperature
- recording or storing the data
- retrieving the data from the device, and
- data analysis, interpretation and recommendations for action.

Until recently, temperature monitoring systems required manual retrieval of a logger, and physical connection to a computer or similar device to download the data from the logger. There are many examples of these systems (e.g. Hastings and LogTag TREX-8 systems) with prices ranging between about $30-$250, depending upon its specifications and logger durability.

The advantages of these systems are that it can support a more expensive logger (more robust with greater storage capacity and battery life) because of the intent to always retrieve the logger to obtain the data. The obvious disadvantage is the need for additional intervention and attention to track and retrieve the logger and download the data.

There are various refinements within the group, e.g. increasing the efficiency of connection to the computer by using a “cradle” into which the logger is inserted, or the data automatically uploaded to a server for data analysis, graphing and reporting. However, the major limitation is the need for logger and manual data retrieval.

## 2.1. Radio frequency identification systems

Automatic data capture technologies, or automatic identification (Auto-ID) technologies can be used to gather information on the food commodities through the supply chain, and identify them using no or minimal hands-on input. There are five major forms of auto-ID technologies currently available: barcode systems, radio frequency identification systems (RFID systems), smartcards, biometric

systems, and optical character recognition (OCR). A number of wireless technologies have been used previously for identification purposes in the food and agriculture industry (Lionel and Yunhao, 2004). However, RFID technology has developed into a quick and reliable system for identification and tracking of produce, as well as data transfer of other parameters.

RFID technology is a flow control technology which allows traceability of goods through all steps of the chain (Costa et al, 2013). The technology uses wireless radio frequency electromagnetic fields to transfer data from tags containing electronically stored information attached to the tray or pallet. RFID is entering a new phase through increased application in a wide range of agri-food processes (Ruiz-Garcia and Lunadei, 2011). Widespread popularity and adaptability of RFID technology will bring significant chain improvements and will eventually replace the ubiquitous universal product code (UPC) identification, mostly known as “barcode” (Michael and McCathie, 2005). The RFID platform can also be used to transmit data from a sensor without the need for retrieval of the sensor/logger and connection to a computer or download device. Combining a sensor (e.g. temperature) on an RFID platform produces a wireless sensor that allows transmission of stored temperature data to a remote reader.

### 2.1.1. RFID components

The basic components of an RFID system are the tag (also known as a transponder) with an antenna, a reader (transreceiver) with an antenna, and a host terminal (Sardroud, 2012; Figure 2). The RFID reader (sometimes called communication unit; CU) can act as both a receiver and transmitter by sending an electromagnetic field that triggers the tag by providing the power required for the tag to function (Sardroud, 2012).


Figure 2: Components of RFID System (Sardroud, 2012)

#### 2.1.1.1. The RFID Tag

The RFID tag is a transportable data storage device located on a chip which is encapsulated in a protective shell and attached to any object (e.g. food product). The tag itself is made up of a small integrated circuit chip which stores important information regarding the object, and an antenna to allow the receipt and response to radio frequency signals from the reader (Sardroud, 2012). The tag three important characteristics: data/memory storage capacity, the way the tag is powered, and the reading and writing ranges. Tags also have varying information storage capacity, life expectancy, recyclable ability, attachment method, usability and cost (Kumar et al, 2009).

Tags can be categorised as Read-Only (RO), Write-Once Read Many (WORM), and Read-Write (RW), based on the nature of the included memory (Jedermann et al, 2009). RFID tags can be further classified into active tags which are battery powered, and passive tags which are powered by the magnetic field emanating from the reader; these have an unlimited life. Tags can be ultra-low-cost, known as chip-less tags, which have a relatively short read range. The reading and writing ranges of tags also depend on radio frequency (low, high, ultra high, and microwave). The typical reading range is between 10 cm and 3 m but can be up to 30-50 m with clear line of sight. RIFD tags suitable for cold chain monitoring operate at several transmission frequencies as shown in Table 1 (Ruiz-Garcia and Lunadei, 2011):

Table 1. Available technologies for radio frequency identification (RFID) operation.

| Frequency band | Common frequency | Tag type | Communication range | | Advantages | Disadvantages |
|---|---|---|---|---|---|---|
| | | | Typical | Max. | | |
| High (HF) | 13.6 MHz | Passive and semi-passive | 10 cm | 1.5 m | Band available worldwide | Inductively powered, so limited range |
| Ultra high (UFH) | 433 MHz | Active | 3 m | 10 m | Band available worldwide;<br>Greater range; | More interference due to shorter wavelength;<br>Battery limitations |
| | 860 MHz | Active and passive | 3 m | 15 m | Higher frequency = more power and greater range | More interference;<br>Not approved in some countries |
| Microwave (MW) | 2.4 GHz | Passive and active | 3 m | 30 m | Band available worldwide;<br>More powerful than UHF | Potential more interference than UHF;<br>Band shared by other technologies (e.g. Bluetooth, Wi-Fi, GSM) |

#### 2.1.1.2. RFID reader and host system

The RFID reader uses an external antenna to read from, and write data to the tag using radio frequency; it can also transmit data to a host computer (Sardroud, 2012). The reader can be configured either as a handheld or a fixed mount device (Finkenzeller, 2010). The host comprises of a system that includes hardware and software that are separate from the RFID tag and reader.

Systems with small reading distance require the manual transfer of the RFID reader into close proximity with the RFID tag to allow data download. This is obviously more efficient than logger retrieval and connection to a host (usually a computer), but still requires intervention for data retrieval.

### 2.1.2. Advantages of RFID

The use of RFID technology is progressively gaining importance in horticulture because (Costa et al, 2013):

- it is a relatively easy way of tracking the product from farm to the retail store through large storage/selling areas
- it is an effective way to identify e.g. temperature problems during distribution, and
- of the ease with which the tags can be programmed to record, retain and display large amounts of information.

Among its many advantages, RFID:

- Reduces or eliminates manual intervention to retrieve sensor data
- Increases versatility of operational environments, e.g. they can be water-proof, antimagnetic, and operate in a wide range of temperatures

- Is available with a range of reading distances between tag and reader
- Has long service life
- Can be miniaturized
- Data may be encrypted on the electronic label, allowing large data storage capacity up to 32 kilobytes. The stored data can be refined by the electronics, providing the possibility to correct eventual errors committed during the information flux or to add information that at the beginning was unknown (Costa et al, 2013).
- They can also transmit data at very high speeds, in some cases in less than 100 milliseconds (Lionel and Yunhao, 2004).

Ongoing and future innovations will lead to larger memory capacities, greater reading distances between the tag and the reader, and faster processing times. Some additional advantages and disadvantages of the RFID technology, over other identification technologies, are outlined in Table 2 (Sardroud, 2012).

Table 2. The advantages and disadvantages of radio frequency identification (RFID) technology.

| RFID Advantages | RFID Disadvantages |
|---|---|
| Non-line-of-sight scanning | Cost of tags and new infrastructure |
| Simultaneous automatic reading | Lack of training and limited knowledge |
| Labour reduction | Still a developing technology |
| Enhanced visibility and forecasting | Concern of return on investment |
| Item level tracking | Lack of ratified standards |
| Traceable warrantees | |
| Reliable and accurate | |
| Information rich | |
| Enhanced security | |
| Robust and durable | |

## 2.2. Wireless sensor networks

Wireless sensor networks (WSNs) build on the wireless sensor platform to provide the greater flexibility often required at packhouse and receival points such as retail distribution centres. It incorporates a network of sensors to collect and transmit the data to the RFID reader or CU (Wang et al, 2006; 2009). The sensors can exchange data with each other and with the RFID reader. This allows data from sensors beyond readable distance to transmit data to sensors within reading distance of the reader, which greatly enhances the total readable distance. A central node such as the RFID reader collects information from the group of spatially related sensors and facilitates communication with the data storage, analysis and reporting system, which is either computer or internet-based. The advantages of WSN are enhanced with the appropriate routing and network topology (Ruiz-Garcia and Lunadei, 2011). The data can be transmitted from the RFID reader to a host computer or system using standard protocols such as Global System for Mobile Communication (GSM) or General Packet Radio Service (GPRS), or through e.g. Local Area Networks (LAN) or Wireless Local Area Networks (WLAN) (Ruiz-Garcia et al, 2009). Recently, low-cost, low-power, multifunctional sensor nodes for WSN have been developed (Costa et al, 2013).

The two most common standard communication technologies between sensors, readers and hosts, available for WSN are (Ruiz-Garcia et al, 2009):

- ZigBee (defined the physical and the Medium Access Control layers for low-rate wireless personal area networks), or
- Bluetooth (developed as a wireless protocol for short-range communication in wireless personal area networks replacing mobile devices).

## 2.3. Implementing RFID using WSN Technology

RFID technology allows the quick and efficient transfer of information about the food product from the tag, via the RFID reader to the end user. WSN technology can enhance the efficiency of the RFID technology by reducing the need for intervention and improving the efficiency of data transfer, especially in large warehouses where the sensors may be some distance from the reader.

Different options for the use of WSN/RFID are proposed in the literature (Jedermann et al, 2009; Costa et al, 2013): These include:

- Intermodal transport containers operating based on the principle of artificial intelligence
- A mix RFID tags and WSN sensor nodes within the same area. A station gathers information from tags and sensor nodes at once then transmits the information to a local host computer or remote server.
- A “smart node,” which uses different types of sensors to detect the required physical data (e.g. temperature), an RFID reader and a radio transceiver which transmits the collected data
- The replacement of RFID tags (which are of active type) by Xbow motes. The active tag is similar to the Xbow mote, but they are not exactly sensor network nodes because they communicate to a centralized mode and cannot cooperate with each other through a formed ad hoc network (Ruiz-Garcia 2008).
- A real time monitoring system using WSN-adapted transport containers. WSN/RFID devices can be placed in transport containers to monitor the environmental conditions in real time, collecting data from the tags and transmitting the data via mobile or satellite networks to an internet-based software application. The possibility of combining RFID with internet-based data storage, analysis and reporting offers valuable opportunities such as (Costa et al, 2013):
    - availability of critical real time information concerning any deviation from a pre-determined set of conditions (or variables) (e.g. temperature, carbon dioxide or ethylene) for any given product at any point in the supply chain, can become a powerful decision making tool for suppliers and retailers
    - reduction in the costs of industrial monitoring technology
    - informative integration
    - optimisation of intra- and inter-company logistics regarding quality preservation and safety implementations.

WSN/RFID integration allows expansion of monitoring possibilities. However, despite its rapid growth and recent technological advances, commercial applications of WSNs are still limited by several technical and economical challenges, including (Kapoor et al, 2009; Ruiz-Garcia and Lunadei, 2011):

- flexibility to be adapted to the complexity and variability of different supply chains
- variations in reading range and accuracy
- fault detection and isolation
- non-uniform and/or incompatible standards
- resistance to information sharing
- computing bottleneck, and
- cost-benefit issues.

## 2.4. Temperature and environment monitoring using WSN technologies

Monitoring the temperature of food products is essential to maintain product freshness and appearance. RFID temperature tags can be just as accurate as traditional temperature data loggers but have the additional advantages of quick data recovery and in some instances having access to the data in real time (Amador et al, 2009). The rapid development of information technology tools has allowed relatively fast implementation of cold chain traceability systems within the agri-food industry although the adoption of these technologies has been relatively slow in Australia.

Temperature and gas monitoring using WSN technologies has been tested on only a few commodities. Wireless sensors have been used for monitoring tomato temperatures from production to retail (Hertog et al, 2008). Oxygen and carbon dioxide conditions have been monitored during transport and distribution of apples, broccoli and lettuce by integrating metal oxide gas sensors into RFID/WSN systems (Vergara *et al*, 2007; Lokke *et al*, 2011; Eom *et al*, 2012).

# 3. Classification of sensor systems and availability in Australia

Sensing technologies can be classified based on the efficiency with which the sensor data are recorded, transmitted to the end-user and analysed/interpreted (Table 3).

Table 3. Summary of characteristics of product and environment sensing technologies

| System | Data retrieval | Manual intervention | Real time capability | Data analysis/availability |
|---|---|---|---|---|
| Traditional | Manual retrieval of the sensing and data storage unit | High | No | Often resides with the individual retrieving the logger, although the system can be integrated with internet-based applications |
| RFID | Wireless | Often requires an operator with an RFID reader | No | As above |
| RFID/WSN | Wireless | Minimal because data can be transferred over longer distances | Yes, assuming the RFID reader/ CU has mobile or satellite network capability | Generally internet-based, username and password access to supply chain members |

Table 4 summarises the key characteristics of available RFID and WSN systems in Australia as of October 2013. Available systems cover the range described in Table 3. Only two WSN systems with potential real time capacity are currently available. However the CU of the CoolTrax system is not battery operated and has to be connected to the power supply of the truck, and preferably hard wired. This is not suitable for containers transported by rail, and is very inconvenient for road transport from the NT because of the time pressures during the peak of the season and the relatively limited choice of transport suppliers and systems. Therefore the Xsense system from Israel was selected for commercial testing in the 2013/14 season.

# 4. Conclusions

Product temperature management is key mechanisms for maintaining quality of perishable products, and is particularly relevant to horticulture. Previous temperature monitoring systems required relatively expensive temperature loggers and logger retrieval to access the recorded data, which limited widespread use of temperature monitoring in the supply chain. Reduced cost of sensors, increased sophistication of RFID/WRN systems, and the advantages these systems offer through ease of data retrieval and access, should allow increased temperature monitoring in Australia. These systems can be successfully applied to manage traceability and monitor supply chain characteristics because of their ability to record and transmit important information about the product and its condition (Ruiz-Garcia et al, 2011). Using this technology, producers and/or suppliers can better maintain the quality of their products, and provide healthy, fresh and quality products to the consumer.

Several RFID/temperature monitoring systems are available in Australia, with varying levels of sophistication. Currently, only two (XSense and CoolTrax) offer WSN capability and real time monitoring using RFID readers/CUs placed in the truck or transport container. At this stage, the CoolTrax CU requires truck battery power and is best hard-wired into the truck electrical system. This is less appropriate in more remote areas such as the Northern Territory because of restricted access to transport infrastructure. On the other hand, the XSense CU is battery-powered and can be placed in any container to allow real-time transfer of data during transport. The unit can also be placed at receival centres such as ripeners for collection of data on arrival from sensors placed in pallets during packing. This system offers the flexibility required for use in mango supply chains. It is currently being tested for mangoes from the Northern Territory during transport to southern markets.

Table 4. Key characteristics of RFID and WSN currently available, or with active plans for release in Australia.

| Component | Feature | Xsense (BT9) (NZ/Israel) | Cooltrax (VIC/USA) | ECEFast (VIC) | CAENRFID (Italy) | Zenatek (EU/USA) |
|---|---|---|---|---|---|---|
| System | Real time | Yes | Yes | Yes | Yes | Yes |
| | Wireless (GSM/LAN/GPS) | Yes | Yes | Yes | Yes | Yes |
| | Reader's battery/operation time | 2-3 months | No battery | ? | ? | 3-6 months |
| | Detect many tags at once | Yes | Yes | Yes | Yes | No |
| | Hands-free data downloading | Yes | Yes | Yes | Yes | Yes |
| | Type of download system/software | Remote via internet | Remote via cloud | Remote via cloud | Remote via internet | Remote via internet |
| Tag | Type/frequency (MHz) | Active, 433 | Active, 433 | Active | SP, 860+ | Active, 850+ |
| | Multi use | Up to battery life | Yes | Yes | Yes | Yes |
| | Battery life | 3-12 mths | ? | Re-chargeable | 1 year | 3-6 mths |
| | Can it measure pulp temperature | No | No | No | Yes | No |
| | Signal range | 100-150 m | ? | ? | 1.5 m | |
| Cost | Tag (each) | $14 | $100-110 | Included in CU | $38 – 55 (w/probe) | 0 (included in CU) |
| | Reader/CU (plus SIM card) | $1,300 | $1,850 | $ 2,000 - 6,000 | $1,950 | $250 |
| | Other (accessories, installation fees) | $1,500 | $100 (+labour) | 0 | $135 | SMS messages |
| | Software | 0 | 1,400 | $50 monthly | ? | No |
| | Data handling fee (per month) | $150 | ? | $30-50 | ? | ? |
| | Cost trip/container (w/ 3 tags) | $1,042 | $2,250 | $ 2,000 - 6,000 | $2,470 | ? |
| Conclusions | Main constraints | | Reader: no battery;<br>Costs: too high. | High cost; it seems unsure about tags | Cost: too high<br>Poor reliability/high interference. | Only one tag placed outside of door. |
| | Main advantages | Best so far; good customer service | | | | Low cost |

Table 4 Cont.

| Component | Feature | Intelleflex (USA) | Sensitech RF (VIC) | Sensitech USB (VIC) | Sensitech Holder (VIC) | SAVI* (USA) |
|---|---|---|---|---|---|---|
| System | Available in Australia | Not currently | Yes | Yes | Yes | Yes |
| | Real time | Yes | No | No | No | Yes |
| | Wireless (GSM/GPS) | Yes | No | No | No | Yes |
| | Reader's battery/operation time | Yes/? | No | No | No | |
| | Detect many tags at once | Yes | No | No | No | |
| | Hands-free data downloading | Yes | Yes | No | No | |
| | Type of download system/software | Remote via internet | Local via PC | Local via PC-USB | Local via PC-holder | |
| Tag | Type/frequency (MHz) | SP, 865-8 | SP, 915-868 | Internal sensor | Internal sensor | |
| | Multi use | Yes | No/Yes | No/Yes | No/Yes | |
| | Battery life | 2 years | Up to 1 year | Up to 1 year | Up to 1 year | |
| | Can it measure pulp temperature | No | No | No | No | |
| | Signal range | ? | 100 m | N/a | N/a | |
| Cost | Tag (each) | US $27 | $30-40/180 | $25/195 | $20/90 | |
| | Reader/CU | US $2,000-2,600 | 0 | No | No | |
| | Other (accessories, installation fees) | $260 p pair | PC p. site | 0 | $100 p. site | |
| | Software | ? | $100 p site | 0 | 0 | |
| | Data handling fee (per month) | ? | 0 | 0 | 0 | |
| | Cost trip/container (w/ 3 tags) | US $2,500 | $90-120/$540 | $75/585 | $60/270 | |
| Conclusions | Main constraints | Not available in AU; reader: high cost | High cost of tag; not real time | High cost of tag; not real time | High cost of tag; not real time | *Needs research (Rod Jordan) |
| | Main advantages | | | | | |

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# Ethylene release from Ripestuff™, an alpha-cyclodextrin complex

## 2015/16 and 2016/17 season results

## In-transit ripening and prediction of outturn quality for mango

## HIA project MG12016

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# Summary

Initiating the ripening of climacteric fruit during road freight from farm to market can save infrastructure and energy costs by reducing the time required to ripen in market. Reliable application of ethylene during transit is an essential component of successful in-transit ripening. The key factors in successful ethylene application is the rate of ethylene release into the trailer, and the leakage rate of ethylene from the trailer. The present study suggested that leakage from difficult refrigerated trailers would result in gas concentrations dropping by half over 5-6 hours. This estimate was used in laboratory trials to understand the ethylene release characteristics from Ripestuff™ (an ethylene encapsulated powder) placed inside 75 mL plastic containers. The trials were conducted in 1 $m^3$ chambers with the required leakage rate. Different Ripestuff™ quantity, number of holes in the container lid, headspace in the container, and moisture content of powder were tested. The holes in each lead were made with a 0.5 mm hypodermic needle.

The results showed that ethylene concentrations in the chamber increase with increasing weight of Ripestuff™ per container, increasing number of containers, and increasing number of holes in the lid of each container. Three containers, each with 12 g Ripestuff™, and with four holes per lid provided up to 60 ppm ethylene in the treatment chamber, and about 70 hours above the minimum 20 ppm. These results were used to estimate the number of 75 mL containers required to provide similar ethylene conditions in a refrigerated trailer with 18-20 mango pallets.

Other important factors influencing ethylene concentration in the chamber included the Ripestuff™ surface area: volume ratio, it's moisture content and the relative humidity inside the container with Ripestuff™. The impact of these factors on commercial application needs to be further researched. In addition, a better understanding of moisture content and particle size effects on ethylene release is required in order to provide more uniform ethylene release characteristics between manufacturing batches.

# Introduction

Climacteric fruit such as mango and banana are often ripen with ethylene, a natural plant hormone that triggers ripening. In some developing countries like India, Pakistan, Philippines and Bangladesh, calcium carbide ($CaC_2$) is used to ripen mango. It reacts with water to produce a settling gas, which in higher concentrations produces similar responses in fruit to ethylene. However, it also contains traces of arsenic and phosphorus hydride which causes significant food safety concerns (Siddiqui and Dhua, 2010).

Ethylene is more difficult to handle, and is usually applied to fruit from pressurised cylinders or from an ethylene generator that catalyses the conversion of ethanol to ethylene. Encapsulated ethylene-α-cyclodextrin (CD) inclusion complex (IC) powder releases ethylene on exposure to high humidity or water, and is easier to handle and transport compared with alternative sources. Methodologies to produce the α-CD encapsulated ethylene inclusion complex (IC) and their physico-chemical characteristics have been reported (Ho et al., 2011a, b), and is being commercialised as Ripestuff™. This has potential for ethylene application during in-transit ripening, as well as a safer alternative to carbide for triggering ripening of climacteric fruit.

The release kinetics of ethylene from the complex should be stable for the long term storage, and predictable to provide consistent responses under commercial conditions. Ho et al. (2011a, b; 2016) describes the release characteristics of the initial IC formulation. A more cost-effective production process produces an IC with likely very different release characteristics. This report describes preliminary research to provide recommendations for testing of Ripestuff™ for in-transit ripening of commercial mango consignments from the Northern Territory to southern markets.

The concentration of ethylene in a chamber with Ripestuff™ is determined by the release of ethylene from the IC, and the leakage rate from the chamber. Most of the Ripestuff™ laboratory trials were conducted in 1 $m^3$ chambers with leakage rates approximating those measured in commercial refrigerated containers. A small trial was also conducted in 2016/17 on the effects of relative humidity (RH) on ethylene release.

# Materials and Methods

## Leak rates from commercial refrigerated containers

Commercial refrigerated trailers used for transporting bananas from North Queensland to Brisbane were tested. Carbon dioxide ($CO_2$) was injected into the trailer with no fruit, and with the refrigeration system on, and the $CO_2$ concentration monitored over about 20 h using a Viasala logger placed inside the trailer. The leakage was measured on several trailers, either stationary or driven around Brisbane for about 3 hours.

The release of ethylene for 4, 8, 12 and 16 gm Ripestuff™ was evaluated in 1 $m^3$ chambers (90 x 90 x 120 cm). The chambers were comprised of aluminium box tubing covered with 1.6 mm aluminium sides and a high density polyethylene lid. Varying leakage rates for each chamber was achieved by pumping out air from the chamber at targeted flow rates using standard fish tank aeration pumps, and with an inlet to allow fresh air into the chamber. 30 cm desk fans were included in each chamber for air circulation. The leakage rate (hours to 50% loss of carbon dioxide ($CO_2$); the half-life) for each treatment was estimated by injecting $CO_2$ into the chambers to about 2% and recording the decrease in $CO_2$ concentration over time. However, the same flow rates of air out of each chamber did not guarantee the same half-life due to slightly differing chamber characteristics. The half-life for each chamber/treatment is given on the graphs against the respective legends.

Air from each chamber was automatically sampled about every 6 min using a proprietary sampling system, with the sample being passed over a $CO_2$ analyser (PP Systems, UK) and an in-house built ethylene logger using an Alphasense Photo-Ionisation Detector (PID). This sampling resulted in about 300 mL of air lost from each chamber every 6 min, which contributed to the overall leakage rate from each chamber. Relative humidity in the chambers was not controlled but was measured at more than 85% using Vaisala HMP50 humidity probes. Standard gases (BOC) of 5, 20 and 97 ppm ($\mu L\ L^{-1}$) ethylene in nitrogen were used to calibrate the PID sensors.

The Ripestuff™ powder was placed in 75 mL, plastic screw top specimen containers. The lid of each specimen container was pierced either once, twice or four times with a Terumo 0.5 x 25 mm gauge needle. Most of the trials were done over about 48-70 hrs, however, a few were stopped after about 24-30 hrs due to significant variation from the expected results (e.g. poor ethylene release or very high leakage rates etc).

Details of the conducted trials are given in Table 1. They included testing the effects of weight per container, numbers of holes in the lid of each container, and number of containers per chamber. About 2% $CO_2$ was injected into each treatment chamber to determine the leakage rate for every chamber.

In addition, the effect of headspace volume in the container above the Ripestuff™, and the Ripestuff™ moisture content of tested in the 1 $m^3$ chambers but with the air pump and gas sampling system disconnected to minimise chamber leakage. Ethylene concentrations in the chambers were monitored using individual in-house built PID loggers in each chamber. The effect of headspace volume was studied by including discs of timber wrapped in aluminium foil, to provide the same free headspace with varying amounts of Ripestuff™ in the 75 mL container, of varying headspace volume with the same weight of Ripestuff™ in each of the 75 mL or 280 mL (62 mm in diameter and 95 mm high) containers. The effect of Ripestuff™ moisture content was studied by adding 1 or 2 mL of water to 16 gm Ripestuff in the 75 mL container. The final moisture content of the Ripestuff was determined by recording the weight loss before and after drying at 65°C for 2 days.

**Table 1.** Overview of trials conducted to understand the release of Ripestuff™ from 75 mL (42.5 mm in diameter x 52.5 mm high and containers inside 1 $m^3$ chambers. 1 x 4 h = one container with 4 holes in the lid, made with an 0.5 x 25 mm gauge needle. Ripestuff™ manufacture dates are given because of variable ethylene content per batch.

| Quantity of Ripestuff™ (gm) per 75 mL container | Leakage Rates (Flow rate of air from the chamber) | | | | | Ripestuff™ manufacture date |
|---|---|---|---|---|---|---|
| | 5.5 L $min^{-1}$ (2hrs HL) | 3.0 L $min^{-1}$ (3-4hrs HL) | 2.0 L $min^{-1}$ (4-5hrs HL) | 1.5 L $min^{-1}$ (5-7hrs HL) | 0.75-0.8 L $min^{-1}$ (14-24hrs HL) | |
| 4 gm | 1 container with x 4 holes<br>1 container with 4 holes + 2 with 1 hole<br>1 x 4h + 4 x 1h | 1 x 4h<br>1 x 4h + 2 x 1h<br>1 x 4h + 4 x 1h | 1 x 4h + 2 x 1h<br>1 x 4h + 4 x 1h<br>1 x 4h + 6 x 1h | 1 x 4h + 2 x 1h<br>1 x 4h + 4 x 1h<br>1 x 4h + 5 x 1h<br>1 x 4h + 6 x 1h | 1 x 4 h<br>1 x 4 h + 2 x 1h<br>1 x 4 h + 4 x 1h<br>1 x 4 h + 6 x 1h<br>- | 20 Sep 15 |
| 8 gm | | | | 3 x 4h | | 21 Apr 15 |
| 12 gm | | | | 3 x 4h | | 21 Apr 15 |
| 16 gm | | | | 1 x 1h<br>1 x 2h<br>1 x 4h<br>2 x 4h<br>3 x 4h | | 20 Sep 15 & 21 Apr 15 |
| 4 +16 gm | | | | 2 x 4 hole(4 gm) + 1 x 4 hole(16 gm) | | 21 Apr 15 & 08 Sep 15 |

*HL: half-life of $CO_2$ used to determine leakage rates; RS: Ripestuff™; hrs: Hours; h: holes in the 70 mL plastic container.

The Ripestuff™ batch dated 08 Sep 2015 did not perform well. The performance of this batch was tested alone or in comparison with other batches (dated 20 Sep 2015 and 21 Apr 2015) at a leakage rate of 1.5L/min.

The effects of relative humidity (RH) around Ripestuff™ was tested using 2 L glass air tight jars (Crafted for quality sealed for freshness™, USA). Tightly fitted and sealed 4 mm PVC inlet and outlet tubes for used to sample for ethylene measurement using a closed loop. The lids also had small 4 cm, 12v fans attached to the tubing inside the jar via cable ties to assist with air circulation inside the jar. 10 mL of saturated salt solutions of magnesium chloride (33% RH), sodium chloride (76% RH) and potassium nitrate (93.5% RH) were placed into separate 70 mL plastic containers. For the control, oven dried sand was placed in the container to maintain a similar headspace to the humidity treatments. The containers were sealed with lids to allow equilibration. About 10 mg Ripestuff™ was accurately weighed into a small plastic cap using a three decimal balance, then the cap quickly floated on the saturated solution in each 70 mL container. The containers were immediately sealed with lids containing four holes made with the 0.5 mm needle. They were then placed in the glass jars, and the jars tightly sealed. An ICA 56 logger was connected to the closed loop to quantify ethylene concentrations in the jars at 1, 3, 5 and 7 hr intervals, then at 24 hr intervals until 10 days (240 hrs). The trial was replicated three times.

# Results and discussion

## Leak rates from commercial refrigerated containers

The results suggested that relatively new trailers with no obvious damage to door seals etc. had similar or lower leakage rates compared with the commercial ripening room, while older trailers with leaky door seals lost 50% of the injected $CO_2$ at least four times faster (**Figure 1**). The same relatively new trailer was injected with $CO_2$ then driven around Brisbane for several hours (repeated twice). The results indicated that leakage was 30-50% faster when the trailer was mobile than when the trailer was stationary, likely because trailer flexing results in door and other seal movement and increased leakage.

Based on these results, leakage rates sufficient to reduce $CO_2$ concentration by about 50% (half life) within 5-6 hours was used in the laboratory trials.

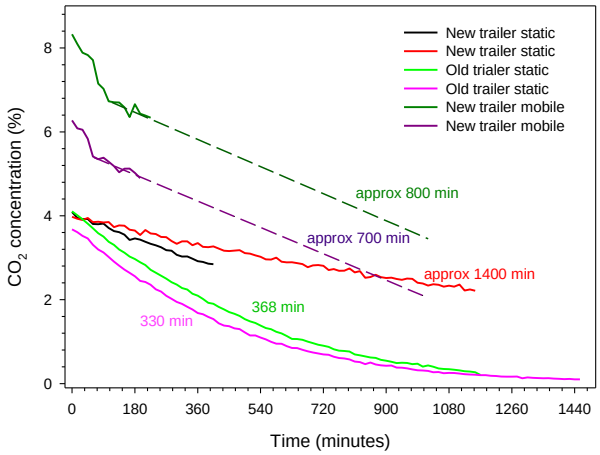


**Figure 1**. Leakage of carbon dioxide from approx. 12 m refrigerated transport trailers. The trailers were either relatively new or older and with visible damage to door seals. Two of the tests were with the same new trailer while being driven around Brisbane for about 3 hours. The numbers indicate the time to losing 50% of the initial CO2 concentration. The dashed lines indicate projected leakage rates.

## Chamber leakage rates (half-life)

Figure 2 illustrates the half-life (hours to 50% reduction in $CO_2$ concentration) achieved with different flow rates of air from the chamber. Results showed that the higher the flow rate, the less time required to reach half-life. It took 16 h to loss 50% of $CO_2$ concentration at flow rate of 0.8 L $min^{-1}$ while it required 1.5 hr to touch half-life with a flow rate of 5.5 L $min^{-1}$.

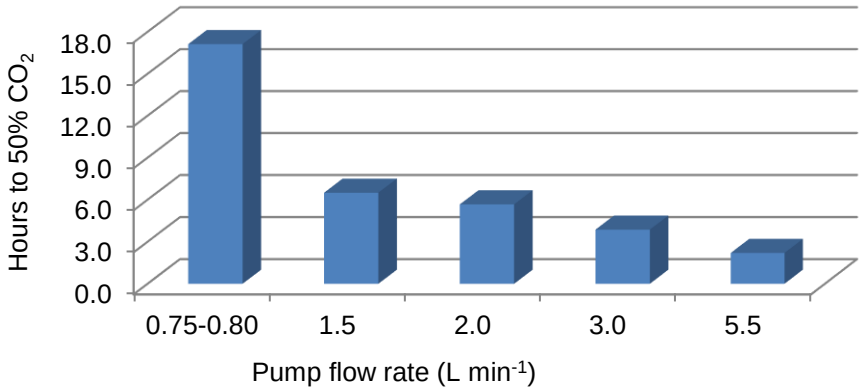


**Figure 2**. Time required to reach half-life (hours to 50% loss of $CO_2$ from of the 1 $m^3$ chamber) at different air flow rates from each chamber.

## 4 gm Ripestuff™ per container and varying leakage rates

Higher leakage rates generally resulted in lower peak ethylene concentrations and less time with ethylene concentrations above 20 ppm (Figure 3). More containers with Ripestuff added to the tent generally produced higher ethylene concentrations. Deviations from these trends were largely due to slightly varying half-life between the four chambers used in these trials.

Future trials concentrated on 1.5 L $min^{-1}$ flow rates which provided a half-life similar to those observed with commercial refrigerated containers.

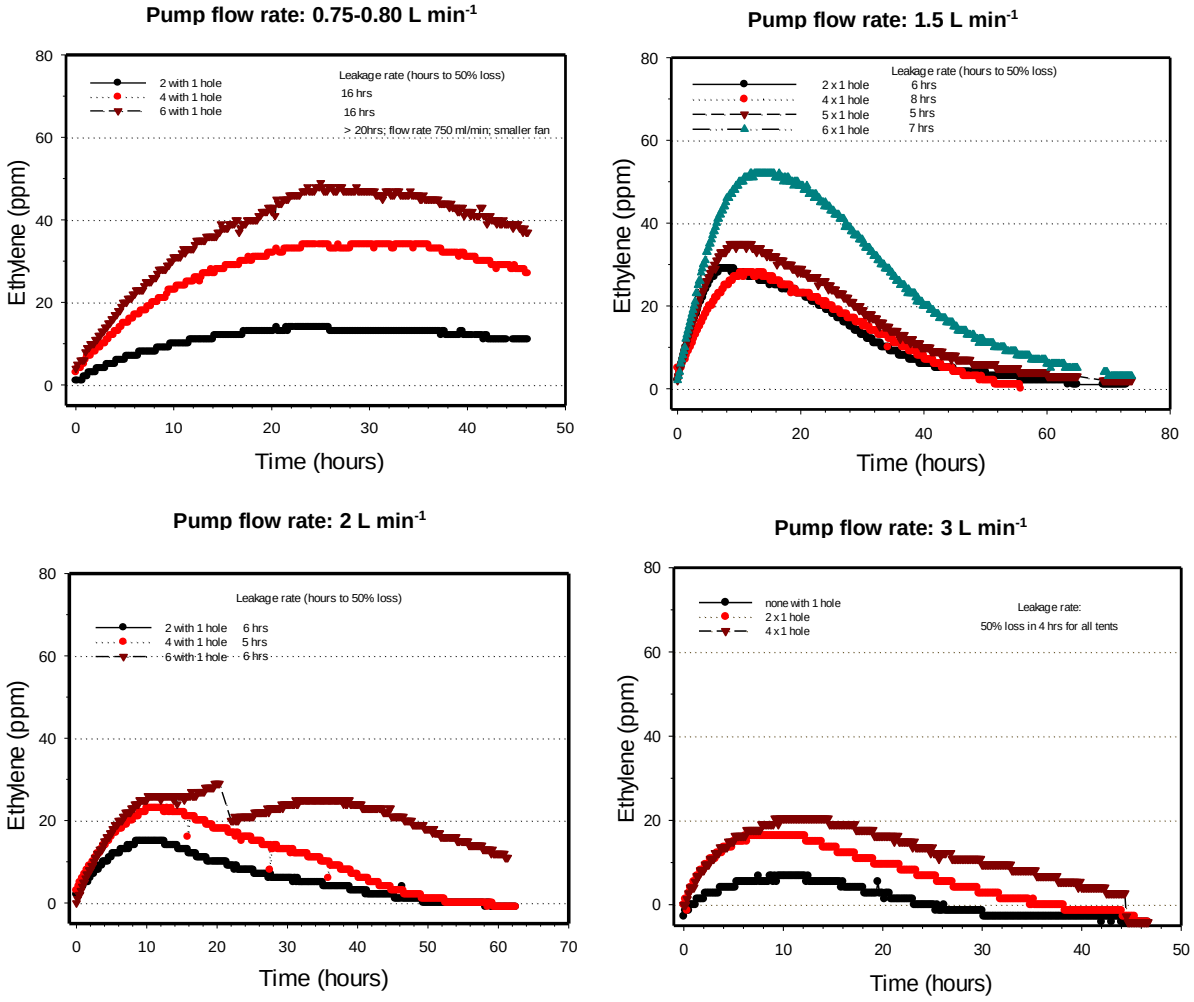


**Figure 3**. Ethylene concentrations in 1 $m^3$ chambers containing differing numbers of 75 mL specimen containers with 4 gm Ripestuff™, and with 1 hole (made with 0.5 mm needle) in each of the lids. Each test was done is a different chambers with leakage rates (half life) as indicated in each figure legend.

## 16 gm Ripestuff™ per container

Four holes in the lid of containers with 16 gm of Ripestuff™ resulted in higher ethylene concentrations within the 1 $m^3$ chambers more quickly than the containers with one hole (Figure 4). With four holes, the ethylene concentration in the chamber was maintained above 10 ppm for about 60 hours. The higher leakage rate (5 hours half-life) from the chamber containing the “two holes” treatment resulted in a lower maximum concentration of ethylene, as expected.

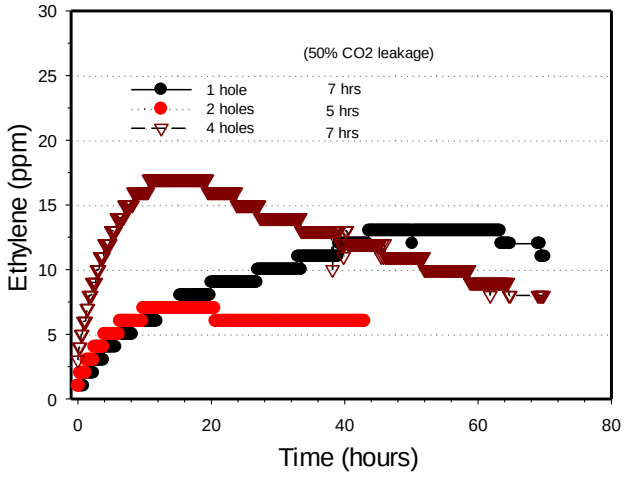


**Figure 4**. Ethylene concentrations in 1 m³ chambers containing one 75 mL container with 16 gm Ripestuff™. The tests were done in separate chambers, and with one, two or four holes (made with a 0.5 mm needle) in the lid of each Ripestuff™ container. Time required to reach half-life for each chamber is indicated.

Increasing the number of containers in the chamber with 16 gm Ripestuff™, also increased then maximum ethylene concentration achieved, and the duration above 20 ppm ethylene (Figure 5).

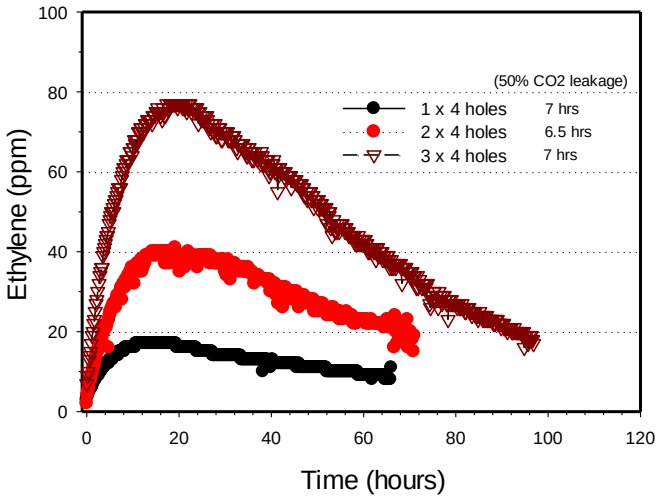


**Figure 5**. Ethylene concentrations in a 1 m³ chamber containing one, two or three 75 mL containers with 16 gm Ripestuff™, and with four holes in each of the container lids. Time required to reach half-life based on carbon dioxide leakage rate for each chamber is indicated.

## Ripestuff™ quantities per container

Increasing the weight of Ripestuff per container (each with four holes in the container lid), proportionately increased the maximum ethylene concentration and the duration above 40 ppm (**Figure 6**). The preferable ethylene concentrations during commercial in-transit are needed to maintain at level greater than 20 ppm for 2-3 days. This result could help to provide some buffer for variable leakage rates from commercial refrigerated containers, 12 g per container with four holes is recommended to use in commercial tests during 2015-16.

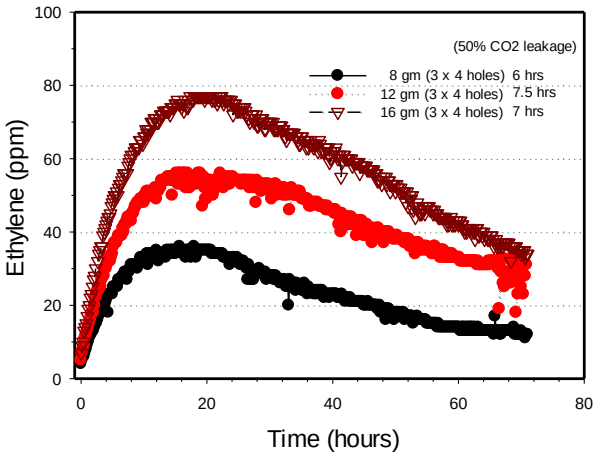


**Figure 6**. Ethylene concentrations in a 1 m³ chamber with three containers each holding either 8, 12 or 16 gm of Ripestuff™ per 75 mL container, and with four holes in the lid of each. Time required to reach half-life for each chamber, based on the reduction in CO2 concentration, is indicated. The occasionally lower ethylene reading is the result of intermittent ethylene sensor power-down.

## Ripestuff™, headspace and surface area

Ethylene concentrations in the chamber from the containers with the same headspace distance of 24 mm was greater at the need of 60 hrs with 16 gm Ripestuff™ in the container compared with 4 and 8 gm (**Figure 7**). The release rate was slower, suggesting that the ratio of head space to Ripestuff™ quantity may affect release rate from the container, although it is possible that the slight leakage from the chambers with 4 and 8 gm (as evidenced by the slow decline after reaching maximum concentrations) may have slightly influenced these results.

16 gm Ripestuff™ in 280 mL containers had more than twice the surface area directly exposed to the headspace, compared with the 16 gm in 75 mL containers. This resulted in ongoing release of ethylene over the 60 hrs of the test, compared to maximum concentrations reached in about 24 hours from Ripestuff™ in the 75 mL containers (**Figure 8**). This suggests more efficient release with larger surface area:volume ratios. These trials should be repeated, and the remaining ethylene in the Ripestuff™ determined after the ethylene concentration in the chamber has reached a maximum.

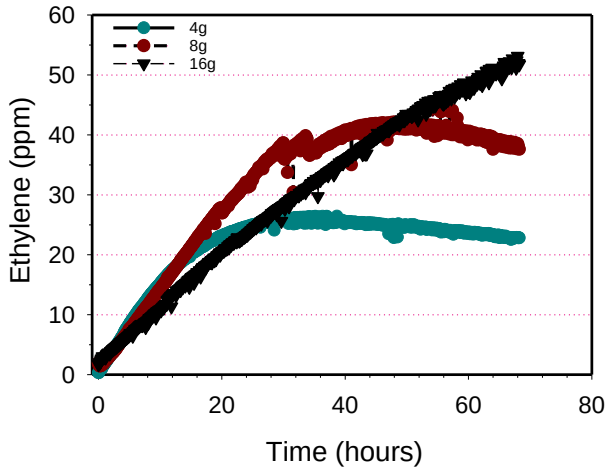


**Figure 7**. Ethylene concentrations in a 1 m³ chamber from different quantities of Ripestuff with the same distance between the top of the Ripestuff and the lid of 24 mm (headspace volume of 34,029 mm³). The slow decline in ethylene concentrations with 4 and 8 gm indicates slight leakage of the chambers.

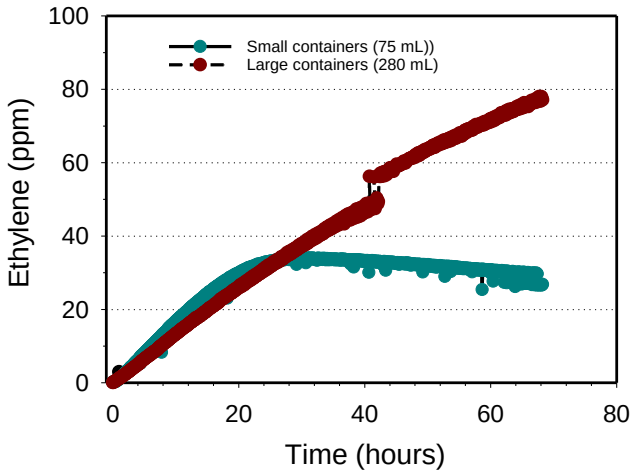


**Figure 8**. Ethylene concentrations in a 1 m³ chamber released from 16g Ripestuff in small (75 mL) or large (280 mL) containers. This relates to a surface area of Ripestuff™ directly exposed to the headspace of 1353.2 mm² with the 75 mL containers, and 2875.8 mm² for the 280 mL containers. Each container had four holes in the lid.

## Batch variability, moisture content and relative humidity

Ethylene concentrations released from different batches of Ripestuff™ is shown in **Figure 9**. The Ripestuff™ batch packed in April 2015 produced more ethylene than that manufactured in Sep 2015 although the same amount of power was used. A 3% increase in the Ripestuff™ moisture content could

almost double the ethylene release rates (Figure 10), and this may be one cause for the batch-to-batch variability. Also, very little ethylene is released when relative humidity around the Ripestuff was 76% or less, but was much greater at 93.5% RH (Figure 11). This indicates the significant role of moisture in ethylene release.

The RH in commercial refrigerated trailers with mango is typically above 80%, and is not controlled, Hence the effects of RH between 80 and 95% needs to be understood since it may have a significant bearing for commercial use. Also, it is likely that commercial application systems will be based on reducing ethylene release using a semi-permeable barrier such as small holes in a container. Hence the trials should study the effects of 80-95% RH in the atmosphere outside the container on ethylene release, since moisture would have to permeate into the container with Ripestuff™ first, then the ethylene released from the Ripestuff™ permeate out of the container into the trailer atmosphere.

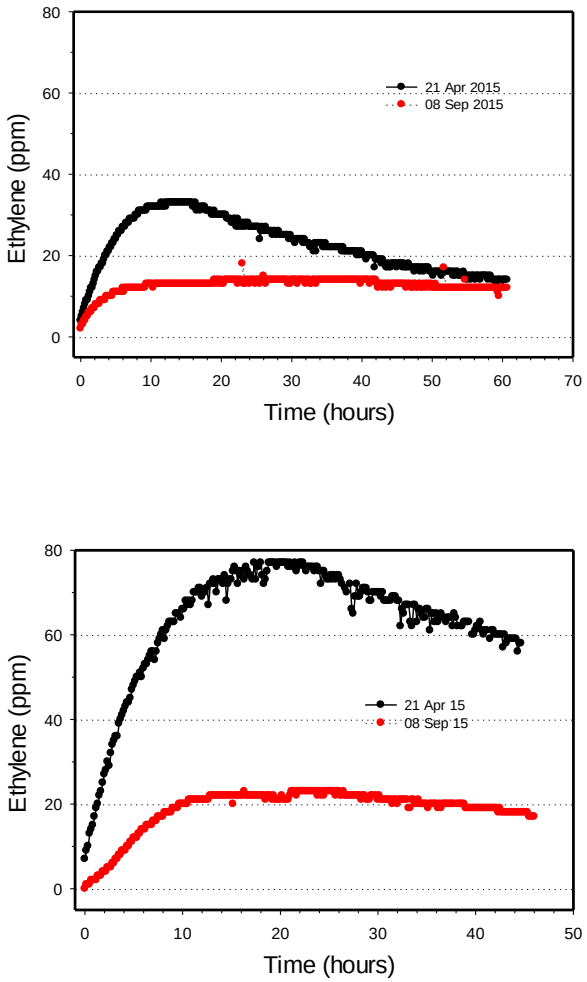


**Figure 9**. Ethylene concentrations in a 1 m³ chamber comparing the ethylene release characteristics of Ripestuff batches manufactured on 21 April and 8 September 2015. The top graph is for chambers with two containers holding 4 gm of Ripestuff™, plus one container with 16 gm Ripestuff™. The bottom graph is for chambers with three containers each holding 16 g of Ripestuff™. All containers had four holes in each lid. The occasionally lower ethylene reading is the result of intermittent ethylene sensor power-down.

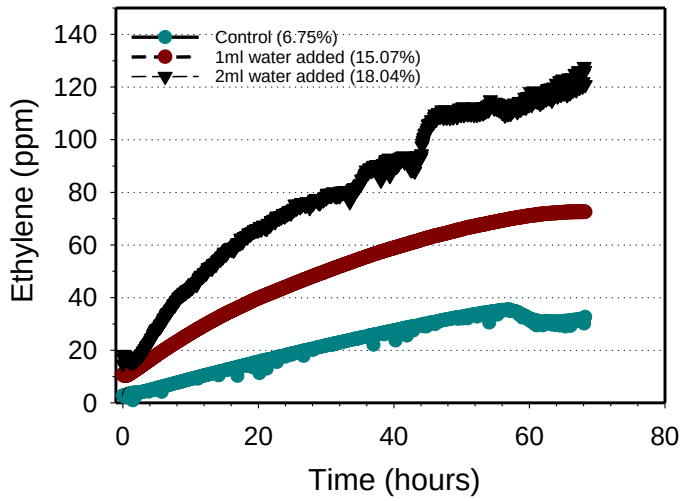


**Figure 10**. Ethylene concentrations in a 1 m³ chamber with 75 mL containers with 16 gm Ripestuff™ at differing various moisture contents. Each container had four holes in the lid.

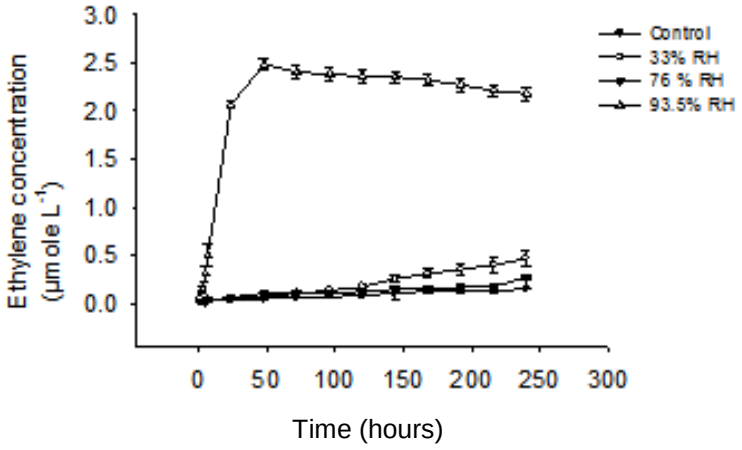


**Figure 11**. Ethylene concentrations in 2 L glass jars, released from 70 mL plastic containers with 10 mg Ripestuff™ and saturated solutions producing relative humidities around the Ripestuff™ of 33, 76 and 93.5%.

# Conclusions and recommendations

- Our tests suggest a half life of 11-22 hours from static trucks, and 5-6 hours from mobile trucks (2 replicates only).
- Adding more 75 mL containers with one hole made with a 0.5 mm hypodermic needle increased maximum ethylene concentration in the outside chamber and maintained concentrations for longer.
- Overall, with a leakage half life of 5-8 hours, the ethylene concentration reached a maximum in 10-20 hours, then decreased because further release from the containers with Ripestuff™ was insufficient to compensate for the leakage from the chamber. Even six of one hole containers in combination with one container with four holes could not overcome the leakage.

- The rate of ethylene release was affected by surface area:volume of Ripestuff™.
- Ethylene release rates increased with higher Ripestuff™ moisture content and RH around the Ripestuff™.
- The trials indicated that three containers with 12 gm Ripestuff™ and four holes per lid would result in up to about 60 ppm ethylene and about 70 hrs above 20 ppm. These factors can be used to estimate the number of containers to be tested in commercial trials.
- Further trials should be undertaken to understand the effects of:
    - 80-95% RH on ethylene release, since release increases significantly between 76-93% RH, and RH in refrigerated trailers with mangoes is not controlled and is typically between 80-95%.
    - Surface area: volume ratios. This will help determine whether several large containers can be used, or whether a large number of smaller containers are required to provide the slow release.
    - Ripestuff™ particle size on ethylene release
    - Moisture content on ethylene release.

  The latter two areas will inform how the manufacturing process can be improved to provide a more uniform ethylene release between batches.

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# Developing effective system for $CO_2$ management under in-transit ripening condition

2015-16 mango season results

In-transit ripening and prediction of outturn quality for mango

HIA project MG12016

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# Summary

In-transit ripening of mangoes during refrigerated road-freighting from northern production areas of Australia to southern markets can provide significant savings in relation to down-stream energy and infrastructure costs associated with ripening. However, commercial trials over the last 6 years have confirmed the risk of excessive accumulation of carbon dioxide ($CO_2$) levels in freight containers due to fruit respiration, and the associated challenges of maintaining the concentrations below the desired 2-4% level. High $CO_2$ concentrations can potentially affect fruit ripening rates, as well as overall quality after removal and at the ripe stage.

Hydrated lime is commonly used in static controlled atmosphere storage facilities, and in the MaxTend seafreight system, to reduce $CO_2$ concentrations. In the MaxTend system, hydrated lime is placed in paper bags with very specific permeability, and placed on top of pallets within the container. The present trials tested lime activity from local suppliers and evaluated the permeability of several paper bags, and confirmed the capability of hydrated lime to absorb $CO_2$.

The results indicated that hydrated lime sources purchased from local suppliers had lime activity over 90%, which was in a recommended range for effective $CO_2$ absorption. Detpak check-out paper bags (540 x 355 x 165 mm) had higher porosity than Maxtend bags. In addition, $CO_2$ absorption by hydrated lime was influenced by the ratio of surface area to volume and the depth of lime. It is advised that the depth of packed hydrated line should not exceed 4 cm.

Based on these results, and the CO2 production rate of 'Honey Gold' mango, the following revised hydrated lime protocol was developed:

- 1.5 kg of hydrated lime evenly distributed within the Detpac checkout bags (total surface area of 1625 $cm^2$) resulting in a surface area to volume ratio of 0.77, and a depth of lime of 1.5 cm
- 128 of these bags placed evenly within cartons on the top of the 18-20 pallets within a standard 10 m refrigerated road container.

# Introduction

In transit ripening of mango requires managing fruit temperatures during transit, treating with ethylene to trigger ripening, and ensuring the carbon dioxide ($CO_2$) produced by fruit respiration does not exceed about 4% because of the risk to worker safety and to fruit quality.

In commercial controlled atmosphere rooms, hydrated lime is used to remove or regulate $CO_2$ during storage. Punctured bags of lime are often loaded on pallets and placed into the store which absorb the $CO_2$ produced by fruit in the course of storage. Hydrated lime [$Ca(OH)_2$] reacts with $CO_2$ to produce lime ($CaCO_2$) [$Ca(OH)_2 + CO_2 \rightarrow CaCO_2 + H_2O$]. The rate of lime formation is thought to be temperature dependent (Dillon and Filippelli, 2012). The higher the temperature the greater the rate of conversion of hydrate to carbonate.

Hydrated lime has been used to remove $CO_2$ during apple storage (Bartsch, 2004; Hoefhn et al., 2009; Thompson, 2010). The amount of lime required depends on the length of storage, type and variety of the crop, the storage temperature and atmospheric composition. Hydrated lime with high calcium content is more active than one significant magnesium concentrations. It is suggested that for efficient $CO_2$ removal, the hydrated lime should contain 70%-75% CaO and less than 20%-25% MgO (Bartsch, 2004; Hoefhn et al., 2009). Only fresh hydrated lime is effective in scrubbing $CO_2$ as lime will gradually lose its freshness over time due to $CO_2$ absorption from the air. The weight of the converted lime increases by about 30% at maximum absorption capacity. For instance, a 23 kg bag will weigh approximately 34 kg once full absorption of $CO_2$ is reached. If the weight of supplied bags of hydrated lime is 10% higher than its nominal weight at the time of purchase/delivery, the batch should be rejected (Bartsch, 2004; Rao, 2015).

In theory 1 kg of lime will absorb 0.59 kg of $CO_2$, but for practical purposes 1 kg of freshly hydrated lime will absorb 0.4-0.5 kg of $CO_2$ (Hoefhn et al., 2009; Thompson, 2010). For apple storage, the amount of hydrated lime required varies with crop and storage time. In general, to maintain < 1% $CO_2$ and 2% $O_2$, the hydrated lime requirement is estimated as 5% of fruit weight or 50 kg of lime per tonne of fruit. However, depending upon the desired $CO_2$ concentration, the amount of lime would be adjusted. For example, to keep $CO_2$ below 2% about 12 kg of hydrated lime per tonne of apples is recommended for apple storage

(Hoefhn et al., 2009), while 7.5 kg of high calcium hydrated lime is needed every 6 to 10 weeks for 1 tonne of apple fruit (Thompson, 2010). No published literature was found on the use of hydrated lime to remove $CO_2$ during fruit ripening, where respiration rates, and therefore $CO_2$ production is considerably higher than under cold storage conditions.

In 2015 we used hydrated lime to absorb $CO_2$ for commercial tests on in-transit ripening in mango. Forty two Maxtend paper bags (Mitsubishi Australia ltd.,), each containing 1.3 kg hydrated lime (Adelaide Brighton Cement Ltd) were used to remove $CO_2$ in refrigerated road containers with 18-20 pallets of mango. The amount of lime used was based on recommendations by Maxtend, (Rod Jorden, pers. com). The results indicated that $CO_2$ concentrations can reach up to 20% in the refrigerated container after four days during treatment with Ripestuff (ethylene releasing agent), despite the inclusion of the hydrated lime. This could have resulted from low lime activity (measured at 60% in a control sample sent to MRF after the trial) or excessively low permeability of the Maxtend paper bags.

The measured hydrated lime activity of 60% is well below the minimum recommended of 85-90% (Duong and Hofman, 2016), and of the manufacturers specification of at least 85-90% lime activity. The reasons for this low activity are not clear, but may be related to long storage times.

A reliable supply of hydrated lime, and of high permeability bags are essential to successful in-transit ripening. Therefore, the following questions were investigated:

- Does locally-sourced hydrated lime contain high lime activity as recommended (>85%)?
- What effect does the bag used to hold the hydrated lime have on $CO_2$ absorption rate, and particularly the bag specifically designed for use in the Maxtend seafreight system as compared other paper bags?
- Is there a reduction in $CO_2$ absorption with greater depth of lime in the bag?
- What is the relation between lime activity and $CO_2$ absorption rate and capacity?

Based on these studies, revised recommendations for hydrated lime to minimise $CO_2$ accumulation in in-transit containers were developed.

# Materials and Methods

## Hydrated lime sources

Hydrated lime was locally purchased as presented in Table 1.

Table 1. Hydrated lime from different suppliers

| Brand | Merchant | Packing date | Date of purchase | Nominal Weight (kg) | Actual Weight (kg) | Origin | Age (month) |
|---|---|---|---|---|---|---|---|
| Bastion | Bunnings | Oct. 15 | 06.07.16 | 20 | 19.75 | Greece | 9 |
| Dingo | Bunnings | 14.09.15 | 06.07.16 | 20 | 20 | Vietnam | 10 |
| Cement Australia | Kawana Hardware & Garden | 05.03.16 | 06.07.16 | 20 | 20.5 | Australia | 4 |
| Sibelco | Landmark Yandina | 04.11.15 | 06.07.16 | 20 | 20 | Gympie | 8 |
| Sibelco (Fresh) | Landmark Yandina | 05.07.16 | 12.07.16 | 20 | 21 | Gympie | <1 |
| Easy Mix | Yandina Hardware | N/A | 06.07.16 | 20 | 19.5 | Vietnam | N/A |

Calcium hydroxide was purchased from Sigma Aldrich and used as a standard (ACS reagent ≥ 95%, calcium carbonate ≤ 3%, density 2.24 g/ml at 25 $^{o}$C).

## Experiment 1: Hydrated lime source

For each commercial source of lime, 4 samples, each representing a replicate (2 from bottom sides and 2 from top sides of the bag, with punctured depth about 10 cm) were taken using a sharp knife and an 18 mm diameter steel tube as shown in Figure 1. All samples were then analysed for lime activity as outlined below and as used by Maxtend. The bag containing the remaining lime was sealed with tape to prevent further exposure to the atmosphere, then used for the other experiments as appropriate. The experimental design was 6 sources of lime (treatment) x 4 samples (reps) per source (bag).

Sibelco hydrated lime was used in the remaining experiments.


Figure 1. Locations of taking hydrated lime samples from the commercial bags

## Experiment 2: The permeability of $CO_2$ through Maxtend and check-out paper bags

Portions of the Maxtend bag (containing 3 layers), and Detpak and another (unknown source) check-out bags of 17 cm diameter was used in two separate permeability cells (Figure 2). Sixty mL of 100% $CO_2$ was injected into compartment I (2415 mL) using a syringe to get approximately 2.4% $CO_2$. $CO_2$ was measured every 10 seconds in compartment II (2550 mL) using a Bridge Analyzer (Bridge Analyser Inc, USA) until equilibrium was reached.


Figure 2. Diagram showing permeability cell (a), Maxtend bag layers (b) and standard check-out bag

## Experiment 3: $CO_2$ absorption with and without check-out paper bags and Maxtend bag covers

*Leakage test*: Chamber No 3 (90 x 90 x 120 cm) was used for the test (Figure 3). Air circulation was achieved by placing two rotary fans (12 mm diameter; Model YX2514 Sleeve Bearing, Sirocco, Taiwan) 15

cm distant from the proposed lime sample and pointing up. Air velocity (0.13m/s) was measured at 40 cm above the fan and over the lime using a hot wire anemometer (AM-4214SD, Taiwan).

The chamber was sealed using the matched door number, and held in a 20°C cold room. $CO_2$ was injected into the chamber to about 5% using an in-house designed RoomControl program and a Vaisala MI70 logger with a $CO_2$ probe (GMP70). The $CO_2$ concentration was recorded every 5 minutes for 5 days to record the leakage rate.

*$CO_2$ absorption test*: Based on the results from experiment 1, Sibelco fresh hydrated lime with a density of 740 kg $m^{-3}$ was used for this and subsequent trials. To maximize the ability of hydrated lime to absorb $CO_2$, a 500 gm sample was evenly spread in an oval-shaped plastic tray without covering (30 cm in length, 22.5 cm width, and 1.2 cm depth) as shown in Figure 4, and four samples (replications) were placed in the tent. This weight of lime was calculated based on commercial in-transit conditions for free air volume in a loaded refrigerated container and the weight of lime placed in the container in the commercial trials in 2015.

In addition, the effect of the Maxtend bag and the standard check-out paper bags on absorption was tested. Maxtend bags (580 x 360 x 160 mm) have two paper layers with a perforated plastic layer (measured in 6 mm line and 0.24 line/$cm^2$) in between, and the inner paper layer also perforated (hole with 1 mm in diameter and 0.26 hole/$cm^2$). The parameter of other paper bags is presented in Table 2. The density (gm $cm^{-2}$; GSM) was taken by weighing a 100 mm x 100 mm section after drying at 60°C for 12 h (four reps each). The hydrated lime samples were prepared as above but covered with an oval-shaped piece of the relevant paper (Figure 4). The layers were securely taped to the edge of the tray to ensure no leakage of air around the contact point between the bag and the tray. The exposed surface area of hydrated lime to the hydrated lime volume was recorded.

Table 2. The size and density (gm $cm^{-2}$; GSM) of check-out paper bags used in the lime absorption test.

| Bag name | Order code | Product | Size (mm) | GSM |
|---|---|---|---|---|
| Detpak bag (old) | A2317 | SOS 20 | 430 x 305 x 175 | 81.95 |
| Detpak bag (new) | C511S0010 | SOS 20 | 430 x 305 x 175 | 63.18 |
| Detpak bag (larger size) | C528S0010 | SOS 25 | 540 x 355 x 165 | 99.26 |
| Unknown bag | | | 430 x 305 x 175 | 81.27 |

After closing the chamber, $CO_2$ was injected into the chamber to achieve 5% within 50 seconds and then monitored at 20°C until it declined to 0.03%. The $CO_2$ concentration, temperature and relative humidity were measured at 5 minute intervals using the above Vaisala $CO_2$ probe and RH-temperature probe. The samples were then removed and weighed. A sample of the hydrated lime from each tray was tested for lime activity as described below. The experimental design was six runs (one for each of the six treatments) x 4 samples per run.

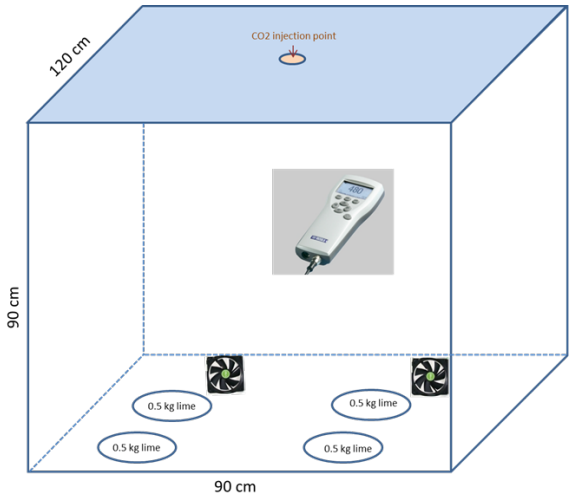


Figure 3. Diagram of the chamber showing number and position of samples and the fan in the chamber.

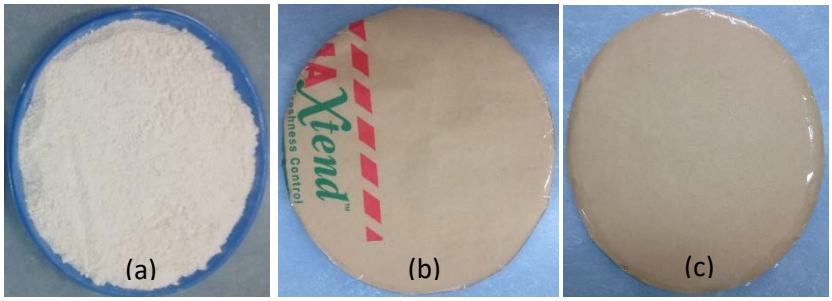


Figure 4. The samples used for the $CO_2$ absorption trials: not covered (a), Maxtend bag (b) and typical check-out paper bag (c).

## Experiment 4: Effect of lime depth on $CO_2$ absorption

### Trial 1

Hydrated lime was placed in pre-marked plastic containers (16.5 cm L x 11 W cm x 6 cm H) as shown in Figure 5. Four containers, each containing a lime source, were fully filled with 570 g hydrated lime without cover and placed inside the chamber. Air circulation was achieved by placing two rotary fans (Model YX2514 Sleeve Bearing, Sirocco,Taiwan) on wooden pallet and 15 cm apart from lime sample and pointing up. Air velocity was measured at 40 cm from the fan the anemometer. The chamber was sealed using the matched door. $CO_2$ was then injected into the chamber to about 5% within 50 second using the RoomControl program and then monitored at 20 $^oC$ until it declined to 0.03%. The $CO_2$ concentration, temperature and relative humidity were measured at 5 minute intervals using a Vaisala MI70 logger with a $CO_2$ probe (GMP70) and RH-temperature probe.

### Trial 2

The samples were prepared as in trial 1, but were kept under a constant environment at 5% $CO_2$ for 5 days using an EC12 system attached to a $CO_2$ sensor (Pacific Data Systems Pty Ltd, Australia). Following exposure to $CO_2$, the lime was removed in 1.5 cm layers by cutting through the dotted lines on the container using a Stanley knife and screeding with a ruler. For each layer, one sample was taken and analysed for lime activity.

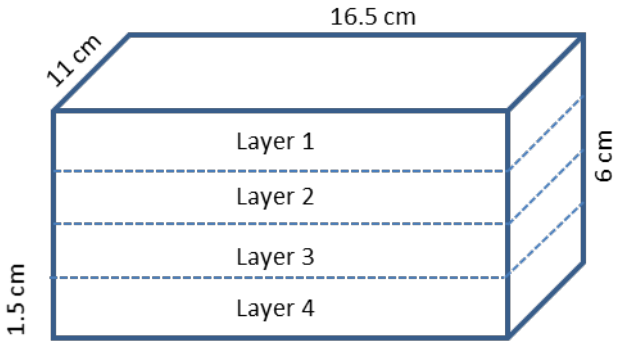


Figure 5. Sample container showing 4 layers of hydrated lime used in experiment 4, trial 1.

## Experiment 5: Effect of lime depth on $CO_2$ absorption over time

The trial was conducted in chamber No 3 (90 x 90 x 120 cm) and air circulation was achieved by placing two rotary fans (12 mm diameter; Model YX2514 Sleeve Bearing, Sirocco,Taiwan) 15 cm above the lime sample and pointing up. Air velocity was measured at 40 cm from the fan using the hot wire anemometer (AM-4214SD, Taiwan).

Sibelco fresh hydrated lime with density of 740 kg/m$^3$ was used for this trial. The samples were packed in frames (100 x 95 x 20 mm) made from grey plastic Perspex.

For the single layer test, hydrated lime was packed in 20 mm depth frames, the bottom was covered by aluminium foil and the top was covered with a 100 x 95 mm piece of Detpak checkout bag (Code A2317). The paper was securely taped to the edge of the frame to ensure no leakage of air. There were 12 frames used for the single layer test.

For the multilayer test, three frames were stacked together to a total depth of 60 mm of hydrated lime (Figure 6). The bottom of the frames were covered with aluminium foil and the top was left open to maximise $CO_2$ absorption. The contact area between the frames was covered by sticky tape to make sure that there was no air coming through. There were 12 stacks of frames (3 frames per stack).

The weight of hydrated lime in both tests was measured before and after removal from the chamber.

All single and stacked frames were placed on a wooden pallet inside the chamber. After closing the door, $CO_2$ was injected into the chamber to maintain 4% using the EC12 system. The trial was run at 20$^o$C for 4 days. Every 24 hours, $CO_2$ injection was temporarily stopped and the chamber opened quickly to remove the samples. Three single layer samples and three stacks were removed daily to measure the weight activity of the hydrated lime (Figure 7). The hydrated lime layer in the stack was separated by removing each frame (Figure 6). The experimental design was three layers/day x 3 reps/layer.

Figure 6. Diagram of the containers used in Experiment 5 to study the effects of depth of lime on $CO_2$ absorption.

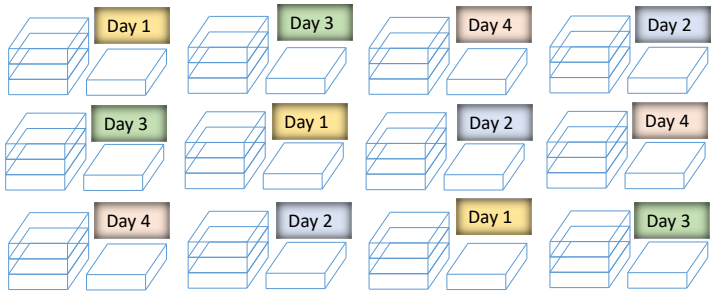


Figure 7. Diagram showing the order of lime sample removal used in Experiment 5

### Experiment 6: Testing maximum $CO_2$ absorption capability of hydrated lime

A sample of 250 g hydrated lime was evenly spread in the oval-shaped plastic trays and the placed inside the chamber. The weight was computed based on literature suggesting that 1 kg of hydrated lime can absorb 0.4 kg of $CO_2$ (Hoefhn et al., 2009; Thompson, 2010). The design and operation of the trial was as described in experiment 2.

### Lime activity test

Lime activity was analysed according to a method provided by Maxtend (Mitsubishi Australia ltd.,). Briefly, a neutralised sugar solution (38.5 % w/w) was prepared by mixing 75 g sucrose into 120 g distilled water. This solution was then homogenised using a magnetic stirrer for 30 min. Ten drops of phenolphthalein and 15 drops of 0.1 M NaOH were added to the solution and swirled. A sample of 3.7 g lime was added to 20 mL of distilled water in a 500 mL erlenmeyer flask, then 50 mL of prepared sugar solution was added. The solution was stirred every 5 min for 25 min. Finally, 4 drops of phenolphthalein was added to the solution before titration. The samples were quickly titrated with 1 M HCL using a 50 mL titration burette until solution colour turned transparent. The volume of acid solution (mL) used was recorded and converted to lime activity (%) on the basis of 1 mL of acid solution used = 1% of lime activity.

## Statistical Analysis

Data analyses were performed using GenStat® (Version 16.1, VSN International Ltd). One way ANOVA was conducted to detect treatment effects at 95% level.

# Results and discussion

### Experiment 1: Hydrated lime source

The lime activity from different sources is presented in Figure 8. In general, hydrated lime purchased from local merchants had lime activity over 92% even though they varied in age from production/packing time to sampling time. Storage time could decrease lime activity. For instance, Sibelco lime packed 8 months ago had 2% lower lime activity as compared to freshly manufactured lime. In addition, the results suggested that lime activity of all samples was in the range recommended by Maxtend.

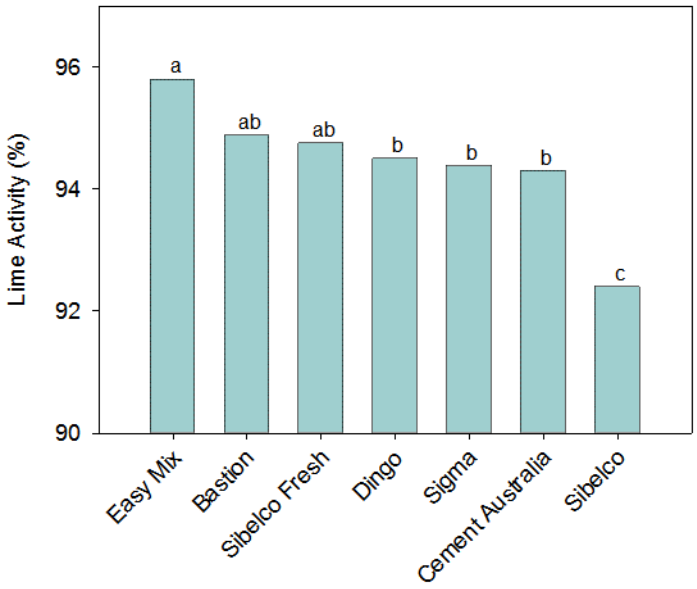


Figure 8. Lime activity of hydrated lime samples obtained from different suppliers. Means (bars) with the same letters above are not significantly different at *P=0.05* (n=4)

## Experiment 2: The diffusibility of $CO_2$ through Maxtend bag and check-out paper bags

The permeability of $CO_2$ through the Maxtend and other paper bags in the permeability cell is presented in Figure 9. Maximum $CO_2$ concentrations were achieved within 3 min with the Detpak and "unknown" paper bags, while almost 2 h was required to reach equilibrium with the Maxtend bag. This suggests a much lower permeability for the MaxTend bag, and could be a significant factor in the excessively high $CO_2$ concentrations in the commercial in-transit trials during 2015-16.

## Experiment 3: $CO_2$ absorption with and without check-out paper bags and Maxtend bag covers

Reduction in $CO_2$ concentration due to leakage from the chamber is shown in Figure 10. This represents a leakage rate of 0.12% per day.

Figure 11 shows reduction in $CO_2$ concentration in the treatment chamber due to absorption by hydrated lime with and without covering the tray with paper or MaxTend bags. Hydrated lime without a cover effectively absorbed all $CO_2$ within 6 h while covering with a Maxtend bag required 120 h to absorb the same amount of $CO_2$. Hydrated lime by paper from a check-out paper bag absorbed all $CO_2$ after 12 hours. The result suggested that check-out bags manufactured by Detpak would help enhance $CO_2$ absorption by hydrated lime due to its greater permeability as compared to Maxtend bags.

Table 3 illustrates the lime activity and weight after absorption of $CO_2$. The lime activity of the samples regardless of cover reduced to from 94% to approximately 80% after complete absorption of $CO_2$, and the sample weight increased from 3.1 % to 4.5% depending on exposure time. The sample covered with the Maxtend bag had the lowest weight increase, possibly because the water produced by the conversion of hydrated lime to lime evaporated during the longer duration of this experiment.

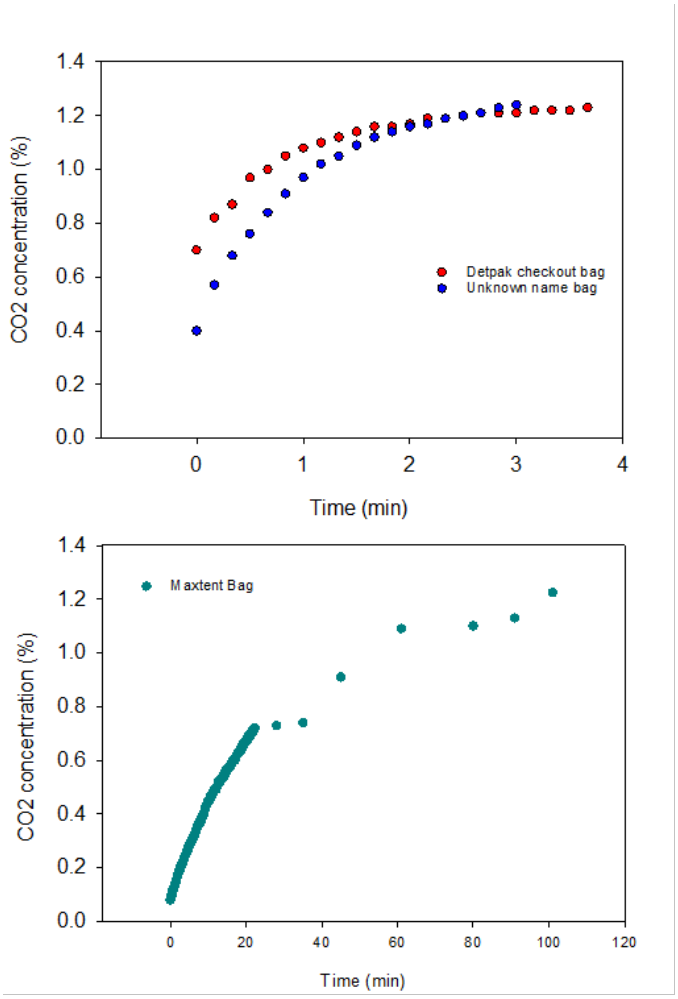


Figure 9. Increase in $CO_2$ concentration in the diffusion chamber when using the paper bags (top graph) and the Maxtend bag (bottom) using the permeability cell (Figure 2).

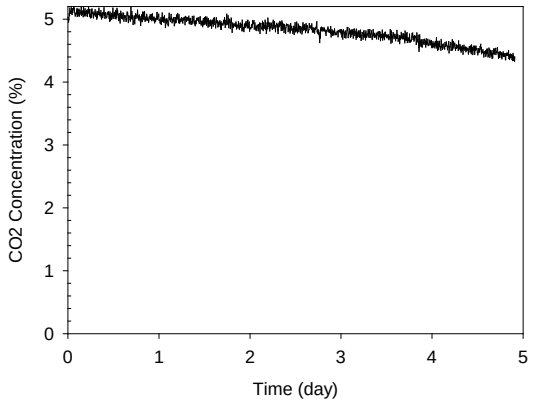


Figure 10. Reduction in $CO_2$ over time from the treatment chamber used in Experiment 3

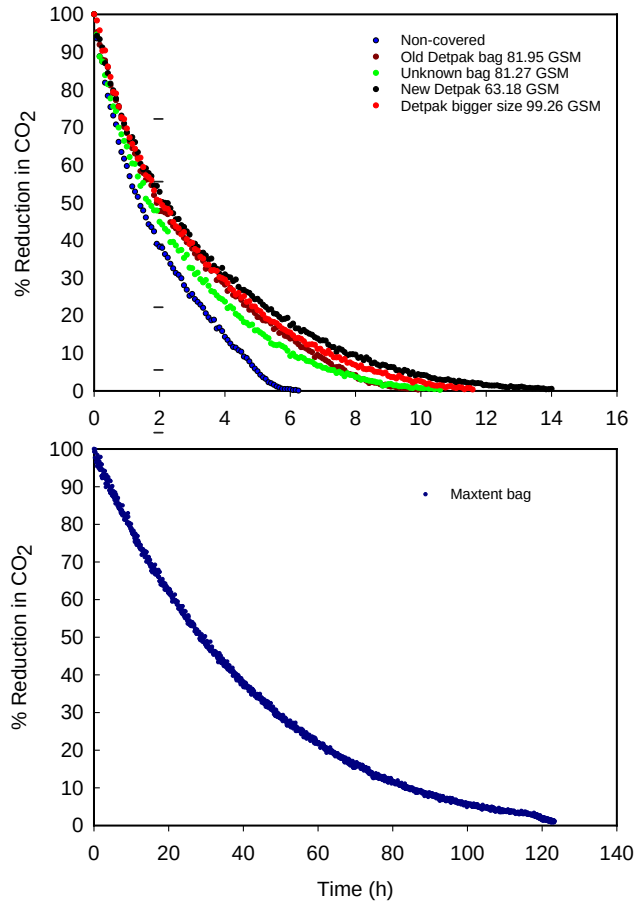


Figure 11. Proportional reduction (relative to the initial concentration) of $CO_2$ in the treatment chamber containing hydrated lime, either without or with Maxtend or paper from typical checkout paper bags.

Table 3. Changes in lime activity and weight of hydrated lime after $CO_2$ absorption

| Sample | Lime activity (%) | Weight increase (%) |
|---|---|---|
| Non-covered | 78.6 a | 4.5 a |
| Detpak bag (old) | 80.5 a | 4.1 a |
| Detpak bag (new) | 80.3 a | 4.5 a |
| Detpak (larger size) | 80.4 a | 4.2 a |
| Unknown | 80.4 a | 4.1 a |
| Maxtend bag | 79.8 a | 3.1 b |

NB: Means with same subscript are not significantly different at the P = 0.050 level

## Experiment 4: Effect of lime depth on $CO_2$ absorption

### Trial 1

Figure 12 shows $CO_2$ reduction after exposure to hydrated lime in the uncovered boxes. It took almost 12 h to absorb all $CO_2$ whereas it took 6 h to absorb fully $CO_2$ in experiment 2 (no cover over the hydrated lime). This could be due to the difference in ratio of surface area of hydrated lime exposed to the air, to the volume (0.78 in experiment 2 vs 0.24 trial 1 in experiment 3). The higher the ratio the faster the lime can absorb $CO_2$. In addition, $CO_2$ absorption was affected by lime layer or depth (Figure 13). The lime activity in the first lime layer declined to 60%. The lime activity was higher in the 2nd layer compared with the top layer, presumably because it absorbed less $CO_2$. The lime activity of the bottom two layers was similar to non-treated hydrated lime (Figure 8). This suggests minimal $CO_2$ absorption, most likely because the top two layers absorbed the available $CO_2$.

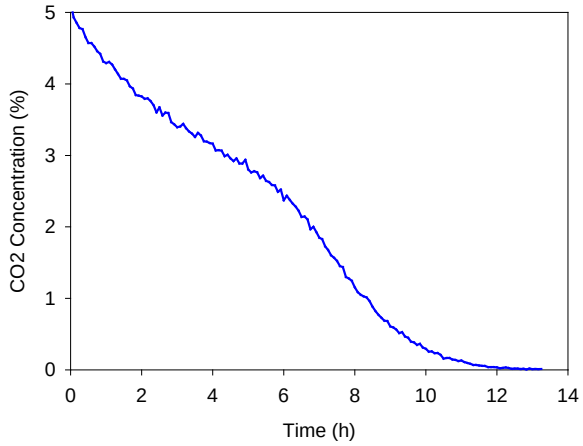


Figure 12. Change in $CO_2$ concentration in the treatment chamber containing hydrated lime with no cover (experiment 4, trial 1).

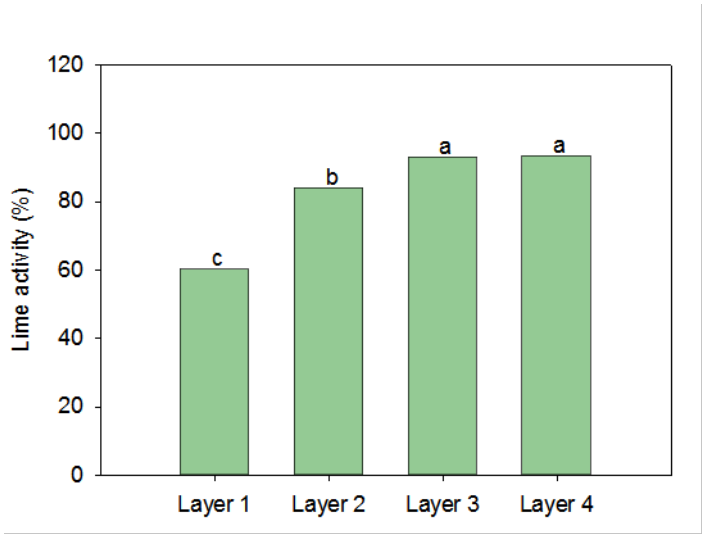


Figure 13. Lime activity in different layers of hydrated lime after exposure to $CO_2$. The lime activity of non-treated hydrated lime was about 94%. Means (bars) with the same letters above are not significantly different at *P=0.05* (n=4)

**Trial 2**

To better understand whether the bottom layer can absorb the same amount of $CO_2$ as the first and middle layers, trial 1 was repeated but with a constant $CO_2$ concentration of 5%. The hydrated lime in layer 2 and layer 3absorbed more $CO_2$ than the top and bottom layers (Figure 14). As a result, lime activity in these middle layers reduced to below 10%. It is apparent that hydrated lime at the bottom layer absorbed the least $CO_2$, and had the highest lime activity. This could be because hydrated lime in layer 2 and 3, after absorbing fully $CO_2$, became crumbling causing the inhibition of $CO_2$ absorption. It is not clear why the top layer had higher lime activity than that in layer 2 and 3.

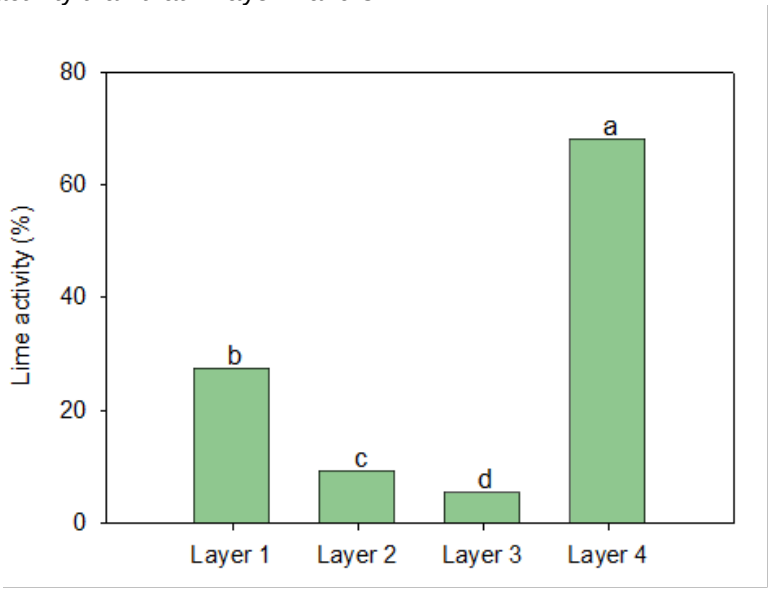


Figure 14. Lime activity in different layers of hydrated lime after exposure to constant 5% $CO_2$. Means (bars) with the same letters above are not significantly different at *P=0.05* (n=4).

## Experiment 5: Effect of lime depth on remaining lime activity over time

Changes in lime activity over the time measured from the single and multiple frame, which was exposed to relatively constant 4% $CO_2$ (Figure 15), is shown in Figure 16. As can be seen from the figure that lime activity in single frame reduced dramatically in the first day and continued decreasing gradually in 2$^{nd}$ and then stayed unchanged until the end of experiment. A similar trend occurred with lime activity in 1st layer of the multiple frame, but its lime activity was slightly higher than that in single frame. It is interesting that lime activity in 2$^{nd}$ (middle) layer in multiple frame reduced steadily over the time and its lime activity was the lowest in the end. There was a somewhat decrease in lime activity at the bottom layer over the period. The results from this experiment was in agreement with the outcome of experiment 4 trial 2, suggesting that hydrated lime in middle layers absorbed the most $CO_2$ and turned into barrier that impede $CO_2$ absorption in the bottom layers.

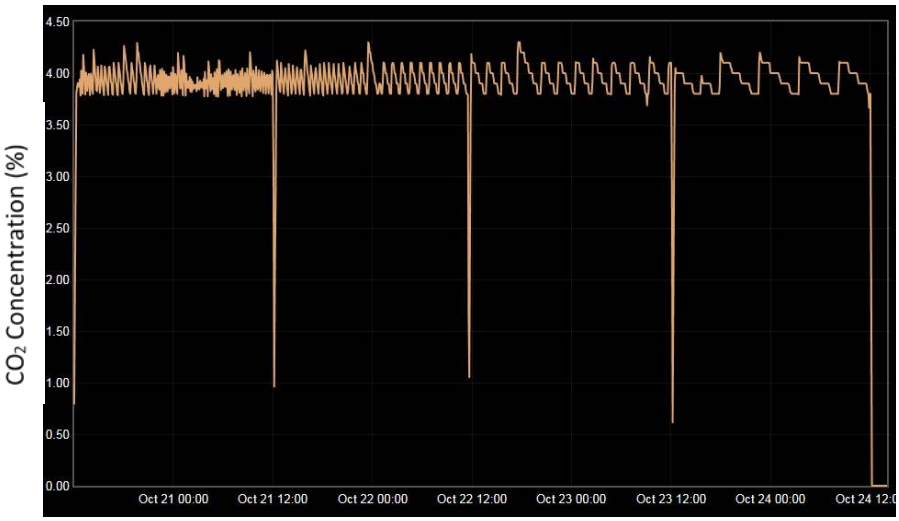


Figure 15. The $CO_2$ concentration in the treatment chamber, maintained at about 4%. The short-term reduction every day was due to removal of a lime sample to test the effect of time of exposure on lime activity..

## Experiment 6: Testing maximum capability of lime to absorb $CO_2$

Figure 17 shows that the $CO_2$ concentration in a chamber with 250 gm hydrated lime was reduced to effectively zero over about 1.8 d. This was equivalent to absorbing 89.4 gr of $CO_2$ after 1.5 d. Additionally, predicted data compared well with the experimental data, suggesting $CO_2$ concentrations could be computed using the equation in Figure 17 . The lime activity after full $CO_2$ absorption was 20.6%, and the weight of the lime increased by 25%. This result was in line with results of Bartsch (2004) and Rao (2015) suggesting that 1 kg of hydrated lime could absorb 0.4 kg of $CO_2$.

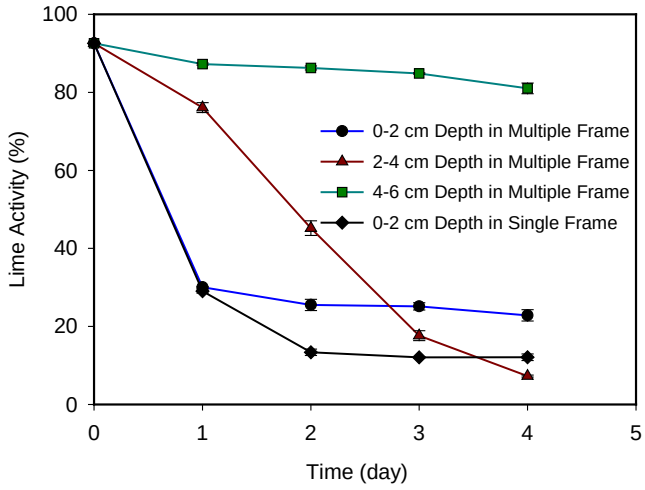


Figure 16. Changes in lime activity over time when exposed to 4% $CO_2$ in single and multiple layer frames (bars represent standard error, n=3) .

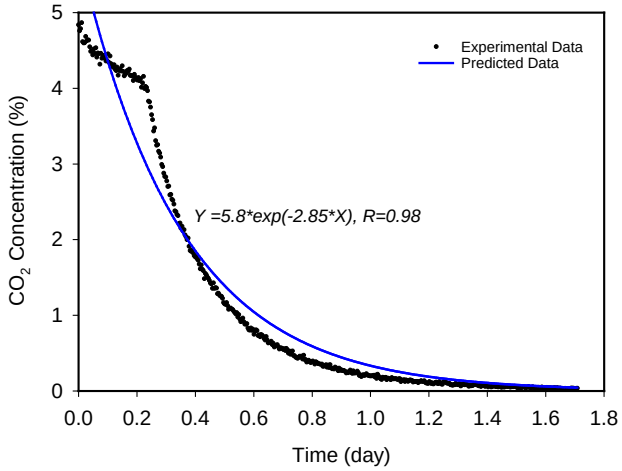


Figure 17. Actual and predicted $CO_2$ concentrations in the treatment chamber containing 250 gm hydrated lime with a surface area of 361 $cm^2$ and a depth of 1.2 cm.

# Commercial implications

Details of calculating air space inside 40 ft refrigerated container is shown in Table 4. The air space was used to compute $CO_2$ concentration released from fruit during the transit and thereby assisting calculation of hydrated lime needed for commercial truck to absorb $CO_2$ to the desired level (below 5%).

Table 5 presents the parameters to calculating $CO_2$ concentration and amount of hydrated needed to absorb completely $CO_2$. In theory, a hundred of hydrated lime is needed to remove all $CO_2$. However, as check-out paper bags prevent partially hydrated lime from absorbing $CO_2$, and actual fruit size and weight could be varied as well as temperature fluctuation during transport, resulting in variation of $CO_2$ production. Therefore, it is suggested that a range of 170-190 kg of hydrated is needed for maintain $CO_2$ concentration below 5% during in-transit ripening.

Table 4. Calculation of air space inside 40 ft refrigerated container for computing $CO_2$ concentration.

| | | | |
|---|---|---|---|
| 40-ft Container ($m^3$) | | | 80.9 |
| Fruit | Number of fruit | 35840 | |
| | Fruit volume ($m^3$) | 0.0005 | 17.9 |
| Tray (430 x 360 x130 mm) | Number of trays | 2560 | |
| | Tray volume ($m^3$) | 0.0028 | 7.2 |
| Pallet (1200 x1200 mm) | Number of pallets | 20 | |
| | Pallet volume ($m^3$) | 0.065 | 1.3 |
| Air space ($m^3$) | | | 54.5 |

Table 5. Parameters used to calculate CO2 concentration and amount of hydrated lime needed for commercial truck during transport of mango fruit.

| Parameters | | Amount |
|---|---|---|
| Respiration rate | (mL/kg/h) | 12.7 |
| Total fruit in container | (kg) | 17,920.0 |
| Total CO2 production | ($m^3$/day) | 5.5 |
| | (kg/day) | 10.1 |
| Air space in container | ($m^3$) | 54.5 |
| CO2 Concentration (%) in 1st day | | 10.0 |
| | 2nd day | 20.1 |
| | 3rd day | 30.1 |
| | 4th day | 40.1 |
| Capability of 1 kg hydrated lime to absorb CO2 amount (kg) | | 0.4 |
| Hydrated lime (kg) needed for 1st day | | 25.2 |
| | 2nd day | 50.3 |
| | 3rd day | 75.5 |
| | 4th day | 100.7 |

# Conclusions and recommendations

- Hydrated lime purchased from local shops had lime actitvity over 90% which was within the recommended range for effective $CO_2$ absorption. However, it is suggested that freshly-manufactured Sibelco hydrated lime should be used for commercial trials. The lime activity should be checked to ensure it is above 85% before commerical use.
- $CO_2$ absorption by hydrated lime was influenced by the ratio of surface area to volume and the depth of lime. It is advised that the depth should not exceed 4 cm.
- Detpak check-out paper bags had higher permeability than the Maxtend bags, making them more suitable for absorption of $CO_2$ under conditions of high $CO_2$ production, such as during ripening.
- Detpak paper retarded $CO_2$ absorption by lime despite its higher permeability. Investigating more permeable bags is recommended, but the bags need to prevent contamination of the fruit from the fine lime powder.
- 540 x 355 x 165 mm Detpak bags were used in commercial tests. Larger bags would reduce handling costs, but this should not be at the expense of reduced permeability in order to increase package strength.
- The results confirmed that 1 kg of hydrated lime without cover could absorb 0.4 kg of $CO_2$ at 20°C.
- Based on these results, the following lime treatment is suggested for in transit ripening of mango held between 16-18°C:
    - Depth of lime (1.5 cm)
    - Total surface area (1625 $cm^2$)
    - 1.5 kg of lime per bag with top surface (1400 $cm^2$) and side surfaces (225 $cm^2$)
    - 128 of these bags per 20 pallet container.

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# In transit ripening of ‘B74’ mango fruit

## Results from the 2013/14 season

In-transit ripening and prediction of outturn quality for mango

HIA project MG12016

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# Summary

Reliable ripening of mango fruit during transport from farm to market requires monitoring and careful control of shipment temperatures and concentrations of carbon dioxide ($CO_2$) and ethylene. The current study further developed initial leads from project MG10008.

During the 2013/14 season, 'B74' mango fruit were commercially picked and packed from farms near Katherine in the Northern Territory and Dimbulah in north Queensland. Palletised fruit were loaded into refrigerated containers and transported 1700-3000 km by rail and road to fruit wholesalers near major markets in Brisbane, Queensland and Adelaide, South Australia. Ethylene was released into containers via diffusion from plastic bags filled with 4% ethylene or from open or punctured bags of encapsulated ethylene within an α-cyclodextrin matrix. Hydrated lime was included in containers to reduce the accumulation of $CO_2$.

Air temperature within containers was monitored by a wireless real-time datalogging system (Xsense) during rail and road-freight. The system performed well and automatically downloaded data to a password protected website either during transit or within 30 minutes of arrival at the wholesaler. Temperatures in containers varied with each shipment, with fruit either being maintained within 2ºC of the set temperature of 16 or 18°C, or increasing to up to 26ºC during transit. The temperature at the rear of the container was always 2-7ºC higher than at the front, suggesting poor air movement inside the container.

Ethylene was released quickly from plastic bags and the α-cyclodextrin-ethylene matrix, but only accumulated to about 6-10 ppm for 1-2 days. Further testing and development is required to attain the right balance of rapid release to establish concentrations above 10 ppm, then slow release to retain the concentration for at least 2 d. The inclusion of paper bags of hydrated lime in containers did not consistently maintain $CO_2$ concentrations below about 3%. The mixed results were possibly because an old batch of lime was used.

When considered overall, these technologies indicate the potential for controlled ripening in transit but further commercial testing is required. Effective monitoring of temperatures, $CO_2$ and ethylene concentrations will also be developed further in the coming seasons.

# Introduction

Current recommendations for Australian mangoes requiring more than 2-3 days transit time from farm to ripener is to cool on farm to 12-13°C within 24 hours of harvest, and transport green fruit at this temperature (Ledger and Barker, 2009; Ledger *et al.*, 2012). The fruit are then exposed to ethylene to trigger ripening in central markets. These recommendations are based on the assumptions that transport containers do not have sufficient refrigeration capacity to cool fruit, or to maintain the temperature of ripening fruit within acceptable limits.

However, there are valid reasons to re-consider these recommendations:

- Farms/packhouses sometimes have insufficient cooling capacity during peak harvest periods, so that mangoes are often loaded at higher temperatures than recommended.
- Modern road/rail containers/trailers possibly have higher refrigeration capacity and airflow, thus allowing limited cooling and better temperature control during transit.
- When market demand for mangoes is strong but supply is low, there can be savings in costs and time by delivering partially ripened fruit to wholesalers.

Controlled ripening during transport by careful temperature management and ethylene exposure is a relatively new concept for the fresh produce industry. This approach has potential cost advantages by reducing on-farm precooling and in-market ripening room floor space, reducing the time from harvest to market, allowing access to higher prices at the start of the season, and reducing energy requirements by not cooling the fruit as much on-farm, running the trucks at higher temperatures, and not requiring warm-up of fruit in market before ripening. While it may be more efficient and cost effective than ripening fruit after transport in market, project MG10008 identified some potential challenges with in transit ripening:

- Ripening fruit produce considerable heat from respiration. Thus, inadequate cooling capacity or airflow in the transport container will allow fruit temperatures to increase, further increasing rates of ripening and heat production, and resulting in “runaway” temperatures.
- Ripening fruit also produce carbon dioxide ($CO_2$) during respiration. High $CO_2$ concentrations can result in green ripe ‘Kensington Pride’ mango fruit with more disease (Nguyen, 2003). Also, $CO_2$ concentrations above about 4% will cause asphyxiation.
- The fruit should be treated with ethylene at the start of ripening. Ethylene treatment on-farm before dispatch is less practical because of the need for increased cool room space, and transporting these ripening fruit will increase the risk of excess heat and $CO_2$ generation as respiration increases rapidly as the fruit ripens. Therefore, fruit should be transported soon after harvest and ethylene is treated in transit. Delaying the ethylene treatment until arrival will reduce the benefits of ethylene treatment.

Several in-transit ethylene release systems have been developed for use inside cartons or containers. These include releasing ethylene from small pressurised canisters (Sharrock *et al.*, 2010 ; Sharrock and Henzell, 2010 ) and from α-cyclodextrin inclusion complexes (Ho *et al.*, 2013). $CO_2$ scrubbing systems utilising hydrated lime (Bartsch, 2004) that are widely used in controlled atmosphere storage rooms may be adapted to in transit ripening.

Project MG10008 evaluated several ethylene release and $CO_2$ control systems. The purpose of the current study is to develop these further, and evaluate monitoring systems for these gases, and temperature.

# Materials and methods

## Fruit material

'B74' mango fruit were sourced from farms near Katherine in the Northern Territory and Dimbulah in Queensland during the 2013/14 season. They were harvested at commercial maturity and packed into cardboard mango trays according to standard commercial practice. The trays were palletised and cooled prior to transport to markets.

## Fruit transport

Palletised fruit were loaded into commercial 40 foot refrigerated rail containers. Depending upon the farm location and truck, 18-20 pallets were loaded into each container. The containers were transported 2700 km by rail from Katherine to Adelaide, South Australia or 3000 km by road to Brisbane or Gatton in Queensland. Consignments from Dimbulah were transported by road to Brisbane or Gatton, a distance of 1800 km. Container temperature set points were 16°C or 18°C, depending upon the particular load. No other alterations to transport conditions were made except for treatments to add ethylene, or reduce $CO_2$ concentrations.

## Treatments

### Ethylene release

The aim was to maintain ethylene concentrations of 10-50 parts per million (ppm) inside containers for at least 2 days. Ethylene was introduced into containers using two techniques:

- An α-cyclodextrin (sugar) inclusion complex (powder) in which pure ethylene was "trapped" in the sugar matrix, developed by The University of Queensland. The sugar matrix dissolves when mixed with water, and liberates the trapped ethylene. Fifty grams of the powder was placed into heat sealed aluminium bags. Ten such bags were placed inside each container. Just before fruit were loaded into containers, the top of the bags were either cut off, or a pinhole was punctured in the bag to allow release of ethylene.
- Semi-permeable membrane bags were constructed from 50 µm thick PVC plastic tube (30 cm diameter). Bags were heat-sealed at both ends and 4% ethylene in nitrogen was injected into the bag through a plug just before loading into containers. Two bags (10 m long) were secured to opposite internal side walls of each container with adhesive tape.

### $CO_2$ control

The objective of this approach was to maintain $CO_2$ concentrations within containers at or below 3% for WH&S reasons. Hydrated lime (Adelaide Brighton, 85-95% available lime index; typically above 90%) was placed in large standard paper bags with a flat footprint the size of a P84 tray. Each bag was placed into an empty tray, and the trays stacked on top of the pallets. Five kg of lime was used per pallet, either as one 5 kg or two 2.5 kg bags.

### Container monitoring

**Temperature**: Air temperatures inside the pallets were monitored with the Xsense system (www.bt9-tech.com). Briefly, wireless RFID tags were placed into a tray on the 4th layer from the top of each pallet located at rows 2 ("front") and 8-9 ("rear") from the front of the container. In some cases a sensor was placed in the top row of the pallet in the 2nd row from the front to measure the temperature of delivery air. A communication unit (CU) was installed at the receivers in Adelaide or Brisbane. The temperature data from the tags was transmitted to the CU when the tags came within reading distance during unloading from the container, and the

data were uploaded to the Xsense server. Stakeholders accessed the password protected website to retrieve temperature graphs of the load and the raw data. E-mail alerts were also sent to the stakeholders on arrival of the tagged pallets at the ripener, and when the pallet was exposed to out of range temperatures.

In two consignments, a portable CU was placed inside the container to collect temperature data in real time. A GPS and mobile aerial were placed on the outside of the truck so the physical location could be tracked and temperature data could be transmitted during transit. This system provided real time data on fruit temperatures and locations.

**$CO_2$ and $O_2$** concentrations were measured using low energy loggers supplied by Mitsubishi. They were placed in a tray on the top of one of the test pallets, then retrieved at market and returned to Mitsubishi for downloading and recalibrating.

**Ethylene** concentrations were recorded with chemical sensor loggers (MSR electronics, Nuremberg, Germany; measuring a range of 0-100 ppm ethylene). A single logger was placed into the same location as the $CO_2$ logger within each container.

# Results and Discussion

## Temperature

The Xsense temperature monitoring system worked well, with good support from the agent. For shipments relying on connection with CUs at the receivers, temperature data from consignments were automatically available on the website for downloading within approximately 30 minutes of consignment arrival. Alerting emails with arrival times and details were very convenient. Some difficulties occurred with CU connection to the internet but this was due to a lack of IT support during the rush of the season.

The temperature data are shown in Figure 1 to Figure 12. These indicate mixed performance in relation to temperature management, with fruit either being maintained within about 2ºC of the set temperature of 16 or 18°C, or increasing to up to 26ºC during transit. Most consignments from Dimbulah in Queensland exhibited poor temperature management, even over the relatively shorter 18 hours of transit compared to shipments from the more distant Northern Territory. The temperature at the rear of the container was always 2-7ºC higher than at the front of the container. The temperatures toward the rear of the container increased the most during transit, suggesting poor air movement inside the container.

Poor container loading practices were observed in previous years (see project MG10008). In the current study, gaps were again left between the front of the container and the first row of pallets, allowing air from the refrigeration unit to short circuit and not reach the back of the container. In addition, the ply sheets used to stabilise the load often blocked the gap in the timber pallet base which can prevent the air returning the to the refrigeration unit. Better training for staff responsible for loading containers is required.

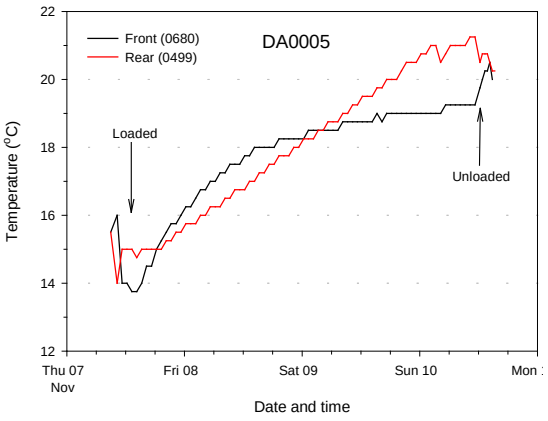



Figure 1 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Darwin to Adelaide. Note: The refrigeration set temperature was 18ºC.

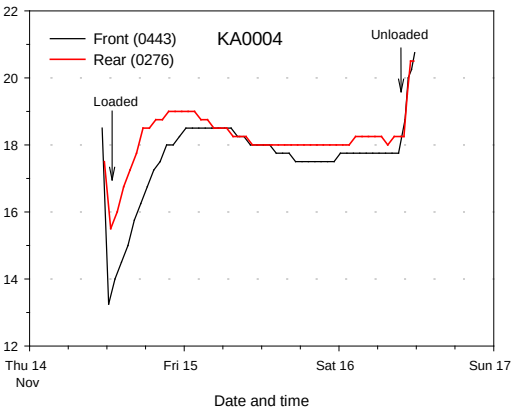



Figure 2 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Katherine to Adelaide. Note: The refrigeration set temperature was 18ºC.

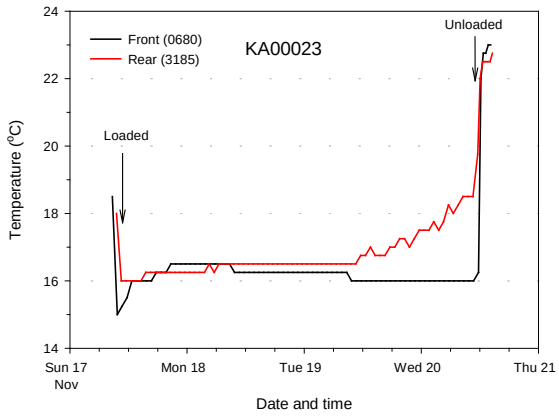



Figure 3 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Katherine to Adelaide. Note: The refrigeration set temperature was 16ºC.

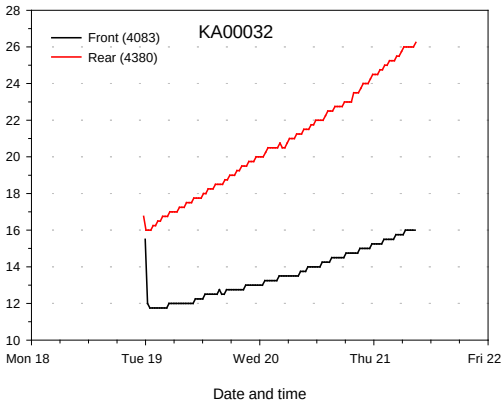



Figure 4 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Katherine to Gatton. Note: The refrigeration set temperature was 18ºC. For real-time temperature and location data see Figure 13.

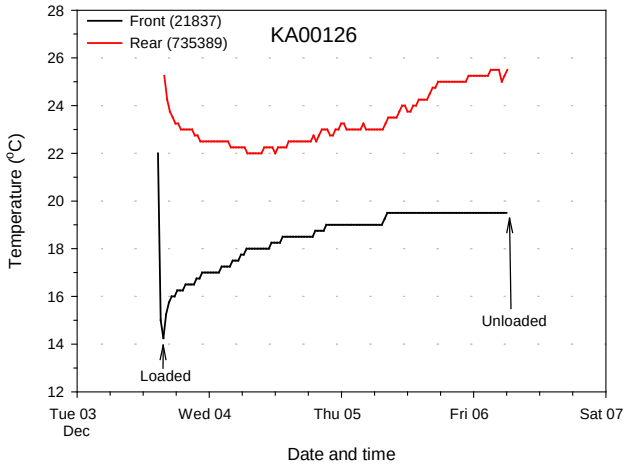



Figure 5 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Katherine to Adelaide. Note: The refrigeration set temperature was 18ºC.

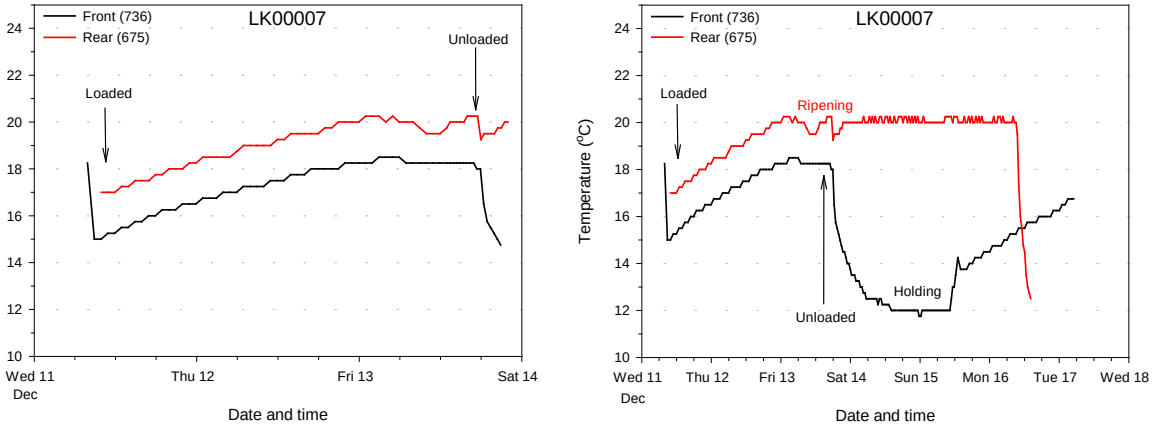
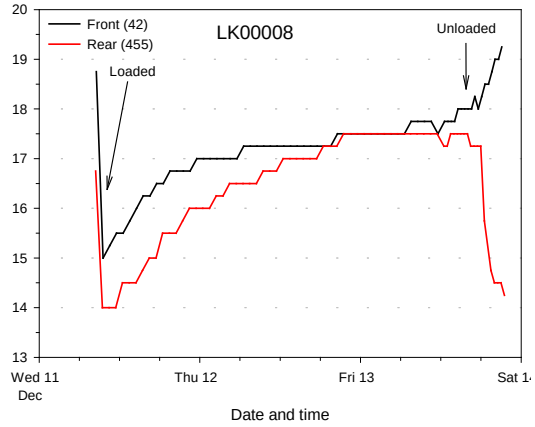



Figure 6 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Brisbane (left). Note: The refrigeration set temperature was 18ºC. Pallet 736 was transported onto a wholesaler in Melbourne, arriving 17 Dec (right).



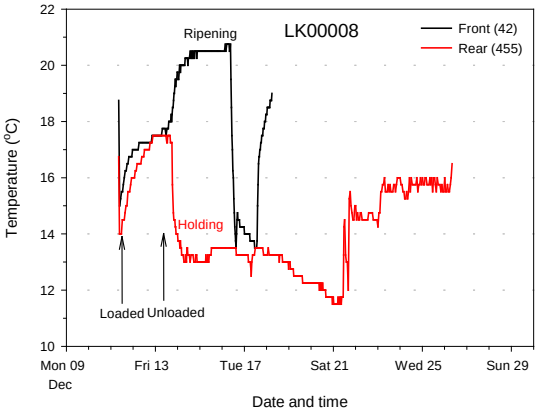




Figure 7 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Brisbane (left). Note: The refrigeration set temperature was 18ºC. Pallet 455 was transported onto a wholesaler in Melbourne, arriving 21 Dec (right).

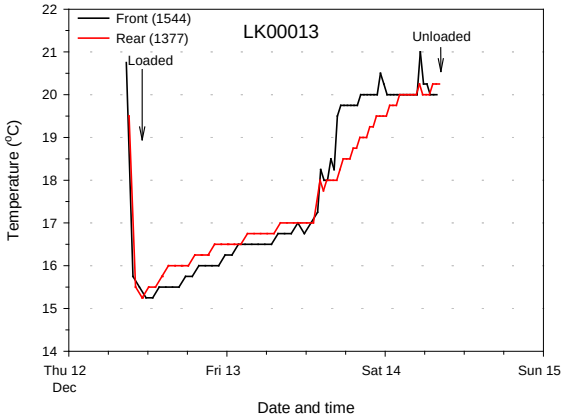



Figure 8 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Brisbane. Note: The refrigeration set temperature was 16ºC.

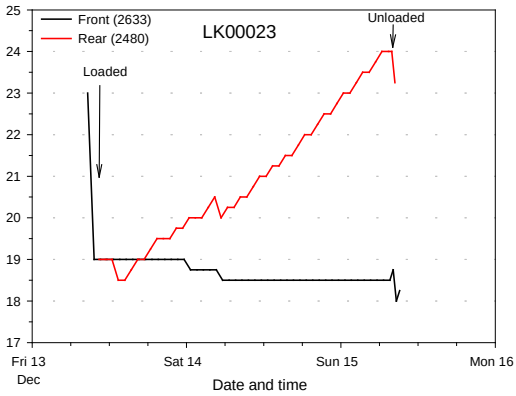



Figure 9 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Gatton. Note: The refrigeration set temperature was 16ºC.

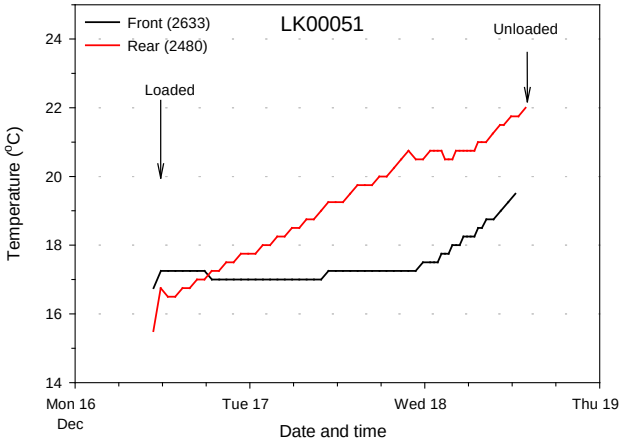



Figure 10 Temperatures at the front and rear of a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Brisbane. Note: The refrigeration set temperature was 16ºC.

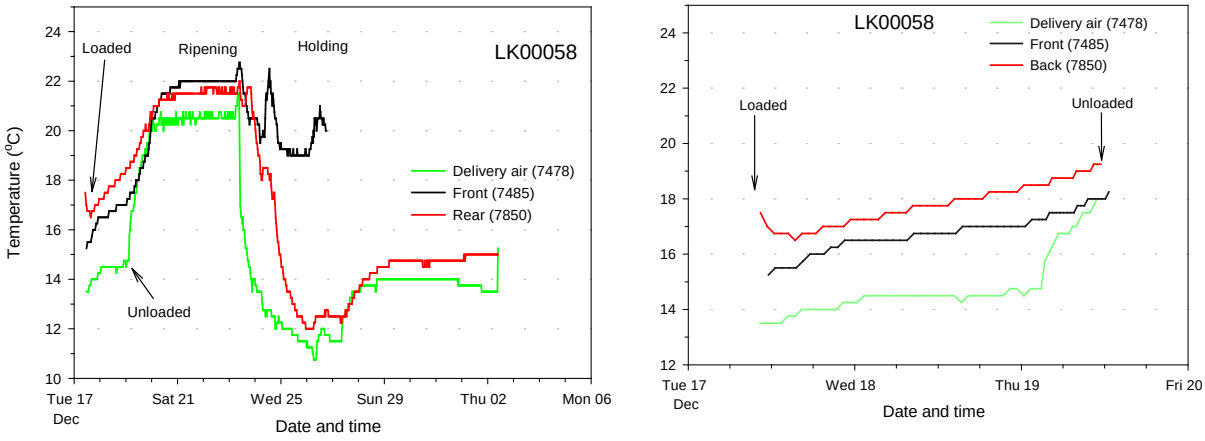



Figure 11 Temperatures at the front, rear and delivery air within a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Brisbane (right) with transport and storage/ripening in Brisbane (left). Note: The refrigeration set temperature was 16ºC.

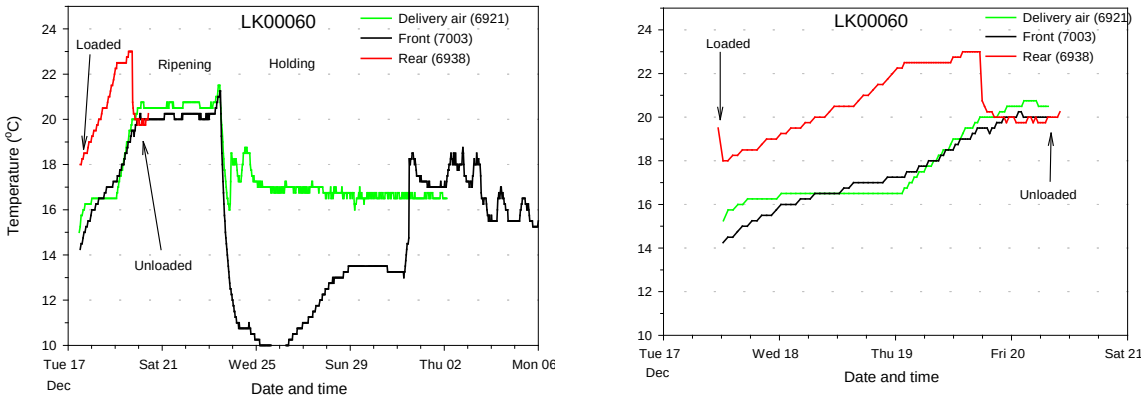



Figure 12 Temperatures at the front, rear and delivery air within a refrigerated container during transport of 'B74' mango fruit from Dimbulah to Brisbane (right) with transport and storage/ripening in Brisbane (left). Note: The refrigeration set temperature was 16ºC.

Two consignments tested the real time capability of the Xsense system. Figure 13 illustrates a screen shot from the XSense website showing progress with consignment number KA00032 from Katherine to Gatton. It was time consuming setting up the CU in the container with the GPS and mobile aerial needing to be fed to the outside of the container. However, having real time data on temperature and location can be valuable.

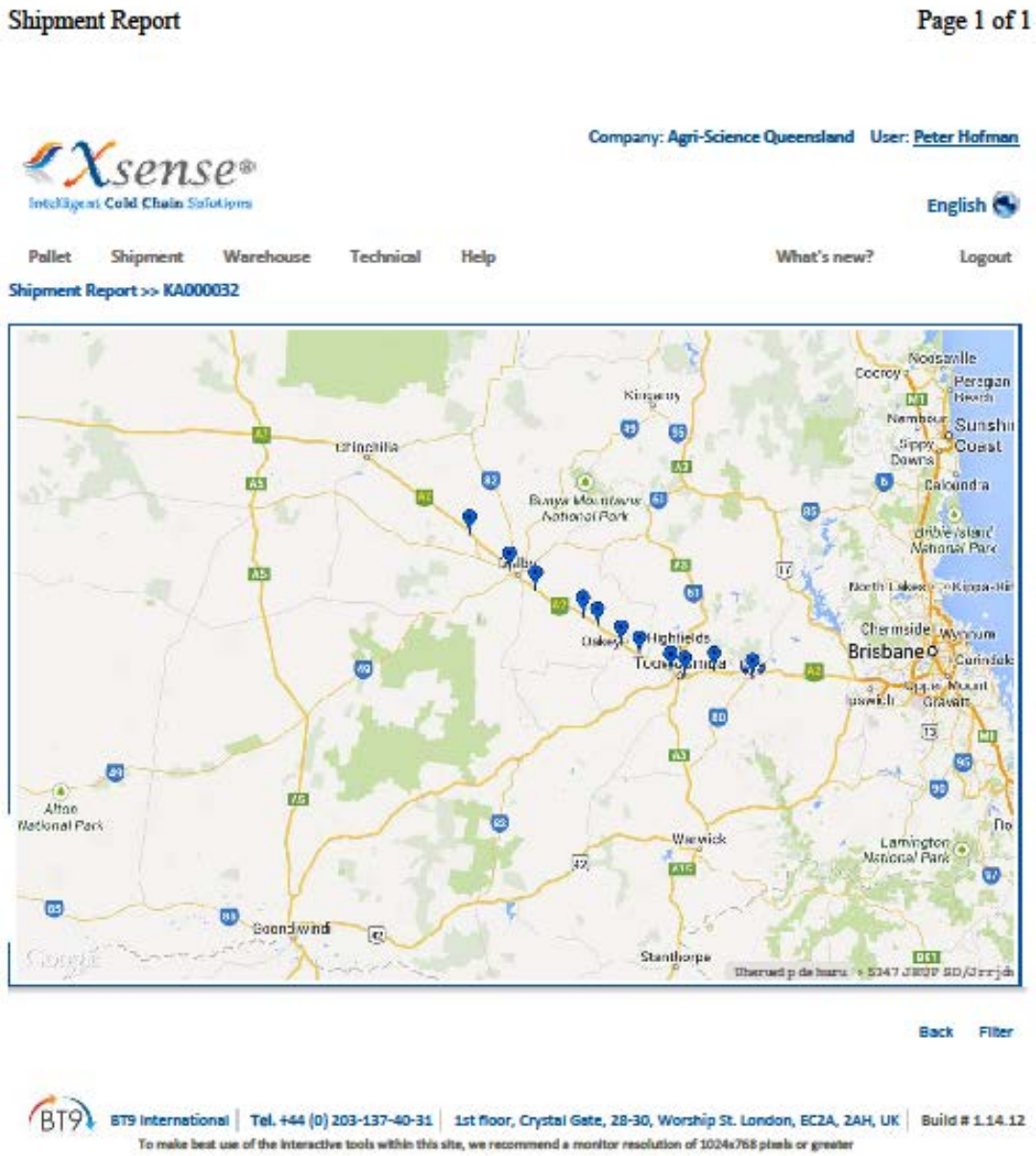



Figure 13 Screen shot of real time monitoring of a consignment from Katherine to Gatton illustrating the Google Maps based GPS location as the consignment travelled to Gatton near Brisbane.

## $CO_2$ control with hydrated lime

The hydrated lime was placed into large paper bags with the surface area of a P84 tray and the bags placed on top of the pallets at loading. All treatments used about 5 kg lime per pallet, either placed in one 5 kg bag or two 2.5 kg bags per pallet. The $CO_2$ concentrations were monitored using loggers from Mitsubishi.

Figure 14 and Figure 15 show the $CO_2$ results from six commercial containers transported from either Katherine to Adelaide, or Dimbulah to Brisbane. The results indicate that maintaining $CO_2$ below 3% was not consistently achieved. However, $CO_2$ concentrations were lower than the 8-10% which was usually observed in similar trials conducted in previous years (see project MG10008). The consignments from Dimbulah (LK00051 and LK00060) to Brisbane only took about 1.5 days as compared with the typical 3 days transit from Katherine, so the time for $CO_2$ accumulation was not long. However, in both these consignments the $CO_2$ concentration plateaued at about 2%, suggesting adequate control.

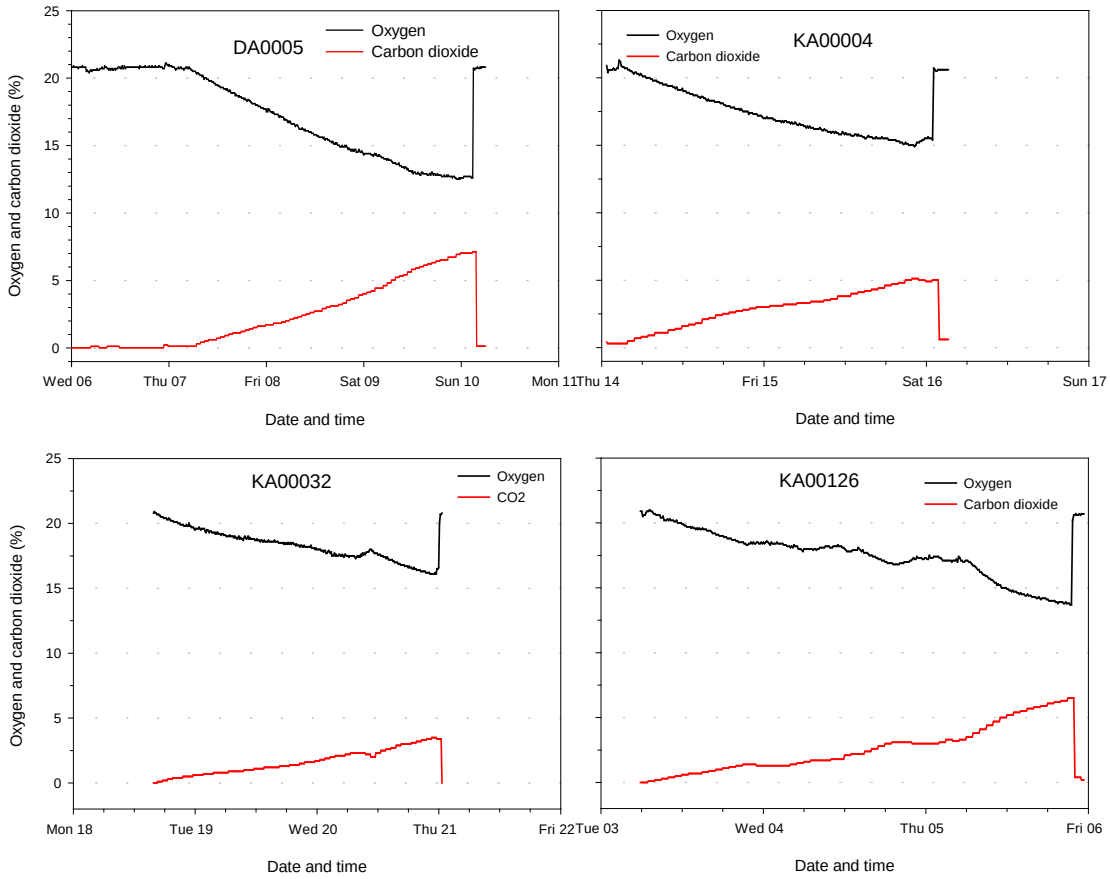



Figure 14 Carbon dioxide and oxygen concentrations in rail containers with 'B74' mango transported from Katherine to Adelaide. The containers held 20 pallets and 20 x 5 kg bags of hydrated lime placed in the container (one bag on top of each pallet).

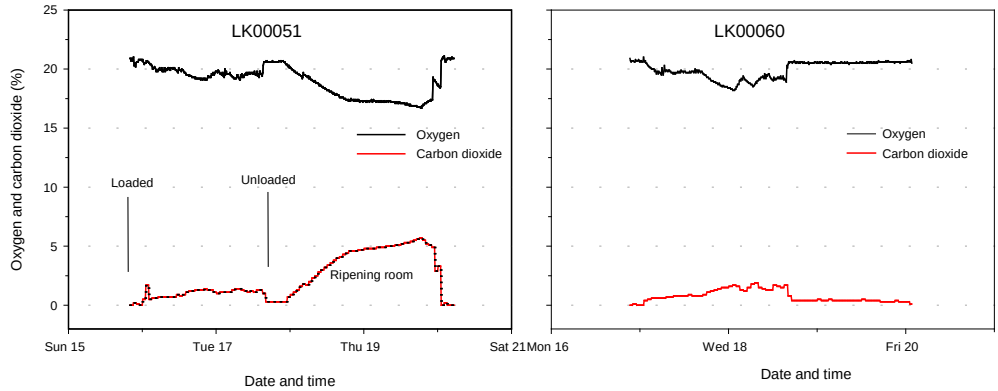



Figure 15 Carbon dioxide and oxygen concentrations in road trailers with 'B74' mango transported from north Queensland to Brisbane. The containers held 22 pallets. Consignment LK00051 had 22 x 5kg lime (left) and LK00060 had 44 x 2.5 kg lime (right).

## Ethylene release and control

Figure 16 shows that the ethylene was released quickly from 2 x 10 m plastic bags in the container, but concentrations did not exceed about 6 ppm. This was in a road trailer and it is possible that the greater vibration from road versus rail transport may have caused greater leakage of ethylene through the door seals etc. In another shipment, concentrations stayed above 10 ppm for about 2 d (left), or only 1 d (right) (Figure 17). However, the ethylene bags in consignment KA00032 (right) had fallen off the walls and blown to the back of the truck trailer by the time it arrived at Gatton, which would have reduced the exposed surface area and therefore the movement of ethylene was out of the bag.

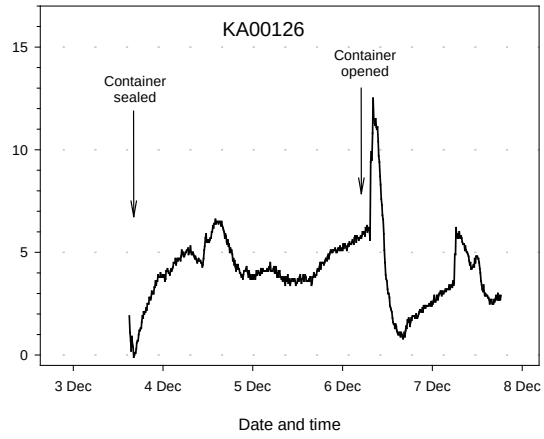



Figure 16 Ethylene concentrations in a road container with 'B74' mango transported from Katherine to Adelaide. The container included 2 x 10 m plastic bags filled with ethylene in nitrogen.

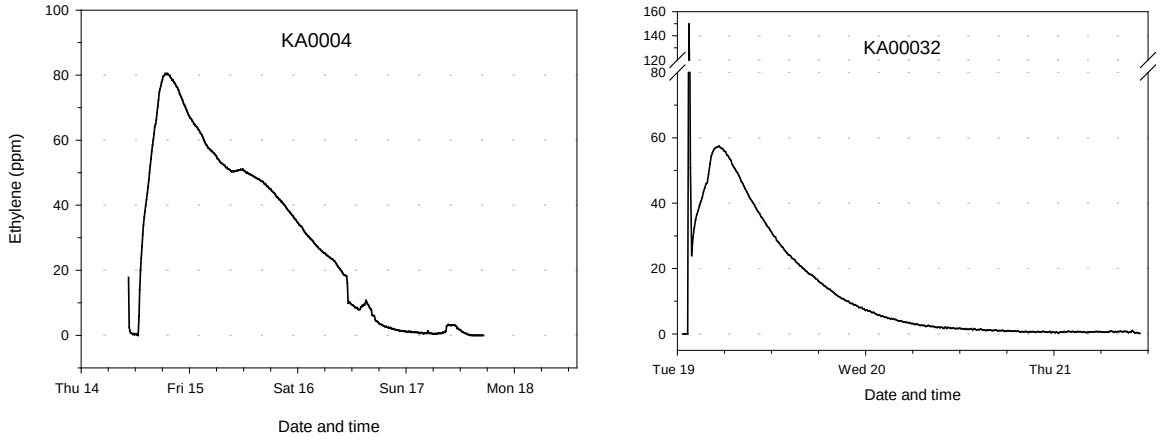



Figure 17 Ethylene concentrations in two rail containers with 'B74' mango transported from Katherine to Adelaide (left) and from Katherine to Gatton (Brisbane; right). Each container included 2 x 5 m bags filled with ethylene in nitrogen.

With regard to the use of ethylene releasing powder, placing a total of 10 bags fully exposed (top of bags cut off) resulted in relatively rapid release of ethylene but concentrations remained above 10 ppm for only 1 d (Figure 18). Leaving four bags open at the top and six bags punctured with pinholes reduced the rapid release but concentrations above 10 ppm only

lasted about 6 h (Figure 19) and 2 h (Figure 20). Using six bags open and four with pinholes resulted in about 1 d above 10 ppm. Further testing is required to attain the right balance of rapid release to establish concentrations above 10 ppm, then slow release to retain the concentration for at least 2 d.

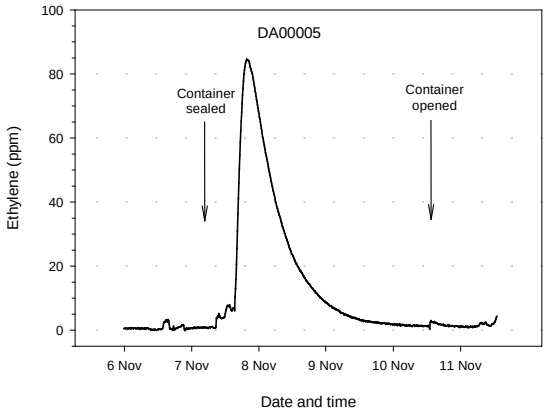



Figure 18 Ethylene concentrations in a rail container with 'B74' mango transported from Katherine to Adelaide. The container included 10 x 50 gm bags of ethylene powder. The bags were fully opened and exposed to the container atmosphere.

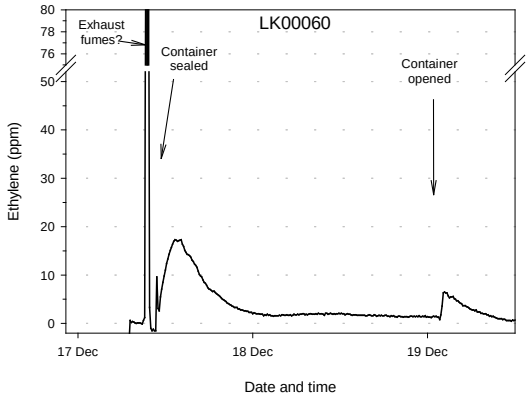



Figure 19 Ethylene concentrations in a rail container with 'B74' mango transported from Dimbulah to Brisbane. The container included 10 x 50 gm bags of ethylene powder of which four of the bags were fully opened and the other six had a small pinprick in the bag to provide slow release of the ethylene.

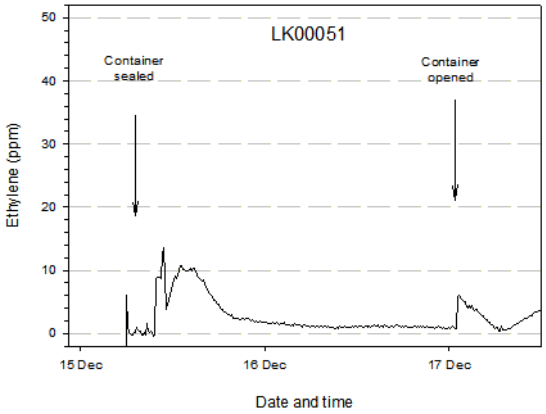



Figure 20 Ethylene concentrations in a rail container with 'B74' mango transported from Dimbulah to Brisbane. The container included 10 x 50 gm bags of ethylene powder of which four of the bags were fully opened and the other six had a small pinprick in the bag to provide slow release of the ethylene.

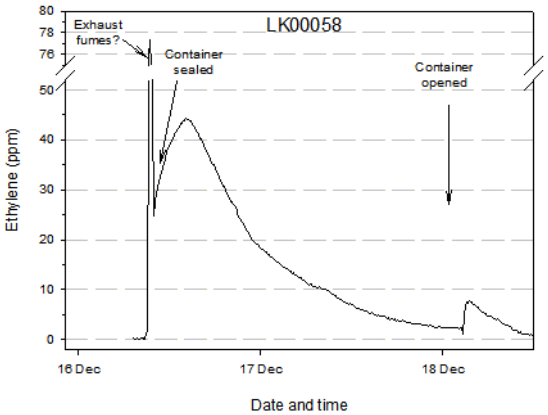



Figure 21 Ethylene concentrations in a rail container with 'B74' mango transported from Dimbulah to Brisbane. The container included 10 x 50 gm bags of ethylene powder of which six of the bags were fully opened and the other four had a small pinprick in the bag to provide slow release of the ethylene.

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Sharrock, K.R. and Henzell, R.F. (2010 ) Ethylene ripening of pears by unconventional means: Use of experimental thimble-sized ethylene capsules inside cartons and clamshells *Acta Horticulturae* 880, 339-346.

# In transit ripening of ‘Honey Gold’ mango

2014-15 season results

In-transit ripening and prediction of outturn quality for mango HIA project MG12016

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This publication has been compiled by Peter Hofman of AgriSciences Qld, Department of Agriculture and Fisheries.

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# Summary

Initiating the ripening of climacteric fruit during road freight from farm to market can save infrastructure and energy costs by reducing the time required to ripen in market. However, temperature, carbon dioxide ($CO_2$) and ethylene concentrations need to be controlled within specified limits to maximise the benefits of in-transit ripening and minimise quality loss. During the 2014/15 mango season, further testing was done in commercial consignments from Katherine to south-east Queensland. These tests included ethylene release from several designs of plastic bag and different configurations of hydrated lime bags for $CO_2$ absorption. Temperatures, and $CO_2$ and ethylene concentrations were monitored during the shipments. The results indicated generally poor temperature management of fruit during transport resulting in fruit temperatures at market of more than 35ºC in some consignments. At least two main issues were involved. Firstly, fruit were often loaded too warm (up to 28°C) because of inadequate precooling with no forced air cooling ability and not enough cold room space. Secondly, poor cooling capacity of the containers resulted in increased temperatures even when fruit were loaded at the correct temperature of about 18°C. It is likely that container loading practices resulted in significant restriction of airflow either to the pallets near the front or near the rear of the container, in some cases resulting in a 10°C difference in pallet temperatures across the load. These temperature fluctuations resulted in excessive $CO_2$ accumulation from the higher respiration rates, so that the hydrated lime was not able to maintain concentrations below about 3%. Better temperature control is critical to effective in-transit ripening and $CO_2$ control. Ethylene release from the plastic bags generally maintained ethylene concentrations above 10 ppm for at least two days. There appeared to be little difference between 50 and 75 µm thick bags.

# Introduction

Current recommendations for Australian mangoes requiring more than 2-3 days transit time from farm to ripener is to cool on farm to 12-13°C within 24 h of harvest, and transport at this temperature (Ledger and Barker, 2009; Ledger et al., 2012). These recommendations are based on the assumptions that transport containers do not have sufficient refrigeration capacity to cool fruit, or to maintain the temperature of ripening fruit within acceptable limits.

However, there are valid reasons to re-consider these recommendations:

- Farms/packhouses sometimes have insufficient cooling capacity during peak harvest periods, so that mangoes are often loaded at higher than recommended temperatures.
- The newer road/rail containers/trailers likely have higher refrigeration capacity and airflow, thus allowing limited cooling and better temperature control during transit.
- Ripening in transit has several significant cost advantages. These include reducing on-farm precooling and in-market ripening room floor space, reducing the time from harvest to market, allowing access to higher prices at the start of the season, and reducing energy requirements by not cooling the fruit as much on-farm, running the trucks at higher temperatures, and not requiring warm-up of fruit in market before ripening.

However, there are several challenges with in transit ripening:

- Ripening fruit produce considerable heat from respiration. Inadequate cooling capacity or airflow in the transport container will allow fruit temperatures to increase, further increasing heat production, and resulting in “runaway” temperatures.
- Ripening fruit also produce carbon dioxide ($CO_2$) during respiration. High $CO_2$ concentrations can result in green ripe ‘Kensington Pride’ mango fruit with more disease (Nguyen, 2003). Also, concentrations above about 4% will cause asphyxiation.
- The fruit should be treated with ethylene at the start of ripening. Ethylene treatment on-farm before dispatch is less practical because of the need for increased cool room space, and transporting these ripening fruit will increase the risk of excess heat and $CO_2$ generation as respiration increases rapidly as the fruit ripens. Therefore, fruit should be transported soon after harvest and ethylene treated in transit. Delaying the ethylene treatment until arrival will reduce the benefits of ethylene treatment.

Several systems have been developed for ethylene release in cartons or containers based on small release canisters (Sharrock et al., 2010 ; Sharrock and Henzell, 2010 ) and from ethylene encapsulated (trapped in a sugar matrix) (Ho et al., 2013) which may have application to transport containers. In addition, hydrated lime with at least 85-90% available lime index can effectively absorb $CO_2$ (Bartsch, 2004), and is used in controlled atmosphere rooms to minimise $CO_2$ accumulation.

In 2013/14 project MG12016 investigated several options for ethylene release from plastic bags, and for $CO_2$ absorption using hydrated lime. These investigations were continued in 2014/15. Similar logging systems were used this season, but with the addition of an in-house ethylene logging system.

# Methods

## Fruit material

'B74' mango fruit were sourced from a farm near Katherine in the Northern Territory during the 2014/15 season. They were harvested at commercial maturity and packed into cardboard mango trays according to standard commercial practice. The trays were palletised and cooled prior to transport to markets.

## Fruit transport

Palletised fruit were loaded into commercial 44 foot refrigerated solid wall truck containers. Depending upon the truck, 18-20 pallets were loaded into each container. The containers were transported 3000 km by road from Katherine to Brisbane or Gatton in Queensland. Containers were set to operate at 18°C. No other alterations to transport conditions were made except to add ethylene, or reduce $CO_2$ concentrations.

# Treatments

## Ethylene

Ripestuff (ethylene releasing powder developed by the University of Queensland) was not tested this season because of the absence of reliable systems to release the ethylene over sustained periods. Hence the main focus was on semi-permeable bags. These bags were constructed from 50 or 75 µm PVC plastic tube (30 cm diameter). The bag was heat-sealed at both ends and 4% ethylene in nitrogen was injected inside through a plug just before loading. Two bags of 5 m in length each were included in each container.

Improved systems for filling the bags and attaching to the container were tested. Small plugs were attached to the bags through which the gun attached to the gas cylinder could be inserted. The new attachment system included taping one end of the bag to the front of the fifth row of pallets, rolling the bag out on top of the remaining pallets as they were loaded, then taping the other end of the bag to the back of the last pallet. A drawstring was included to assist rolling the bag out on top of each pallet as they were loaded.

## Carbon dioxide

The target was to maintain $CO_2$ concentrations below 3% for WH&S reasons. Hydrated lime (Adelaide Brighton, 85-95% available lime index; typically above 90%) was placed in large standard paper bags with a flat footprint the size of a P84 tray. Each bag was placed into an empty tray, and the trays placed on top of the pallets. Each bag was filled with 5 kg or 2.5 kg to provide a thickness of lime of approximately 10 and 5 cm per bag. Combinations of 20 x 5 kg, 40 x 2.5 kg or 30 x 5 kg bags were placed in each container.

# Container monitoring

**Air temperature** inside the pallets was monitored with the Xsense system (www.bt9-tech.com). Basically, wireless RFID tags were placed into a tray on the 4th layer from the top of each pallet, and inside pallets in rows 2, and 8-9 from the front of the container. In some cases a sensor was placed in the top row of the pallet in the 2nd row from the front. A communication unit (CU) was installed at the receivers in Gatton or Brisbane. The temperature data from the tags was transmitted to the CU when the tags came within reading distance during unloading from the container, and the data uploaded to the Xsense server. Stakeholders accessed the website with username and password to retrieve temperature graphs of the load and the raw data. Email alerts were also sent to the stakeholders on arrival of the tagged pallets at the ripener, and when the pallet was exposed to out of range temperatures.

**$CO_2$ and $O_2$** was measured with low energy loggers supplied by MaxTend (Mitsubishi). They were placed in a tray on the top of one of the pallets, then retrieved at market and returned to Mitsubishi for downloading.

**Ethylene** was recorded with Gas Alarm Systems (Australia) loggers containing electrochemical cells (MSR electronics, Nuremberg, Germany; measuring range of 0-100 parts per million; ppm ethylene). In-house built ethylene loggers using electrochemical cells (WinSense, China) and the Arduino platform for control and data logging were also used. The loggers were placed in the same location as the $CO_2$ logger (above).

# Results and Discussion

## Temperature

The XSense tags measured air temperatures in the middle of the load. Previous experience indicates that air temperatures in these locations are close to or slightly lower than pulp temperatures under conditions of relatively low air movement such as experienced in transport containers. Hence, in the following discussion, air temperatures are assumed to estimate fruit temperatures.

Container temperature management was variable but generally poor. In some instances, fruit temperatures were well maintained when fruit were pre-cooled and loaded close to the container set temperature of 18ºC (Figure 5). However, when other consignments were loaded with pulp temperatures of > 18°C, the container temperatures increased during transit to more than 28°C (Figure 1, Figure 6, Figure 7). In consignment NI00045, the measured pallet temperatures at loading were between 19-28°C (Figure 3) and pulp temperatures increased up to 35°C during transit. In other instances, measured pulp temperatures at loading were 29°C, but temperatures declined significantly in fruit toward the back of the container resulting in a temperature difference of about 10°C between pallets on arrival (Figure 4).

The higher transport temperatures were associated with lower relative humidity (Figure ), which may result in increased weight loss and lower saleable weight.

These results and other observations suggest two main causes for poor temperature control:

- Inadequate pre-cooling, resulting in most consignments having temperatures in excess of the targeted 18°C when loaded. The Katherine packhouse had no forced air cooling and cold room capacity was not always adequate. Under these conditions, pallets were sometimes air-cooled with less than the 30 cm gap between pallets, and likely did not remain in the cold room long enough. This typically results in the outside fruit on the pallet cooling down more quickly than inside fruit, so that temperature checks of the outside fruit before loading may indicate acceptable temperatures while the bulk of the fruit in the pallet are still well above the required temperature.
- Poor air movement through the pallets in the container results in inadequate removal of heat generated by the fruit. Figure 1 indicates that air temperature in the head space above the container was close to the container set temperature of 18°C, but air temperature within the bulk of the load increased from about 18 to 25°C over 2 days. Diverging temperatures between the front and the back (Figure 4) also indicates uneven airflow.

Efficient airflow is critical to effective temperature maintenance of transported fruit. Under good transport conditions, fruit temperatures near the front of the container are 1-2°C lower than near the back of the container. However, this gradient can be more than 8°C if fruit are loaded considerably warmer than the container set temperature. In most instances, this results in warmer fruit toward the front of the container compared to the back because of less cold air reaching the back of the container. This is likely to be worse when the pallets are loaded with:

- A pallet space against the front bulkhead that would allow cold air to travel directly from the delivery air vent to the return air vent, and/or
- Tall plywood sheets obstructing the space between the top of the pallet and the roof, and also blocking the openings in the pallet itself (where the forklift picks up the pallet). This prevents air from returning along the floor of the container to the return air vent.

However, Figure 4, Figure 5 and Figure 7 indicated an opposite pattern this season. We observed the following loading patterns that likely contributed to warmer fruit near the front compared to the back (see Plate 1 and Plate 2):

- A pallet space against the front bulkhead and
- Tall plywood sheets at the front of the load obstructing the space between the top of the pallet and the roof, and blocking the openings in the pallet itself, and
- Roof shoots that channelled cold air to the back of the container, further restricting cold air availability to the fruit at the front.

Combined, these factors would reduce cold air flow to fruit near the front of the container and result in larger temperature differences.

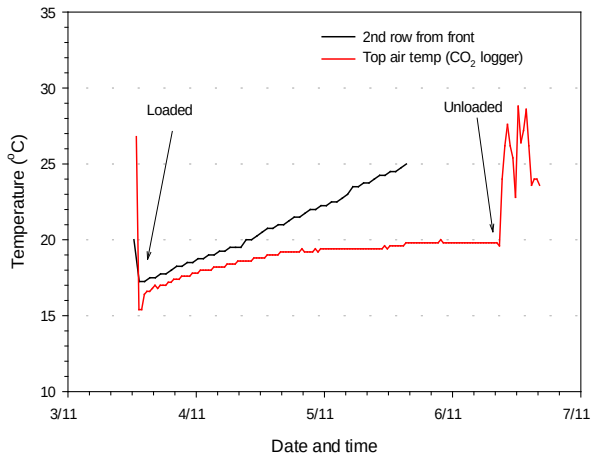


Figure 1. Temperatures recorded in consignment DA00016.

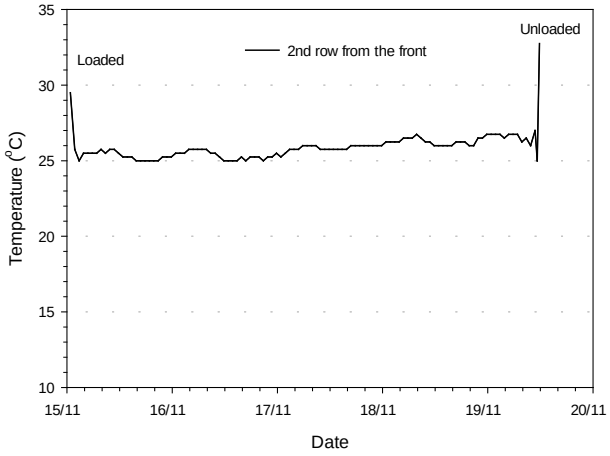


Figure 2. Temperatures recorded in consignment NI00028.

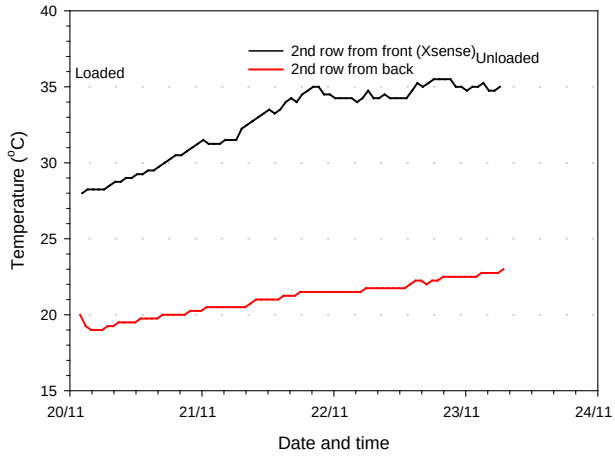


Figure 3. Temperatures recorded in consignment NI00045.

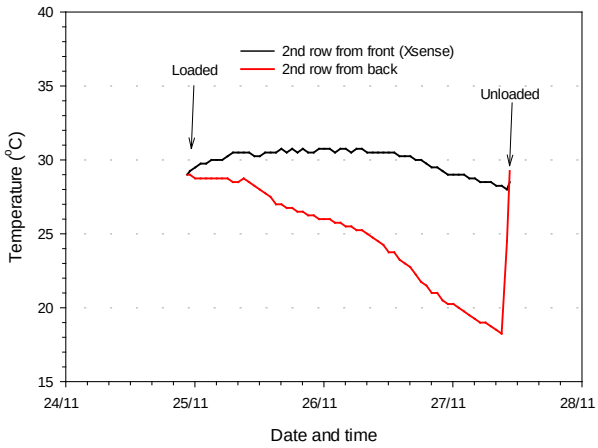


Figure 4. Temperatures recorded in consignment NI00073.

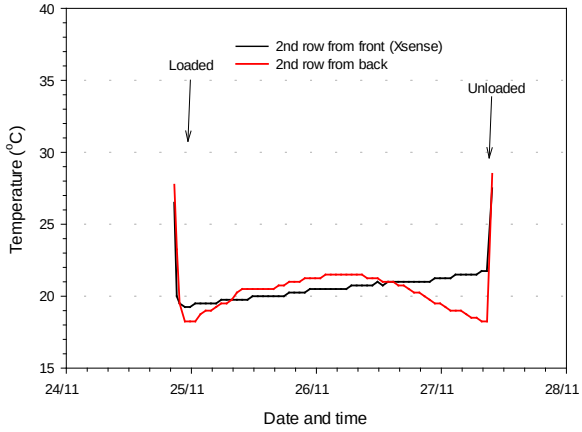


Figure 5. Temperatures recorded in consignment NI00074.

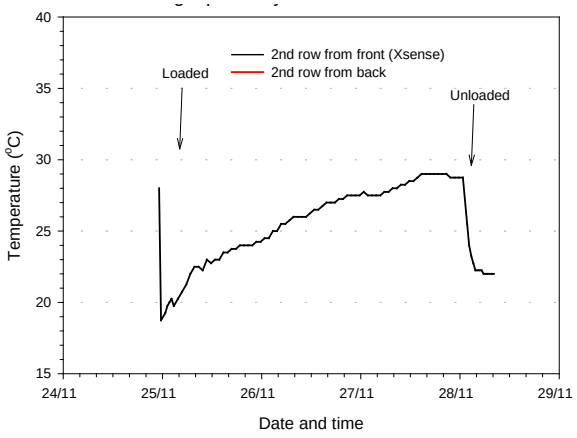


Figure 6. Temperatures recorded in consignment NI00076. The logger in the rear of the container failed.

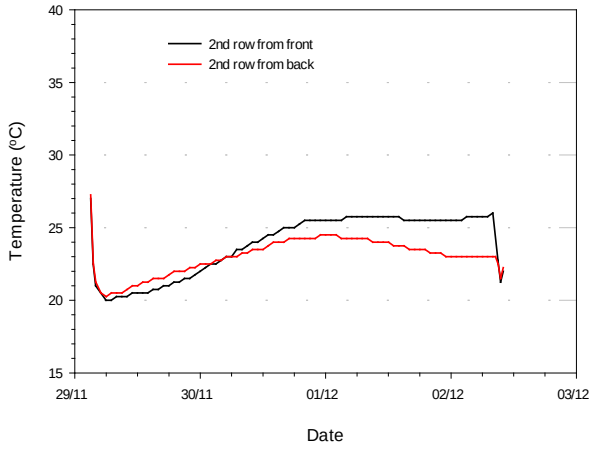


Figure 7. Temperatures recorded in consignment NI00100

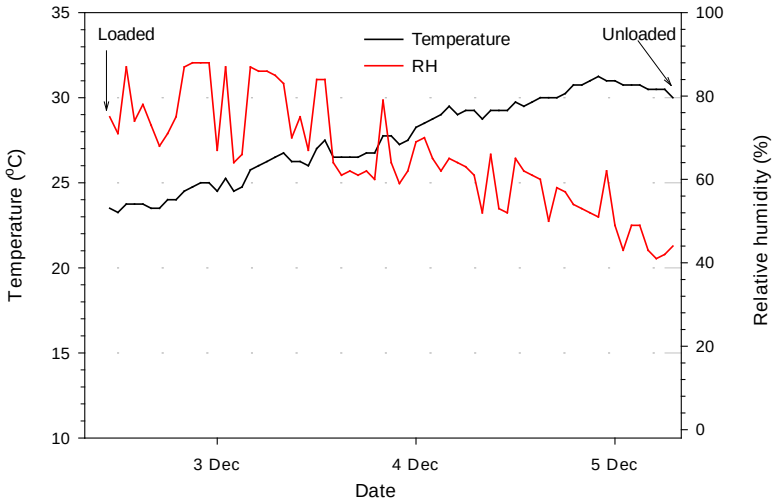


Figure 8. Temperatures and relative humidity recorded in consignment NI00115.

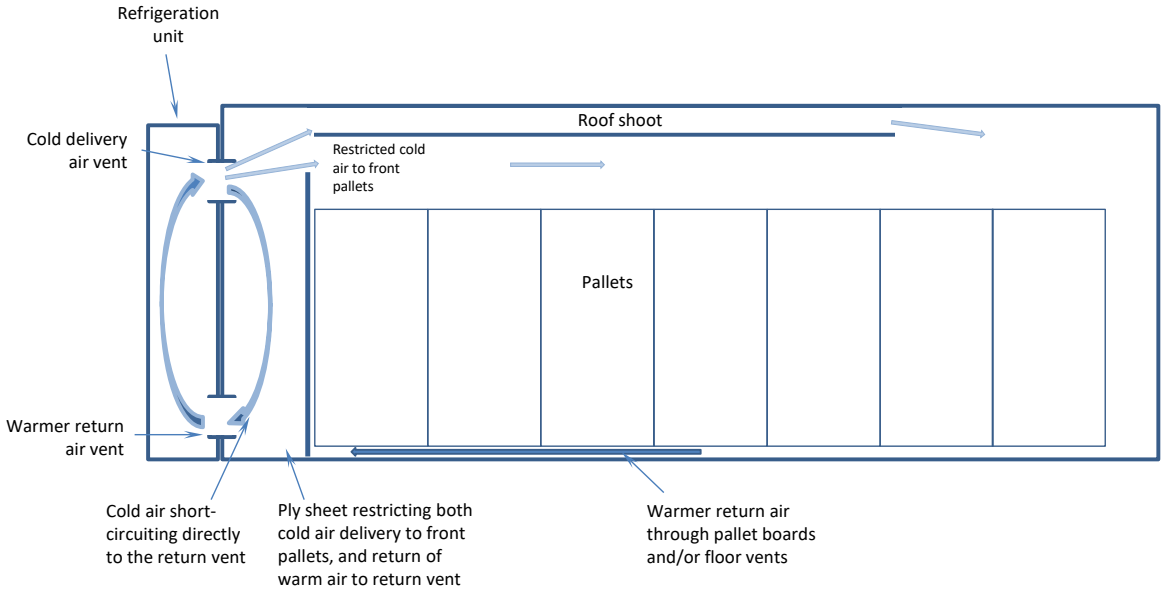


Plate 1. Diagram of likely cold air flow in refrigerated transport containers loaded with a gap at the front, a tall plywood sheet, and a roof shoot. Air availability to the front pallets was likely restricted, resulting in considerably warmer fruit temperatures near the front of the container compared with fruit near the back.


Plate 2. Loading of a typical trailer with a gap at the front of the container to reduce the load over the front axles, the excessively high plywood sheet and the ceiling shoots all combining to significantly reduce the availability of cold air to the fruit, and particularly in the first few pallet rows.Figure 1

# Ethylene

The plugs attached to the plastic bags did not hold under the more robust commercial conditions, resulting in excessive leakage from the bags. A better design is required. None of the bags detached from the pallets during transit, unlike the previous season when the bags were attached to the side of the container. However, more thought is required on how to more easily roll the bags over the top of the pallets during installation.

The in-house loggers generally performed well but several of the loggers failed mid-way through the journey, likely from intermittent electrical faults.

The ethylene bags maintained concentrations above 10 ppm for at least 2 days in most of the containers monitored (Figure -14). There appeared to be little consistent effect of plastic thickness on ethylene concentrations.

Figure 8 likely indicates significant leakage of ethylene from the container, perhaps from the air vent (if fitted) remaining open. Similar consistent patterns were identified for $CO_2$ concentration in the same container (Figure ).

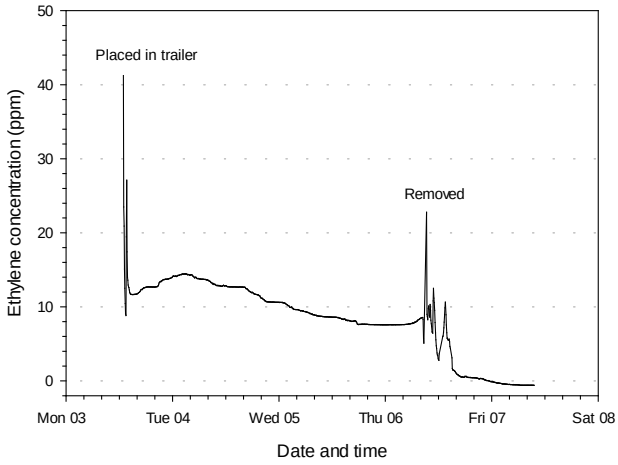


Figure 9. Recorded ethylene concentrations in consignment DA00016, with 2 x 5 m bags of 50 µm plastic.

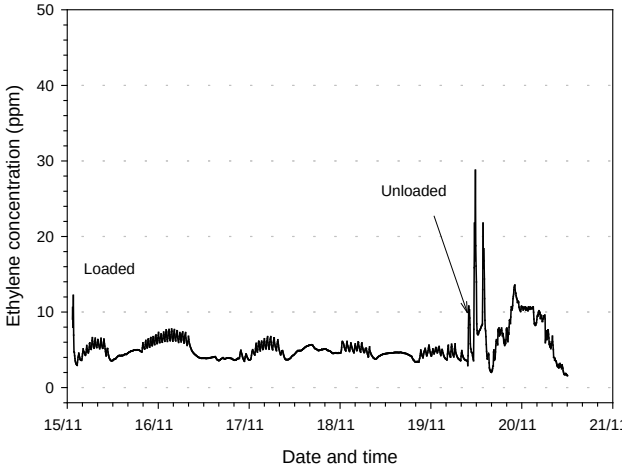


Figure 8. Recorded ethylene concentrations in consignment NI00028, with 2 x 5 m bags of 75 µm plastic. The bags were about half deflated on arrival at Gatton.

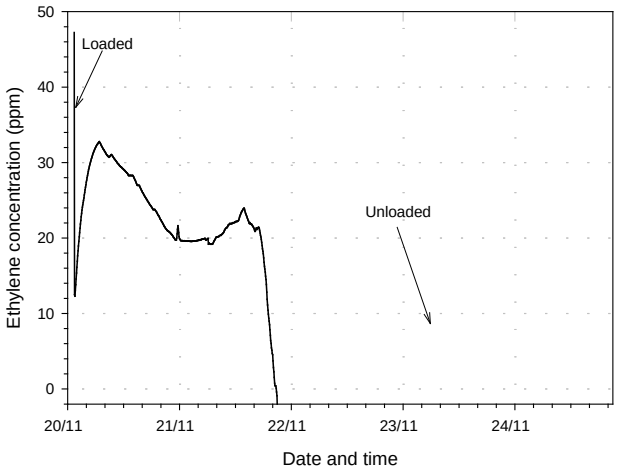


Figure 9. Recorded ethylene concentrations in consignment NI00045, with 2 x 5 m bags of 50 and 75 µm plastic. The logger failed after 2 days.

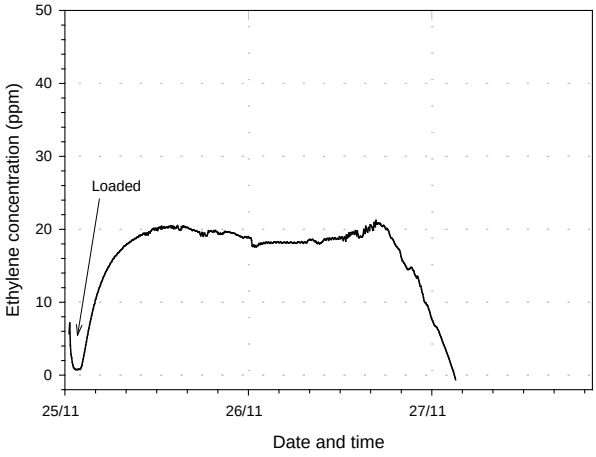


Figure 10. Recorded ethylene concentrations in consignment NI00073, with 2 x 5 m bags of 75 µm plastic. The logger failed after 2 days.

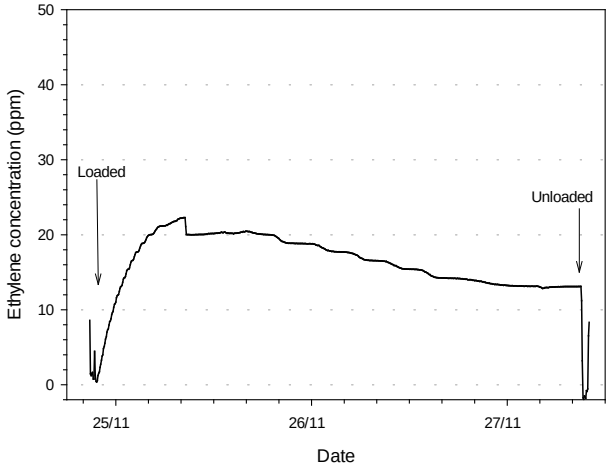


Figure 11. Recorded ethylene concentrations in consignment NI00074, with 2 x 5 m bags of 50 µm plastic.

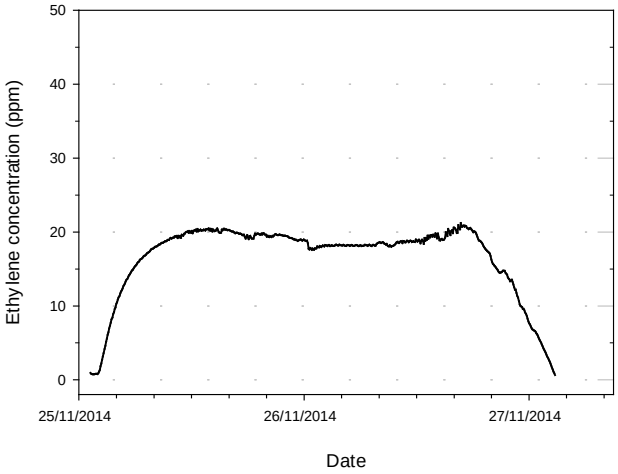


Figure 12. Recorded ethylene concentrations in consignment NI00076, with 2 x 5 m bags of 50 and 75 µm plastic.

# Carbon dioxide

The total amount of hydrated lime per container and the amount per bag was aimed at maintaining $CO_2$ concentrations below 3% based on the respiration rate of fruit at about 18°C. The considerably higher temperatures experienced in most of the loads compromised these studies, since respiration rate, and therefore $CO_2$ production would be higher at these high temperatures. As a result, $CO_2$ concentrations in all but one consignment (Figure ) were above the preferred maximum, and in this case it was likely due to high container leakage. In one instance (Figure 17) the $CO_2$ concentration increased above 20% and the $O_2$ concentration declined to below 2%. This would have resulted in anaerobic respiration and off flavours. The temperatures in this consignment were higher than preferred (Figure 7), although not considered high enough to generate such high $CO_2$ concentrations. It is possible that the fruit had already started the climacteric rise in respiration rate on loading, thereby generating considerably more $CO_2$ than unripe fruit.

Further studies should concentrate on using 2.5 kg bags to reduce the depth of the lime and increase the surface area:weight ratio. These studies will only be useful if fruit temperatures are maintained at around 18°C, since practical quantities of hydrated lime are not able to maintain $CO_2$ concentrations with the increased respiration rates at higher temperatures.

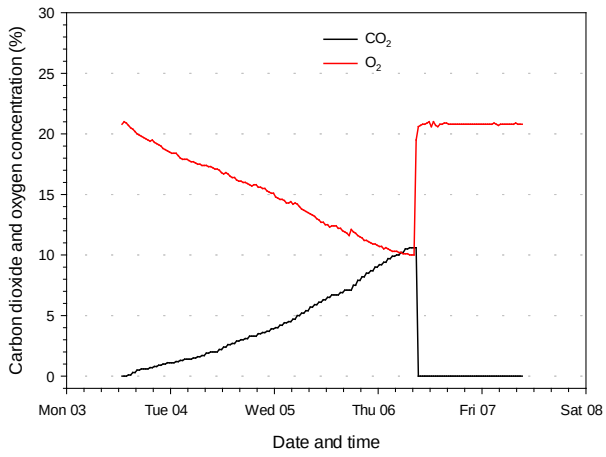


Figure 13. DA00016 (956979), dispatched 3/11/14, from Darwin to Gatton. 20 x 5 KG lime

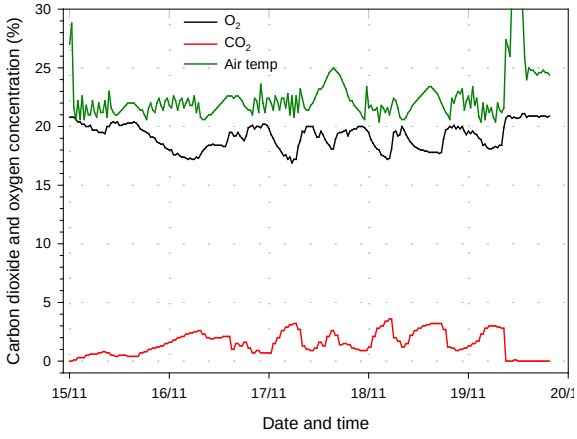


Figure 16. NI00028. 40 x 2.5 KG lime

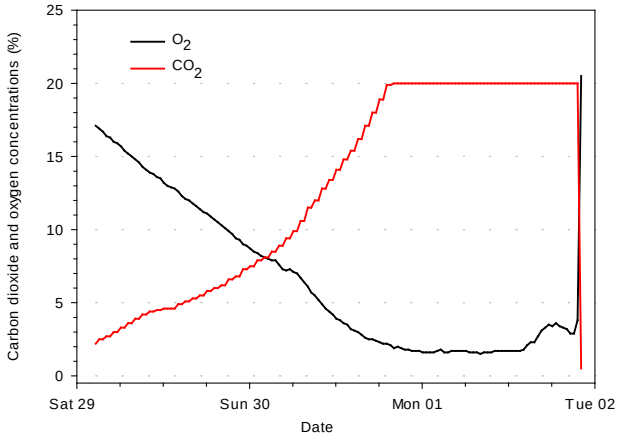


Figure 17. NI00100. No info on lime bags

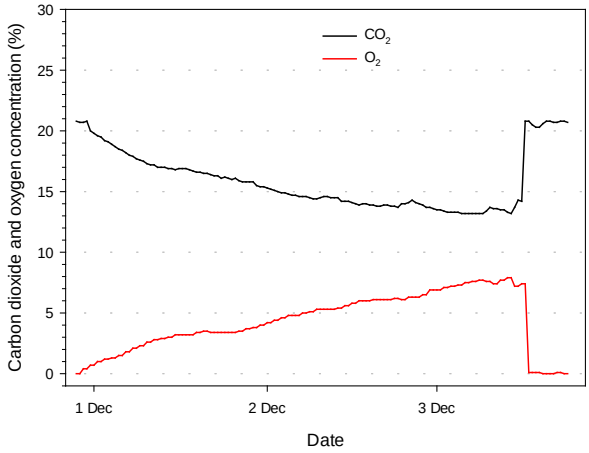


Figure 18. NI00112. No info on lime bags

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Sharrock, K.R. and Henzell, R.F. (2010 ) Ethylene ripening of pears by unconventional means: Use of experimental thimble-sized ethylene capsules inside cartons and clamshells *Acta Horticulturae* 880, 339-346.

# Evaluating in-transit ripening of 'Honey Gold' mango fruit in refrigerated road containers

## 2015/16 season results

## In-transit ripening and prediction of outturn quality for mango

### HIA project MG12016

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Note: the preliminary results in this report are part of a research higher degree program, and are therefore confidential until published.

This publication has been compiled by Peter Hofman of Horticulture and Forestry Sciences, the Department of Agriculture and Fisheries.

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# Summary

Initiating the ripening of climacteric fruit during road freight from farm to market can save infrastructure and energy costs by reducing the time required to ripen in market. The current study evaluated the efficacy of Ripestuff™ (ethylene encapsulated powder) and carbon dioxide ($CO_2$) control on ripening of 'Honey Gold' mango fruit during transit. The trials were conducted in six trailers, three with Ripestuff and three remaining ones acting as control. About 2 kg Ripestuff was packed in polypropylene specimen containers (75 mL capacity). The specimen containers were then placed in each 40 ft refrigerated trailer containing 2560 mango trays to maintain ethylene at concentrations above 10 µL $L^{-1}$ inside containers for 2-3 days. Approximately 54 kg hydrated lime (Adelaide Brighton) was packed in 42 Maxtend bags (1.3 kg each) and placed inside the containers to maintain $CO_2$ levels below those that are injurious to fruit and industry workers. Ethylene concentrations, air and pulp temperatures, and $CO_2$ and oxygen concentrations were monitored during the shipments. Samples of fruit from the Ripestuff-treated and control trailers were taken upon arrival for initial fruit quality assessment and quality at ripe.

The results indicated that ethylene concentrations in Ripestuff-treated containers were maintained above the desired concentrations for most of the journey. Average pulp temperatures over the 3-4 days was 1-4°C above the container set temperature and there were significant variations in temperature across the load and over time. Roof chutes in the container affected the temperature gradients. $CO_2$ concentrations in control container reached only 1-5% on arrival at the ripener, but $CO_2$ concentrations in the containers with Ripestuff increased dramatically during transport, reaching 13 to more than 20%. Hydrated lime did not maintain $CO_2$ concentrations within the desired concentration. The results from post-shipment assessment showed that the ripening time of Ripestuff-treated mango fruit was 2 days shorter than control fruit. The presence of high $CO_2$ concentrations in the container during transit did not affect the loss of green colour during ripening.

To explain the causes for the higher $CO_2$ concentrations in the Ripestuff-treated fruit, 'Honey Gold' mango fruit were treated with Ethephon® (releases ethylene) and ripened at 20 °C. The respiration rate in these fruit increased rapidly within 1 day of treatment, compared with about 4 days in non-treated fruit, and maximum respiration was double that of the non-treated fruit. This explained the higher $CO_2$ concentrations observed in the Ripestuff-treated fruit. Further research is required to reduce $CO_2$ accumulation in these containers.

# Introduction

Mango production in Australia includes orchards in the Northern Territory, Queensland, Western Australia, New South Wales, Victoria and South Australia. Mango cultivars such as 'Kensington Pride', 'Calypso', 'R2E2', 'Honey Gold' and 'Keitt' are grown for the domestic and export markets. The average production volume in Australia for the last 5 years has been ~66,000 tonnes per annum with a gross value of production at the farm gate of ~$190 million per annum (Australian Horticulture Statistics Handbook, 6/2015).

Typically mango fruit are harvested at a mature hard-green state and are generally pre-cooled to 12°C within 48 hours of harvest. These practices prolong storage life and can prevent premature ripening (Meurant et al., 1999). Refrigerated containers are used to transport fruit by road and/or rail to ripening facilities located up to 4000 km away in urban centres. Current controlled ripening techniques in Australia involve exposing mangoes to ethylene for 2-3 days (Ledger et al., 2014). The target concentrations are 10 µL $L^{-1}$ continuous ethylene using a trickle systems or 100 µL $L^{-1}$ ethylene every 8 to 12 hours using a shot system. After gas ripening, most mangoes are sold through large supermarket chains and wholesale agents.

To avoid physical and quality losses when transporting mangoes by refrigerated road or rail containers, it is important to establish and maintain the desired environmental conditions. These environmental conditions include optimum air and pulp temperatures (°C), relative humidity (RH), and ethylene and carbon dioxide ($CO_2$) concentrations.

Previous in-transit ripening work by Hofman et al. (2014) investigated ethylene release in refrigerated road and rail containers of 'B74' (Calypso™) mango consignments travelling from Katherine, Northern Territory (NT) to distribution centres in Adelaide and Brisbane. The ethylene was released from two sources including semi-permeable tubing filled with 3.8% ethylene in nitrogen, or sachets of Ripestuff™, an ethylene encapsulated powder (Ho et al., 2013). The Ripestuff™ provided adequate ethylene concentrations and was easier to apply compared with the tubing.

The following commercial trials evaluated the application of Ripestuff™ in refrigerated truck containers of 'Honey Gold' mango fruit from Katherine, NT to Wamuran, south east Queensland. Hydrated lime was included to absorb excess $CO_2$ produced by the fruit. Air and pulp temperatures, as well as $CO_2$, oxygen ($O_2$) and ethylene concentrations were monitored during transit, and fruit quality was assessed at arrival and at eating soft (ES).

# Materials and Methods

## Commercial trials

### Fruit

'Honey Gold' mango fruit were picked and packed according to standard commercial procedures from a commercial orchard in Katherine, NT. These included de-stemming in a mango wash solution to prevent sapburn injury, treating with a postharvest and insecticide spray, grading for uniform size and quality and packing into cardboard trays.

After packing all fruit and pallets were handled according to typical commercial practices, except for the ethylene and $CO_2$ control treatments applied in the containers. For each container, 15 trays of count 14 fruit were obtained from the same fruit batch off the packing line and temperature loggers inserted as described below. Each of the trays were re-inserted into targeted pallets during pallet stacking, and these trays removed from the container on arrival at Wamuran (south east Queensland). They were then transported to the Maroochy Research Facility (MRF), Nambour (about 1 h drive) for further assessment.

For the 2015 mango season, five truck consignments were monitored. With three containers including Ripestuff™ and hydrated lime, and two containers without Ripestuff (Table 1). Control container 2 had air temperature, ethylene and $CO_2$ loggers included, but no Ripestuff™ or hydrated lime and fruit was not collected for quality assessment.

# Treatments

## Ethylene release

Based on a standard refrigerated container size of 45 ft (13.38 m long, 2.4m wide, 2.5m high) and the head-space volume within the loaded container, it was estimated that-2 kg of Ripestuff™ powder was sufficient to produce the target concentration of between 20-80 µL $L^{-1}$ ethylene over at minimum of two days. 12 g of Ripestuff™ was placed into 75 mL screw top specimen containers the day before consignment departure (Plate 1). The lid of each specimen container was pierced four times with a Terumo 0.5 x 25 mm gauge needle, about two hours before container departure. All lids were covered with clear masking tape to prevent the premature release of Ripestuff™. The tape was removed just prior to Ripestuff™ being placed on the top of a pallet at the rear of the container, and before closing of the container doors.


Plate 1. Ripestuff™ powder in 75 mL specimen containers just before placement in the refrigerated container.

## Carbon dioxide absorption

To minimise fruit quality loss from excess $CO_2$, 1.3 kg hydrated lime powder (Adelaide Brighton) was placed into each of the 42 MaxTend paper bags. Each bag was placed into a six-per layer tray, making sure that the lime layer was evenly spread to minimise layer thickness and maximise the surface area to volume ratio. A total of 54 kg of lime was placed in each container. A sub-sample of the lime was tested for its capacity to absorb $CO_2$. Briefly, a 38.5% (w/w) neutralised sucrose (AR grade; Chem-Supply Pty Ltd, Gillman, SA, Australia) solution was prepared in distilled water. Ten drops of phenolphthalein (1% in methylated spirit; Ajax Finechem, Taren Point, NSW, Australia) and 15 drops of 0.1 M sodium hydroxide (AR grade; Ajax Finechem, Seven Hills, NSW, Australia) was swirled into the sucrose solution. A sample of 3.7 g of lime was mixed into 20 mL of distilled water and added to 50 mL of the sucrose solution. The solution was stirred every 5 minutes for 25 minutes. Finally, four drops of phenolphthalein was added to the solution before titration. The samples were titrated with 1 M hydrochloric acid (AR grade; Chem-Supply Pty Ltd, Gillman, SA, Australia) until the solution becomes transparent. One mL of acid solution used is equivalent to 1% of lime activity.

## Temperature

Each truck consignment contained 18 pallets with and 15 I-button temperature loggers. \One I-button was placed into each of five trays and positioned in an ‘X’ pattern, when two pallets were side-by-side (Plate 2). This included placement of I-buttons in the top left, bottom left, centre, top right and bottom right of the two pallets. This was repeated in the 2nd row from the refrigeration unit and the cab of the truck), middle (about row five) and the 2nd last row and near the rear doors; Figure 1). Each pallet was 16 trays high.


Plate 2. Two pallets (numbers 3 (left hand side) and 4 (right hand side)) loaded next to each other near the front of the container. Sample tray locations (T=top, B=bottom, L=left, R=right, C=centre) are indicated, and each contained an I-button air temperature logger. The centre tray (C) also contained a Hobo pulp temperature probe.

The I-button temperature loggers were placed in the centre of each of the 15 mango trays, with logger number and tray/pallet placement recorded. The flagging tape attached to each logger was secured to the outside of the tray to prevent movement and for easy retrieval. Fruit pulp temperature was recorded using a Hobo temperature logger probe placed in position three (centre) of each of the three rows for each container. The fruit probe was inserted into the cheek of a fruit in the centre of the tray. Temperature and RH in the delivery air flow on the top of the pallet was also recorded using an I-button temperature/RH logger placed next to the Ripestuff™ containers at the rear of the container.

## Gas atmospheres

In-house manufactured, arduino-based loggers were used in the trials. Each logger contained a photoionization (PID; Alphasense, UK) and electrochemical (EC) cell for ethylene, and an infrared-based sensor (CO2meter, USA) for $CO_2$. The PID sensor is reportedly more sensitive and less prone to drift over time, but is responsive to a wider range of volatile compounds compared with the EC sensor. A ethylene logger (Gas alarm MSR, Germany) was placed in Ripestuff container 1. A Maxtend (Mitsubishi Australia) logger was also used to record $O_2$ and $CO_2$ concentrations. The gas loggers were placed in a tray on top of a pallet near the door of the container.

# Quality assessment

Upon arrival at MRF, 10 ‘Honey Gold’ fruit were randomly chosen from each tray location and numbered from 1-600 (150 fruit from each of the four containers). All numbered fruit were assessed for weight, skin colour, firmness and external defects at arrival, then five random fruit were destructively assessed for total soluble sugars (TSS) and titratable acidity (TA). The remaining five fruit from each tray were ripened at 20°C, assessed daily for firmness until reaching eating ripe (ES) for each fruit, then assessed for quality as above.

Front of truck

| | |
|---|---|
| 1 | 2 |
| **3 x** 3 | **2 x** 4 |
| 5 | 6 |
| 7 | 8 |
| **3 x** 9 | **2 x** 10 |
| 11 | 12 |
| 13 | 14 |
| **3 x** 15 | **2 x** 16 |
| 17 | 18 |

Rear doors of truck

Figure 1. Plan view of 18 pallets loaded into a refrigerated road container. The location of pallets with I-button temperature logger are highlighted and are located in the front (pallet numbers 3 and 4), middle (pallet numbers 9 and 10) and rear (pallet numbers 15 and 16) of the container.

## Fruit dry matter content and weight loss

Upon arrival at the MRF laboratory, five fruit were randomly sampled to determine dry % matter (DM). The cheek flesh from each mango was removed and grated. The flesh was weighed, then placed into a dehydrator at about 65°C until a steady weight was reached. The DM % was calculated as: Dry Weight (g)/Fresh Weight (g) x 100. The physiological weight loss was expressed as the % loss from individual fruit based on the original fruit weight.

## Skin colour

Visual skin colour was rated using a scale of 1-6 (1: 0-10% yellow, 2: 10-30% yellow, 3: 30-50% yellow, 4: 50-70% yellow, 5: 70-90% yellow, 6: 90-100% yellow on the fruit skin) as per the Mango Quality Assessment Manual (Hofman et al., 2010).

The skin colour was also assessed using a Minolta Chroma Meter (Minolta Corp, Ramsey, NJ) calibrated to a white standard plate (L* 97.31, a* -0.18, b* 2.45), in the L*a*b* colour system as L* value (lightness), a* value (redness or greenness) and b* value (yellowness or blueness). Three locations that were free of blush, blemish and sunburn were marked on each fruit and measurements were taken on the same locations upon arrival, and removal and at ES.

## Fruit firmness

Individual fruit firmness was measured using an EZ Test Shimadzu, EZ-SX, 100 N (Kyoto, Japan). Firmness was assessed as the Newtons (N) force required to push a 12 mm spherical probe to a depth of

2 mm into the non-peeled fruit at a rate of 10 mm $min^{-1}$. The probe was applied to the cheek area located at the middle to lower stem end of each fruit. Subsequent tests at removal and at ES were done on different locations on the fruit.

In addition, individual fruit were rated for hand firmness using the scale of 0-4 (0 = hard, no 'give' in the fruit; 1 = rubbery, slight 'give' in the fruit; 2 = sprung, flesh deforms by 2-3 mm with extreme thumb pressure; 3 = firm soft, whole fruit deforms with moderate hand pressure; and 4 = soft, whole fruit deforms with slight hand pressure) (Hofman et al*.,* 2010).

### Total soluble solids and titratable acidity

At ES, the peel of each fruit was removed, and a portion of the flesh from each fruit used for total soluble solids (TSS) content using an ATAGO hand refractometer (PAL – 1). The remaining flesh was frozen and the titratable acidity (TA) of the thawed sample determined by titrating the puree to a pH endpoint of 8.2 using a Mettler Toledo G20 compact titrator with 0.1M sodium hydroxide (NaOH). Results are presented as the % citric acid equivalents.

## Experimental design

The trials were not replicated because it is not possible to control the required conditions of temperature, duration and atmosphere composition in commercial consignments. The fruit quality data were presented as the average across the 15 sampled trays per container.

## Fruit respiration

About 40 commercially picked and packed 'Honey Gold' fruit from Katherine were air-freighted to MRF within about 36 hours of harvest. Half the fruit were dipped in a 0.1% solution of Ethephon® (2-Chloroethylphosphonic acid) for 10 minutes as an ethylene treatment, and the remaining 20 fruit were not dipped. The fruit were then placed into individual 1 L sealed Tupperware containers, with a carefully regulated and recorded air flow of 40-50 mL $min^{-1}$ passing through each container. The outlet from each container was connected to a gas sampling system that channelled each outlet at predetermined intervals into a single outlet connected to a flow meter (Honeywell) and an infrared gas analyser (Horiba) for $CO_2$ analysis. The $CO_2$ concentration from each container was recorded about three times per day. Fruit firmness and skin colour was assessed daily as described above.

## Statistical analysis

Data analysis was performed using GenStat® (Version 16.1, VSN International Ltd). Two–way ANOVA and LSD comparison at 95 % level were conducted.

# Results and discussion

## Dry matter content

The % DM of fruit in containers 1-4 were 18.0, 19.1, 18.7 and 19.3 %, respectively. Fruit samples were not collected for container 5.

## Temperatures

Average fruit pulp temperatures over the 3-4 day road freight was 1-4°C above the container set temperature of 16°C (Table 1). However, there were significant variations in temperatures across the load and over time (Figure 2). In control container 1 (with no roof chute), fruit temperatures were higher toward the back of the container compared with the middle or front. However, in Ripestuff container 2 (with a roof chute; Plate 3), fruit temperatures closer to the front of the container were significantly warmer than those toward the back, which also increased during the journey. These results indicate varying

performance of refrigerated container. The purpose of the roof chute is to capture some of the cold delivery air from the refrigeration unit and channel the air toward the rear of the container. However, the results suggest insufficient cold air was received by pallets near the front of the container.

There was little to no consistent pattern in air temperatures within a row (tray location in Figure 3.).

More research is required on refrigerated container design to optimise consistency of temperature management throughout the load, although it is possible that ethylene may reduce the impact of temperature differences on fruit performance after arrival.

Table 1. Container number, presence of a roof chute in the container, Ripestuff for ethylene, and hydrated lime for carbon dioxide ($CO_2$) absorption, and the average pulp temperature and ethylene concentration (based on PID) monitored during transit of 'Honey Gold' mango from Katherine (Northern Territory) to Wamuran (South East Queensland) and $CO_2$ concentration in the container at arrival.. Control container 2 only recorded ethylene and $CO_2$ concentrations. It contained no Ripestuff, hydrated lime or in-tray temperature loggers.

| Container | Chute | Temp (°C) | Ripestuff™ | Hydrated lime | Ethylene (uL $L^{-1}$) | $CO_2$ at arrival (%) |
|---|---|---|---|---|---|---|
| Control 1 | No | 16.9 | No | No | 6.6 | 1 |
| Control 2 | N/A | N/A | No | No | 6.2 | 4.4 |
| Ripestuff 1 | Yes | 20 | Yes | Yes | 129.8 | >20 |
| Ripestuff 2 | Yes | 19.4 | Yes | Yes | 123.3 | 14.3 |
| Ripestuff 3 | Yes | 18.1 | Yes | Yes | N/A | 13.3 |

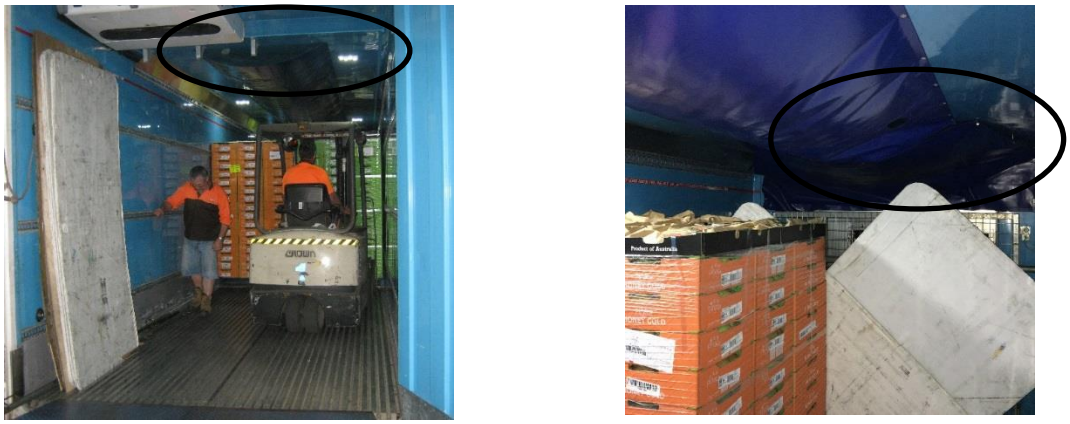

Plate 3. A roof shoot in one of the refrigerated containers, designed to channel the cold top delivery air to the middle and rear of the container. The chute completely covered the cold delivery air vent which likely restricted delivery of cold air to pallets near the front of the container.

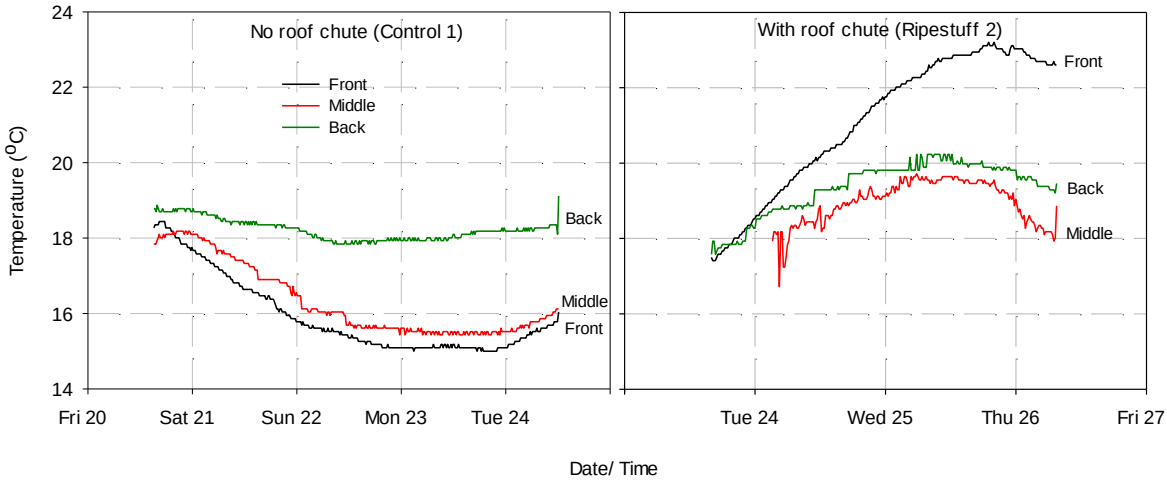


Figure 2. Pulp temperatures of 'Honey Gold' mango fruit in the middle of a pallet in the second row from the front, the middle, and the second row from the back of a commercial, 18 pallet refrigerated load during road-freight from Katherine (Northern Territory) to Wamuran (South East Queensland). Control container 1 had no roof chute while Ripestuff container 2 had a roof chute to channel air from the top delivery cold air vent toward the rear of the container.

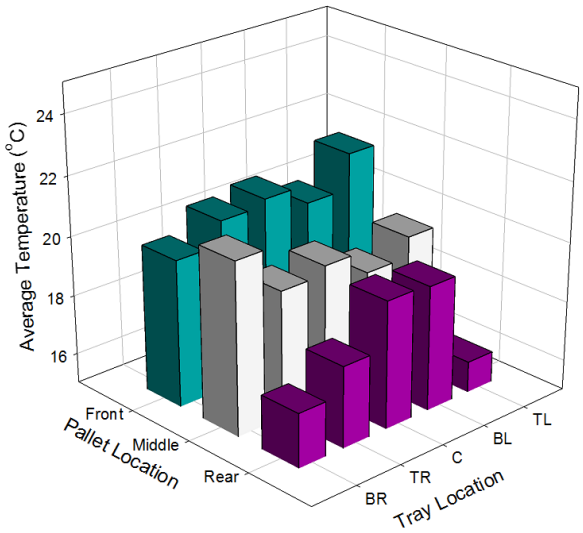


Figure 3. Air temperature during the 3-4 day road freight (Ripestuff container 2) journey of 'Honey Gold' mango fruit from Katherine (Northern Territory) to Wamuran (South East Queensland). Tray location is the location in the container row as illustrated in Plate 2, and pallet location is either in the 2nd row from the front (near the refrigeration unit), or in the middle or in the 2nd row from the back (near the doors; Figure 1). Abbreviations: T = top, B = back, C = central, L = left and R = right.

# Atmospheres

## Carbon dioxide and oxygen

Carbon dioxide concentrations in containers without Ripestuff (control containers 1 and 2) reached only 1-5% on arrival (Table 1 and Figure 4). In contrast, $CO_2$ concentrations in containers with Ripestuff increased significantly during transport, reaching 13-14% in Ripestuff containers 2-3 (Table 1Figure 4), and more than 20% in Ripestuff container 1 (Figure 4). The hydrated lime included in the Ripestuff-

treated containers did absorb $CO_2$ as its lime activity decreased from 60% to 20% (Figure 5). However, the starting lime activity of 60% was well below the recommended 90%, and the amount of lime per container was not enough to absorb the $CO_2$ produced by the fruit. In container 1, $O_2$ concentrations rapidly declined to about 2% within 40 hours.

The higher $CO_2$ concentrations with Ripestuff treatment were largely because ethylene enhances the ripening process and reduces the time between harvest and the start of the rapid increase in $CO_2$ production by the fruit (see below).

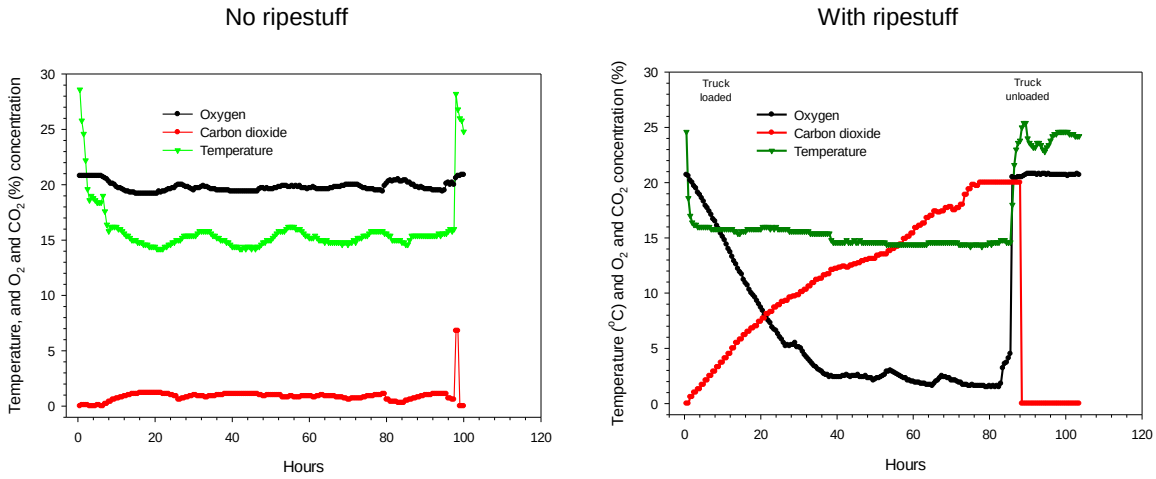


Figure 4. Top delivery air temperature, carbon dioxide and oxygen concentrations in a refrigerated container without Ripestuff (control container 1; left) and with Ripestuff (container 1) with 'Honey Gold' mango fruit during road freight from Katherine (Northern Territory) to Wamuran (South East Queensland). The maximum measuring range of the $CO_2$ sensor in container 1 was 20%. An additional $CO_2$ sensor in the same load registered 25% $CO_2$ on arrival.

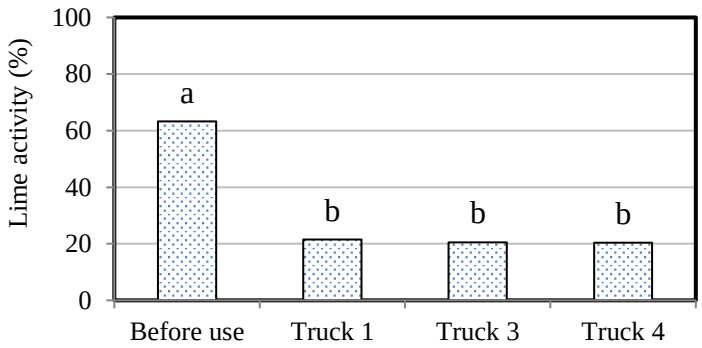


Figure 5. Lime activity before and after use in three refrigerated containers with 'Honey Gold' mango, and with Ripestuff. Means with same letter are not significantly different at the *P* =0.05 level (n=6).

## Ethylene

Two ethylene sensors were tested (PID and EC). In the absence of Ripestuff, the PID detector recorded about 5 µL $L^{-1}$ ethylene, and the electrochemical cell detected effectively nil (Figure 6). In the presence of Ripestuff™, the PID sensors detected about 170 µL $L^{-1}$ for Ripestuff 1 and 2 (Table 1), and the EC sensors, 35-50 µL $L^{-1}$. The differences in sensor responses is a concern, however it is likely that the

Ripestuff™ produced sufficient ethylene in the containers to trigger ripening. The relatively small difference between the two sensors in control containers 1 and 2 (without Ripestuff™) suggests only minor interference of the PID sensor by fruit or other volatiles, however it is possible that the fruit volatiles released as ripening progressed (which was more rapid in the presence of Ripestuff™) may have been a larger contributing factor to the higher PID response in these containers, compared to the EC sensor.

Further research is required to identify response differences between the sensors, and improved ethylene sensor technology evaluated as it becomes available (e.g. www.c2sense.com).

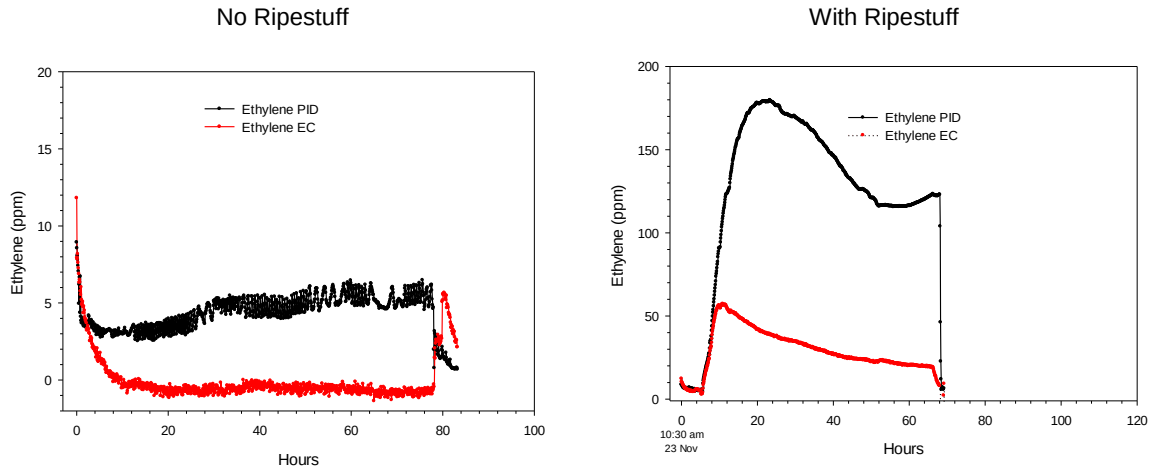


Figure 6. Ethylene concentrations (µL $L^{-1}$) recorded during refrigerated road freight of 'Honey Gold' mango fruit from Katherine (Northern Territory) to Wamuran (South East Queensland). A photo-ionisation detector (PID) and electrochemical (EC) sensor was used in both consignments. The container included either no Ripestuff (left; control container 2) or 2 kg Ripestuff (right; Ripestuff container 2) to release ethylene during transit.

# Fruit quality

The presence of Ripestuff, or high $CO_2$ concentrations in the container during transit did not affect the loss of green colour during ripening, with all treatments resulting in effectively full yellow colour at eating soft (Table 2). The presence of Ripestuff™ (containers 2 and 3) resulted in the fruit reaching ES three to four days earlier compared with no control container 1). The longer days to ES with Ripestuff container 1 was likely associated with the very high $CO_2$ and very low $O_2$ concentrations in this container. The results suggest that Ripestuff™ can reduce the ripening time required at market, as long as $CO_2$ concentrations in the container during transit are controlled.

The average firmness of fruit on arrival was influenced by the average air temperature around the fruit (Figure 7), but this did not affect the average days to reach ES once they had been removed from the container and all the trays ripened at the same temperature of 20°C. This may be partly because of the container-applied ethylene reducing the variation in days to ES between fruit and consignments.

Fruit from trucks with Ripestuff were much softer on arrival than those from control truck 1 (Table 3). Firmness of Ripestuff-treated fruit varied from truck to truck. Fruit from Ripstuff 1 were softer than those from Ripestuff 2 and 3 because of higher temperatures in truck 1. Fruit firmness declined significantly during post-transport. Similarly, Brix of Ripestuff-treated fruit at arrival was higher than that of non-treated fruit. There was effect of Ripestuff on Hue value of fruit colour on arrival, meaning that Ripestuff-treated fruit have lower green colour as compare control fruit, except for Ripestuff-treated fruit in Ripestuff 1 because high CO2 concentration was recorded this container, resulting in reduction of loss of green colour. At ripe, control fruit had lower hue angle compared with the Ripestuff fruit. This could be because

of the higher CO2 in the Ripestuff trailers. However, based on results of colour rating at eating ripe (Table 2). There was no commercially significant difference in appearance at ripe, but the hue angles indicate a risk of this if CO2 is not controlled.

Table 2. Fruit skin colour of 'Honey' mango at arrival and eating ripe, (1=green, 6=yellow), and the days to eating soft (ES).

| Container | Colour scale at arrival | Colour scale at ER | Days to ES* |
|---|---|---|---|
| Control 1 | 1.9 d | 6.0 ab | 11.9 b |
| Control 2 | N/A | N/A | N/A |
| Ripestuff 1 | 2.0 d | 6.0 a | 12.3 a |
| Ripestuff 2 | 1.6 e | 5.9 ab | 8.8 c |
| Ripestuff 3 | 2.4 c | 5.8 b | 8.1 d |

* Day counted from dispatch to eating ripe. Means with same letter in each column are not significantly different at the P = 0.05 level. (n= 75)

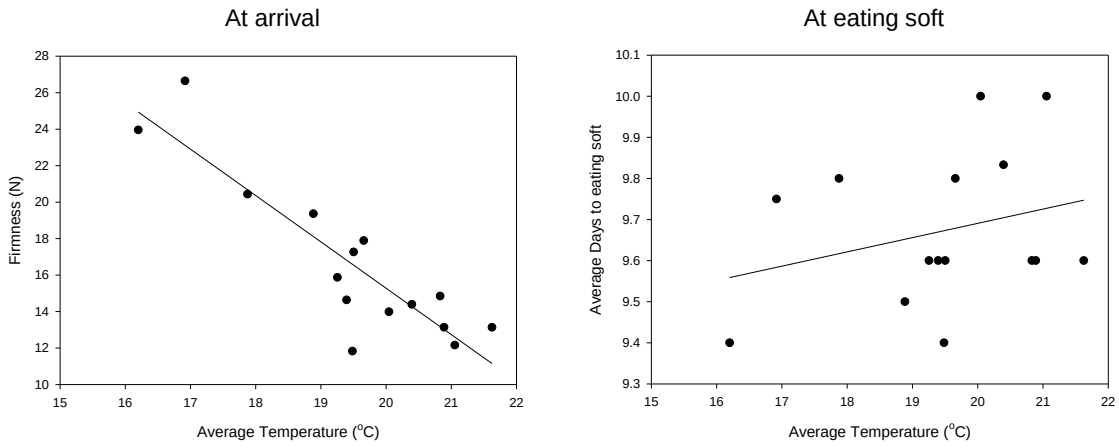


Figure 7. The relationship between average air temperature around 'Honey Gold' mango fruit during road freight from Katherine (Northern Territory) to Wamuran (South East Queensland), and fruit firmness on arrival at Wamuran (left) or the days to eating soft following removal from the container and holding at 20°C until ripe (right). The data are from Ripestuff 3. Each data point represents the results for each of the 15 trays per container.

**Table 3.** Effect of Ripestuff treatment on 'Honey Gold' mango fruit quality during the transport and post-transit.

| Treatment | Firmness (N) | TSS (Brix) | | Titratable Acid (%) | |
|---|---|---|---|---|---|
| | Arrival | Arrival | Eating ripe | Arrival | Eating ripe |
| Control Truck 1 | 35.7 a | 10.44 d | 15.89 a | 1.11 a | 0.64 d |
| Ripestuff Truck 1 | 10.73 c | 11.15 c | 15.25 b | 0.96 b | 0.58 e |
| Ripestuf Truck 2 | 16.64 b | 11.24 c | 15.81 a | 1.15 a | 0.78 c |
| Ripestuff Truck 3 | 17.73 b | 10.76 cd | 15.16 b | 0.93 b | 0.80 c |

N = Newton force; TSS = total soluble solids. Means with same letter in each column are not significantly different at the P = 0.05 level. (n= 75)

**Table 4**. Effect of RipeStuff treatment on 'Honey Gold' mango colour during the transport and post-transit.

| Treatment | Hue | | Chroma | | Lightness | |
|---|---|---|---|---|---|---|
| | Arrival | Eating ripe | Arrival | Eating ripe | Arrival | Eating ripe |
| Control Truck 1 | 110.75 a | 77.85 f | 47.89 c | 55.88 a | 63.36 d | 67.36 c |
| Ripestuff Truck 1 | 111.13 a | 80.08 de | 45.07 d | 57.60 a | 62.39 e | 67.95 c |
| Ripestuf Truck 2 | 108.87 b | 79.37 e | 45.01 d | 51.40 b | 67.18 c | 69.42 b |
| Ripestuff Truck 3 | 106.53 c | 81.01 d | 46.63 cd | 26.68 e | 67.71 c | 70.30 a |

Means with same letter in two columns of each attribute are not significantly different at the P = 0.05 level. (n= 75)

## Fruit respiration

'Honey Gold' fruit respiration rate (determined as $CO_2$ production rate) increased as the fruit ripened (Figure 8). Treatment with Ethephon resulted in more rapid increase in $CO_2$ production compared with no treatment, and also increased the rate of yellow skin colour development, and softening (Figure 9). These results explain the higher $CO_2$ concentrations in the refrigerated containers that included Ripestuff™ compared with no Ripestuff™ (Figure 4).

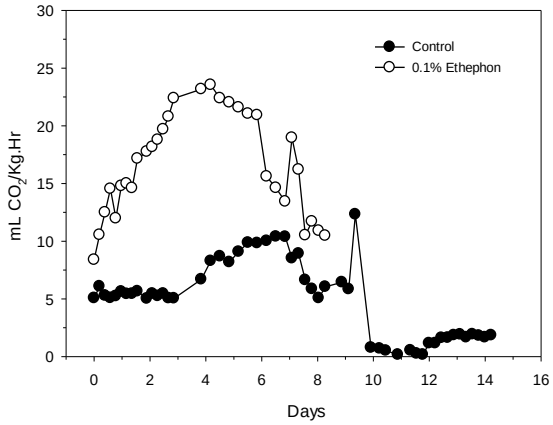


Figure 8. Rate of respiration (mL $CO_2$ $kg^{-1}$ $hr^{-1}$) of 'Honey Gold' mango, either treated with 0.1% of Ethephon, or not treated, then ripened at 18°C.

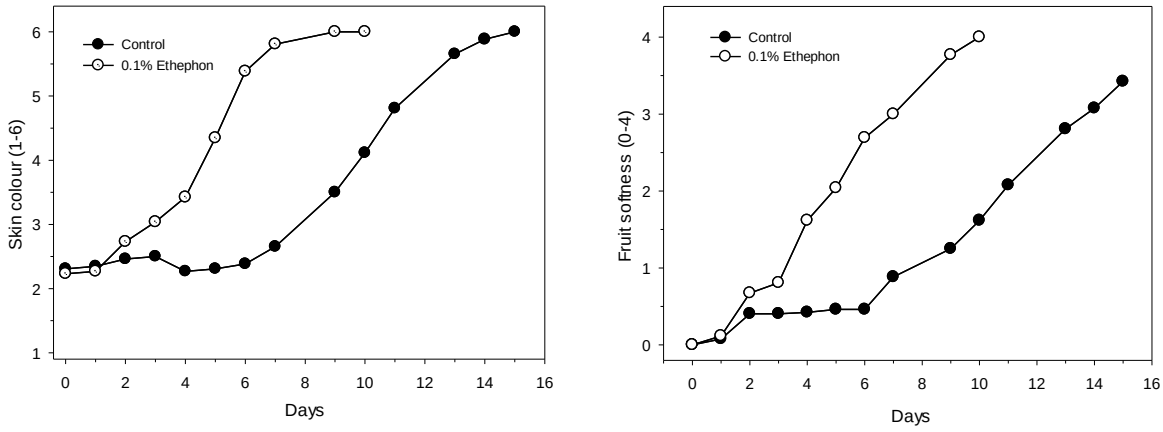


Figure 9. Change in skin colour (1= green, 6= yellow, n=20) and fruit softness (0= hard, 4= eating soft) of 'Honey Gold' mango fruit, either dipped in 0.1% Ethephon or not, then ripened at 18°C.

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# In transit ripening of ‘Honey Gold’ mango

## 2016/17 season results

## In-transit ripening and prediction of outturn quality for mango

HIA project MG12016

Hung van Duong, Andrew Macnish, Peter Hofman, Daryl Joyce

This publication has been compiled by Peter Hofman of Horticulture and Forestry Sciences, the Department of Agriculture and Fisheries.

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# Summary

Initiating the ripening of climacteric fruit during road freight from farm to market can save infrastructure and energy costs by reducing fruit cooling costs and the time required to ripen in market. The current study evaluates the efficacy of Ripestuff™ (ethylene encapsulated powder) to trigger the ripening of 'Honey Gold' mango fruit during road freight, and treatments to maintain carbon dioxide ($CO_2$) concentrations in the container to below 4%. The trials were conducted in six commercial, 20 pallet refrigerated road trailers, three with Ripestuff and three as non-treated controls. A total of about 2.4 kg Ripestuff was placed in 200 polypropylene specimen containers (70 mL capacity). The specimen containers were then placed in each refrigerated trailer containing 2560 mango trays to maintain ethylene concentrations above 10 µL $L^{-1}$ for 2-3 days. Approximately 190 kg hydrated lime (Sibelco) was packed in 128 check-out paper bags (1.5 kg each) and placed inside the treatment containers to maintain $CO_2$ concentrations below those that are injurious to fruit and industry workers. The control and treated containers were set at 16°C, with average fruit temperatures of 18.7 °C. Ethylene, $CO_2$ and oxygen ($O_2$) concentrations, and air and pulp temperatures were monitored during the shipments. Samples of fruit from the control trailers were taken upon arrival at the ripener and were treated with ethylene as per commercial practice.

The results indicated that ethylene concentrations increased above 170 µL $L^{-1}$, and were maintained above 10 µL $L^{-1}$ for about two days. Average fruit temperatures for the whole journey were 18.7 °C, but were usually 4-5 °C higher in the front and middle of the trailers compared with fruit near the back. $CO_2$ concentrations were maintained below 5% using hydrated lime, with average $CO_2$ concentrations during the journey of just above 2%. However, $O_2$ concentrations declined to about 2% in several of the treated containers. The results from post-shipment assessment showed that the ripening time of Ripestuff-treated mango fruit was 3 days less than control fruit. Ripestuff-treated fruit had higher total soluble solids (TSS) content and titratable acidity (TA) than control fruit. Ripestuff did not affect skin colour upon arrival, but increased the % of the fruit skin with attractive yellow colour at eating ripe. There was no difference in ripening time and fruit quality between Ripestuff-treated fruit and post shipment ethylene-treated fruit. This was likely because of the low $O_2$ concentrations in the treated containers, the short, two day transit period, and not using lower fruit temperatures for the control fruit.

In summary, the Ripestuff and hydrated lime systems proved effective. Further testing and development in the following areas are recommended:

- Optimising ethylene management using less Ripestuff per container, and with slower release systems to provide concentrations between 10-100 µL $L^{-1}$ over 2-3 days.
- Reducing labour costs of the hydrated lime system.
- In conjunction with transport companies, identify procedures and practices to maintain more consistent fruit temperatures across the load.

# Introduction

Mango fruit production in Australia is predominantly located in northern tropical and sub-tropical regions. 'Kensington Pride', Calypso™, 'R2E2', and 'Honey Gold' are popular cultivars. About half of all mango production in Australia (ca. $70 million) originates from orchards in the Northern Territory (AMIA). The fruit are typically harvested at a mature hard-green stage and pre-cooled to 13°C to maximise storage life. Thereafter, they are transported 3,000-4,000 km in refrigerated (e.g. 12-14°C) containers by road and/or rail from rural production districts in northern Australia to ripening facilities and retailers in metropolitan areas (1999). Survey work by Hofman et al (2014) showed that Calypso™ mango fruit from northern Australia could spend 15-20 days in the distribution system. Given the rise of the 'buy local' and 'farm fresh' consumer movements, the fresh produce industry in Australia is looking at strategies to reduce the time that fruit remain in the system.

Triggering ripening with ethylene applied during transport at elevated temperature (e.g. 18°C) could reduce both on-farm and in-market infrastructure and energy costs. Previous work by Hofman et al. (2014) evaluated the potential to treat Calypso™ fruit with ethylene during refrigerated road and rail transport from Katherine

in the Northern Territory to ripening centres in Brisbane. Ethylene was applied using Ripestuff™, an ethylene encapsulated powder (Ho et al., 2013) that rapidly releases ethylene on exposure to moisture. The results indicated that ethylene concentrations can be maintained above 10 µL $L^{-1}$ inside containers for 2-3 days, but more development and testing was required with Ripstuff quantities and slow release systems. Follow-on research during the 2015 mango season confirmed that the application of Ripestuff™ in truck containers of 'Honey Gold' mango fruit could generate and maintain biologically-active ethylene concentrations during a three day transit from Katherine to south east Queensland (Mott et al., 2016). Fruit generally ripened quicker in response to in-transit ethylene treatment by 2-3 days relative to non-treated fruit. However, an accumulation of > 20% $CO_2$ and variation in temperatures at different positions inside trailers reduced the consistency of the ripening response.

The purpose of the current study was to:

- Confirm that exposure of 'Honey Gold' mango fruit to ethylene during interstate road transport would initiate ripening and thereby reduce the time that fruit remain in the distribution system than conventional practice.
- Refine the recommendations for Ripestuff amount per container, and slow release systems.
- Test improved hydrated lime recommendations to manage CO2 concentrations.

# Materials and Methods

## Plant material

'Honey Gold' mango (*Mangifera indica* L.) fruit were harvested from orchards near Katherine (14°27'S, 132°16'E) and Mataranka (14°55'S, 133°4'E) in the Northern Territory during November and December 2016. A trained harvest crew selected and picked fruit at commercial maturity (e.g. ≥ 1500 heat units, fullness of fruit shoulders, light yellow flesh, ≥ 15% dry matter content (Winston et al., 2014)) during 22:00 – 06:00 hours. The fruit were de-stemmed in a proprietary mango wash solution (2.5 g $L^{-1}$ mango desapping powder; Harvey Distributors, Winnellie, NT, Australia) on a motorised harvest aid to prevent sapburn injury. De-sapped fruit were placed into field bins and transferred to a nearby shed. The bins were then transported by truck at ca. 25-35°C from the orchard to a pack house at 7 Field in Katherine within 6-10 hours of harvest. At the packing shed, the fruit were treated with combined insecticide (Dimethoate at 1 mL $L^{-1}$, Nufarm Ltd.,), fungicide (Sportak at 0.5 mL $L^{-1}$, Bayer CropScience Pty Ltd.,) and an acidifying and penetration surfactant (Spraybuff 700 at 0.5 mL $L^{-1}$, Agricrop). Fruit were then graded for uniform size and quality and packed into single layer trays with bubble wrap inserts.

## Chemicals

### *Ethylene*

Encapsulated ethylene gas in the form of an inclusion complex with α-cyclodextrin (designated as Ripestuff™) was prepared in a laboratory in Brisbane, Queensland according to the methods of Ho et al. (2011). A sub-sample of the Ripestuff™ batch was placed into a sealed glass container and dissolved in water to release ethylene. The concentration of ethylene released was quantified by gas chromatography. 12 g samples of Ripestuff™ was added to individual 70 mL polypropylene specimen containers (Techno Plas Pty Ltd, St Marys, SA, Australia). Polyethylene screw-on lids was immediately secured onto the containers. Each lid had four 0.5 mm-diameter perforations made by hand using a 0.5 mm-diameter x 25 mm-long 18 gauge hypodermic needle (Terumo Corp., Somerset, NJ, USA). Twenty sealed containers wereplaced in each of 10 aluminium foil trays. Each tray was placed inside an aluminium foil bag and vacuum-sealed. The sealed bags were transported by truck at ca. 20-40°C to the packing shed in Katherine in 4-5 days and held for 4 weeks before use. A total of 2.4 kg of Ripestuff was used for each truck. The weight of Ripestuff required to maintain concentrations above about 40 µL $L^{-1}$ was calculated using the free air space in the refrigerated container (**Table 1**), the expected leakage rate, and the volume of ethylene per kg of Ripestuff.

**Table 1**. Parameters used to calculate free air space inside the refrigerated container

| | | | |
|---|---|---|---|
| 40-ft Container ($m^3$) | | | 80.9 |
| Fruit | Number of fruit | 35840 | |
| | Fruit volume ($m^3$) | 0.0005 | 17.9 |
| Tray (430 x 360 x130 mm) | Number of trays | 2560 | |
| | Tray volume ($m^3$) | 0.0028 | 7.2 |
| Pallet (1200 x1200 mm) | Number of pallets | 20 | |
| | Pallet volume ($m^3$) | 0.065 | 1.3 |
| Air space ($m^3$) | | | 54.5 |

### *Calcium hydroxide*

Fresh calcium hydroxide or hydrated lime with a density of 740 kg $m^{-3}$ was purchased from an industrial processing facility (Sibelco, Gympie, QLD, Australia). Equal quantities of lime (1.5kg) were placed into 540 mm long × 355 mm wide × 16.5 mm deep Detpak brown paper checkout bags (Concord Paper Bags, Kingsway West, NSW, Australia) to a depth of 1.5 cm to maximise $CO_2$ absorption (Duong et al., 2016b). The bag opening was stapled closed. The bags of lime were wrapped in plastic film and transported to the packing shed in Katherine as described above. A sub-sample of the lime was tested for its capacity to absorb $CO_2$. Briefly, a 38.5% (w/w) neutralised sucrose (AR grade; Chem-Supply Pty Ltd, Gillman, SA, Australia) solution was prepared in distilled water. Ten drops of phenolphthalein (1% in methylated spirits; Ajax Finechem, Taren Point, NSW, Australia) and 15 drops of 0.1 M sodium hydroxide (AR grade; Ajax Finechem, Seven Hills, NSW, Australia) was swirled into the sucrose solution. A sample of 3.7 g of lime was mixed into 20 mL of distilled water and added to 50 mL of the sucrose solution. The solution was stirred every 5 minutes for 25 minutes. Finally, four drops of phenolphthalein was added to the solution before titration. The samples were titrated with 1 M hydrochloric acid (AR grade; Chem-Supply Pty Ltd, Gillman, SA, Australia) until the solution was non-coloured. One mL of acid solution used is equivalent to 1% of lime activity.

## Fruit sample preparation

Eighteen packed trays of count 14 'Honey Gold' fruit, prepared as described in section 2.1, were sampled at random from the end of the packing line. A HOBO temperature logger (U12-012; Onset Computers Corp., Bourne, MA, USA) were inserted into six randomly selected sample trays. The dataloggers were fitted with an internal temperature sensor with an accuracy of ± 0.3°C, and an external needle style temperature probe to record fruit pulp temperature. The air temperature loggers were calibrated with a certified thermometer (Model 376 data logger, Centre Technology Corp., Taiwan)w while pulp temperature loggers was calibrated by placing each needle probe into an ice water bath before shipment. The sample trays were maintained in a coldroom set at 16°C pending insertion of the trays into pallets. An additional 10 fruit were sampled for determination of dry matter content (see below).

## Pallet assembly

Three pallets in each consignment contained sample fruit and the temperature loggers. The sample trays for each consignment were removed from the packing line at the same time to reduce variability ensure they

were harvested at the same time and from the same farm and block. Eight packed trays were assembled as a single layer on a 1.2 × 1.2 m wood pallet base. A total of 16 layers of eight trays each was stacked onto each pallet. Sample trays were built into six randomly selected pallets during their assembly. For the non-treated (control), four sample trays were inserted in each of the three sample pallets per consignment at layers 8 -11 in one of the eight stacked columns of trays while two sample trays were inserted in each of three remaining pallets per Ripestuff-treated consignment at layers 8 and 9 (Figure 1). The trays containing the temperature loggers were positioned at layer 9.

The test pallets were labelled then forced-air cooled for 12 hours to a pulp temperature of about 16 °C. The fruit were held in a 16 °C room for 1-3 days until dispatch.

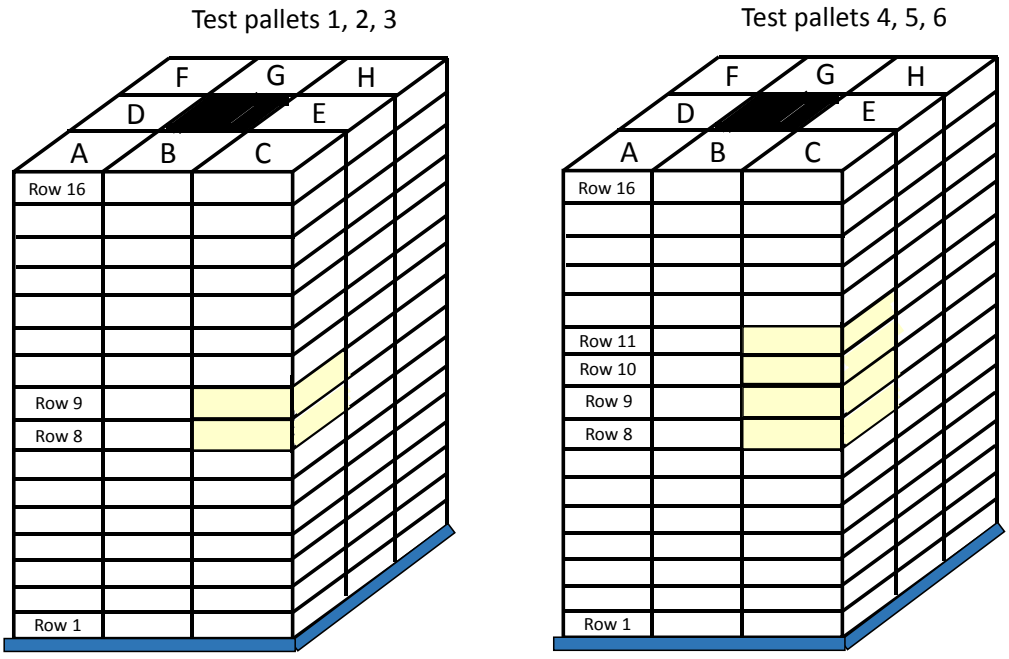


Figure 1**.** Side view of mango pallets consisting of 16 layers of eight (A, B, C, D, E, F, G, H) trays each. Sample fruit were placed in rows 8 and 9 of column C in sample pallets for the treated containers, and in rows 8-11 of column C for sample pallets for the control containers (no Ripestuff™).

# In-transit treatments

## *Ethylene gassing*

Ten aluminium foil bags that each held 20 containers of Ripestuff™ were included in each treated refrigerated trailer. This quantity of Ripestuff™ was estimated to generate 20-80 µL $L^{-1}$ ethylene inside the trailer with fruit. Two foil bags of Ripestuff™ were packed into individual empty cardboard mango trays. Five such trays were placed at random on the top of two pallets at the rear of the trailer. The sealed foil bags were opened once the last row of pallets were loaded into the trailer and within 30 seconds of closing the trailer doors. An additional foil bag holding containers of Ripestuff™ was air-freighted to Brisbane to re-confirm the ethylene concentrations within the Ripestuff™.

## *$CO_2$ removal*

One hundred and twenty eight paper bags containing fresh Sibelco hydrated lime (1.5kg/bag) were included in each treatment trailer containing Ripestuff™. Preliminary laboratory experiments (Duong et al., 2016b) suggested that this quantity and surface area of lime would maintain $CO_2$ concentrations < 3-5% inside the closed trailers. Briefly, the plastic film was removed from the outside of each hydrated lime bag. Individual

bags were placed into single empty cardboard mango trays. Eight trays were placed on top of each of 16 pallets in the trailer.

## *Trailer loading and transport*

For each trailer, three test pallets each containing four (for control) or two (for treated trailers) sample trays were consolidated with an additional 17 pallets of pre-cooled fruit. The pallets were configured for loading into a 13.7 m long × 2.2 m wide × 3.2 m high solid wall refrigerated truck trailer equipped with air-bag suspension. The sample pallets were loaded on the left side of the trailer and positioned at rows 2 (from the front), 5 (middle) and 9 (two from the rear) (Figure 2). The other three test pallets containing four sample trays each were loaded into a second trailer at identical positions on the same day. When the two trailers could not be dispatched on the same day, fruit for each trailer were sourced from separate harvests (Table 2). The refrigerated trailers set at 16°C before loading. Two ethylene dataloggers (ADL-51-1189-E-X101; MSR Electronics GmbH, Germany) and $CO_2$ and $O_2$ (Mitsubishi) were placed on top of a pallet at row 10 (the rear) of each trailer. A 3 m length of 3mm ID plastic tubing was secured to the last pallet and the end fed through the rubber lining around the doors to the outside. The tubing allowed gas sampling for ethylene, $CO_2$ and $O_2$ measurement when the trailers arrived at Wamuran.

**Table 2**. The date and times of harvest, packing, dispatch from Katherine and arrival at Wamuran for control and in-transit treated trailers with 'Honey Gold' mango.

| Container | Date/Period | | | |
|---|---|---|---|---|
| | **Harvesting** | **Packing** | **Dispatch** | **Arrival** |
| Control 1 | 21st and 22nd night 11/16 | 12 am 22/11/16 | 6 pm 24/11/16 | 10 am 27/11/16 |
| Treatment 1 | 21st and 22nd night 11/16 | 12 am 22/11/16 | 6 pm 24/11/16 | 10 am 27/11/16 |
| Control 2 | 23rd and 24th night 11/16 | 10 am 25/11/16 | 9 pm 25/11/16 | 3 pm 28/11/16 |
| Treatment 2 | 26th and 27th night 11/16 | 9 am 27/11/16 | 8 pm 28/11/16 | 9:30 am 01/01/17 |
| Control 3 | 28th and 29nd night 11/16 | 4 pm 29/11/16 | 6 pm 01/12/17 | 9:30 am 04/01/17 |
| Treatment 3 | 28th and 29nd night 11/16 | 4 pm 29/11/16 | 6 pm 01/12/17 | 9:30 am 04/01/17 |

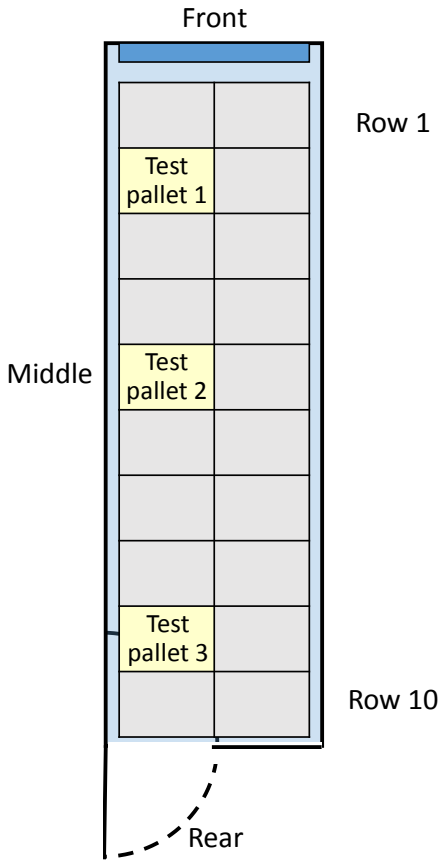


Figure 2. Top view of a mango trailer consisting of 20 pallets as used in the test consignments. Fruit were sampled from pallets at rows 2 (two rows from front), 5 (middle) and 9 (two rows from rear) on the left side of the trailer. This

The trailers were equipped with double refrigeration and roof chute on the top to deliver the cold air from the refrigerated unit and channel air toward the rear of the trailer. The fruit were transported by road from the packing shed in Katherine to a ripening and packing facility near Wamuran, Queensland (27°2'S, 152°52'E) in 2-3 days,

## Post-shipment handling and treatment

Upon arrival at Wamuran, ethylene and $CO_2$ concentrations inside each trailer was determined using Kitagawa precision gas detection tubes (Komyo Rikagaku Kogyo K.K., Kanagawa, Japan). Briefly, a hand-held pump was used to withdraw the trailer air via the plastic tubing through the doors and into detection tubes 108B (0.1-100 µL $L^{-1}$ ethylene) and 126SH (1-20% $CO_2$) according to manufacturer instructions. Ethylene and $CO_2$ measurements was made in duplicate. The sample pallets were then removed and the sample trays transported in and air-conditioned carto the Maroochy Research Facility near Nambour, a distance of approximately 70 km.

Upon arrival at the laboratory, fruit in the sample trays were labelled. Two trays from each control pallet were maintained in a coldroom at 20°C and ca. 80% relative humidity until ripe. The remaining two sample trays per control pallet were transferred to a ripening room for treatment with 10 µL $L^{-1}$ ethylene (provided as Ripegas™) at 20°C for 3 days using an EC12 control system (Pacific Data Systems Ltd.,) connected to a ripening gas transmitter (PDS-EX-TX-RG1000) to mimic commercial ripening procedures. Thereafter, the fruit were maintained at 20°C until ripe as described above.

# Assessments

## *Fruit dry matter content*

The dry matter content of fruit was determined at a laboratory at the Katherine Research Station. Briefly, the skin on opposite cheeks of each fruit was removed with a peeler. Flesh samples were grated, weighed and then maintained in an oven at about 65°C until a steady weight was reached. The dry matter content was expressed as the proportion (%) of dry weight to fresh weight.

## *Skin colour*

Skin colour (% of the skin with red, yellow and green) was subjectively assessed at arrival at the laboratory and when individual fruit were ripe. Skin colour was also determined using a colorimeter (Model CR-400; Konica Minolta, Inc., Osaka, Japan) at arrival at the laboratory and again when ripe. The colorimeter was linked to CR-400 utility software via a computer. The instrument was calibrated to a white standard plate (X=91.34, Y=93.24 and Z=106.2). Colour as the X, Y, Z value was measured at three locations on each fruit that are free of blush, blemish and sunburn. The colour data was then converted to lightness (L*), chroma (C) and hue ($H^o$) using the following equations:

$L^* = 116(Y/Y_n)^{1/3} – 16$; $a^* = 500\ ((X/X_n)^{1/3} - (Y/Y_n)^{1/3})$; $b^* = 200\ ((Y/Y_n)^{1/3} - (X/Z_n)^{1/3})$ where $X_n$, $Y_n$ and $X_n$ is calibration standard value.

$C^* = (a^{*2} + b^{*2})^{1/2}$

$H^o = \arctan(b^*/a^*)$.

## *Fruit firmness*

Individual fruit firmness was measured using an EZ Test (EZ-SX; Shimadzu Corporation, Kyoto, Japan) arrival at the laboratory and as they reached ripe. Firmness was determined on non-peeled tissue on opposite cheeks at the equatorial region of each fruit. The firmness as Newtons (N) was determined as the force required to push a 12 mm-diameter spherical probe 2 mm into the fruit surface at a speed of 10 mm $min^{-1}$.

## *Days to ripen*

The fruit was monitored daily to check when they reached eating ripe (around 4 N) for final assessment. The time (in days) from arrival at the laboratory to reaching eating soft was recorded.

## *Total soluble solids and titratable acidity*

When individual fruit reached the eating ripe stage (4 N), the skin was removed with a knife. The fruit flesh from opposite cheeks was removed and placed into plastic sandwich Snap Seal bags (Coles Supermarkets Australia Pty Ltd; Hawthorn East, VIC, Australia). The bags were sealed and the samples placed in a freezer operating at -20°C pending analysis. The frozen samples were thawed at room temperature (e.g. 22°C). The juice from each sample was extracted by hand and filtered through two layers of cheesecloth to remove fibres. The total soluble solids (TSS) of juice samples was determined using a hand-held digital refractometer (PAL-1; Atago Co., Ltd, Tokyo, Japan). The titratable acidity (TA) of the juice was determined by titrating a sample with 0.1 N sodium hydroxide to a pH endpoint of 8.2 using a compact titrator (G20; Mettler-Toledo AG, Greifensee, Switzerland). TA data were expressed as the % citric acid equivalents.

# Statistical analysis

Data analysis was performed using GenStat® (Version 16.1, VSN International Ltd). Two–way ANOVA and LSD comparison at 95 % level were conducted.

# Results and discussion

## Dry matter content

The % dry matter of fruit in treatment 1 and control 1 was 19.6%.

## Ethylene and CO2 measurement on arrival

No ethylene was detected in the control trailers upon arrival at Wamuran (**Table 3**). This could be because the leakage rate was greater than the ethylene production rate by the fruit, or the detection limit of the Kitagawa tubes was not sufficient to detect the ethylene in the container. Ethylene measured in treatment trailer 2 and 3 was 50 µL $L^{-1}$ and 5 µL $L^{-1}$ in treatment container 1.

**Table 3**. Carbon dioxide and ethylene concentrations in control and in-transit treated trailers containing 20 pallets of 'Honey Gold' mango following 2-3 days road freight from the Northern Territory to Southeast Queensland. Gas concentrations were determined using Kitagawa tubes.

| Container | $CO_2$ (%) | Ethylene (µL $L^{-1}$) |
|---|---|---|
| Control trailer 1 | 5.1 | 0 |
| Control trailer 2 | 5.9 | 0 |
| Control trailer 3 | 7.5 | 0 |
| Treatment trailer 1 | 3.5 | 5 |
| Treatment trailer 2 | 2.8 | 50 |
| Treatment trailer 3 | 3.3 | 50 |

## Fruit temperatures

Air and pulp temperatures of 'Honey Gold' mango fruit from packing to arrival at Wamuran are shown in Figures 3-8. In most cases, the fruit were cooled to 16 $^{o}$C at the packhouse before dispatch. Temperatures in all trailers increased gradually during the journey. Fruit temperatures on arrival was 4-6 $^{o}$C above the trailer set temperature of 16 $^{o}$C. There were significant variations in temperatures across the load. Fruit temperatures near the front and middle were always 4-5 $^{o}$C higher than that in the back.

With control trailer 2, fruit were not sufficiently pre-cooled before loading. Sample fruit in the front and middle of the container were loaded at about 20°C and fruit temperatures increased to about 24°C during transport. In contrast, sample fruit near the rear of the container were loaded at about 28°C, but fruit temperatures decreased to about 18°C during transport.

The temperature gradients from front to rear of the trailer is likely because the roof chutes that completely covered the top cold delivery vent channelled excess air to the rear of the container, which resulted in insufficient cold air at the front and middle to remove the heat generated by fruit respiration. More research is required on refrigerated trailer air flow systems to improve consistency of temperature management throughout the load.

Air and fruit pulp temperature were similar in all trailers (Figures 3-8). Average fruit pulp temperatures over the 2-3 day road journey are presented in Table 4. Mean pulp temperatures for control trailer 2 was 2-4 $^{o}$C warmer than that of the other trailers.

## Ethylene release by Ripestuff

Recorded ethylene concentrations in the three treatment trailers are illustrated in

Figure 9. In general, ethylene in all three treatment trailers exceeded the 170 µL $L^{-1}$ limit of the loggers within 6 h of trailer closing. Concentrations declined to 33.6, 29.2 and 66.1 µL $L^{-1}$ at arrival time for treatment trailers 1, 2 and trailer 3, respectively. These results indicate that Ripestuff likely exceeded the desired ethylene concentrations in the trailers. However, the final ethylene concentrations recorded by the loggers were different to those measured with the Kitagawa tubes (**Table 3**). These apparent differences are not surprising, since there are no cost-effective systems for accurately measuring ethylene concentrations during transit. Ongoing evaluation of new ethylene monitoring technology is required to improve confidence in ethylene monitoring under these conditions.

**Table 4**. Average pulp temperature, oxygen and carbon dioxide concentration in control and in-transit treated trailers containing 20 pallets of 'Honey Gold' mango following 2-3 days road freight from the Northern Territory to Southeast Queensland.

| Container | Pulp temperature ($^{o}$C) | $O_2$ (%) | $CO_2$ (%) |
|---|---|---|---|
| Control trailer 1 | 18.4 | 17.0 | 6.3 |
| Control trailer 2 | 21.5 | 16.6 | 4.7 |
| Control trailer 3 | 17.0 | 12.8 | 8.6 |
| Treatment trailer 1 | 19.5 | 10.7 | 2.4 |
| Treatment trailer 2 | 18.0 | 10.1 | 2.1 |
| Treatment trailer 3 | 17.9 | 9.9 | 2.4 |

Data is averaged cross all positions in each trailer

## Oxygen and carbon dioxide

There was a fluctuation in $O_2$ and $CO_2$ inside control trailer 1 and 2 during the journey. Oxygen concentrations generally decreased to 8-13% during the journey (Figure 10). In contrast, $CO_2$ concentrations increased to 9-14%. The temporary increase in O2, and decrease in CO2 in control trailers 1 and 3 is likely because the trailer doors were opened for a short period.

The average $O_2$ concentration in control trailer 3 was much lower than those in control trailers 1 and 2, while $CO_2$ concentration in control trailer 3 was higher than those in other control trailers (**Table 4**). This suggests either higher respiration rates from fruit in control trailer 3, or lower gas leakage from the trailer. A higher respiration rate in trailer 3 fruit may be related to the later harvest and more mature fruit.

Similar patterns in CO2 and O2 concentrations were observed in the treatment trailers, but to varying degrees. Oxygen concentrations decreased to about 2% in all treatment trailers. This was significantly lower than concentrations recorded in the control trailers, which likely reflects high respiration rates due to the ethylene treatment.

The CO2 concentrations were generally maintained below 5% by the hydrated lime (Figure 11). Lime activity decreased from 92.5% to 23% with treatment trailer 1 and 3, but declined to only 54% with treatment trailer 2 (Figure 12). This variation could be because the fruit in treatment trailers 1 and 3 were

held at the packinghouse for 3 days before transport, and may have started ripening more quickly during transit, with higher $CO_2$ production, than the fruit in treatment trailer 2.

The increase in $CO_2$ concentration toward the end of the trailer 3 journey was likely due to CO2 production significantly exceeding the absorption capacity of the hydrated lime. This may suggest that the higher respiration rate of the more mature fruit in treatment trailer 3 resulted in more rapid "exhaustion" of the hydrated lime (as indicated by the low lime activity after the journey; **Table 2**), compared with treatment trailer 1 containing earlier-harvested fruit.

Oxygen and $CO_2$ concentrations were similar near the front, middle and back of treatment trailers 2 and 3, (Figure 11), suggesting sufficient air circulation to also provide even ethylene concentrations throughout the load.

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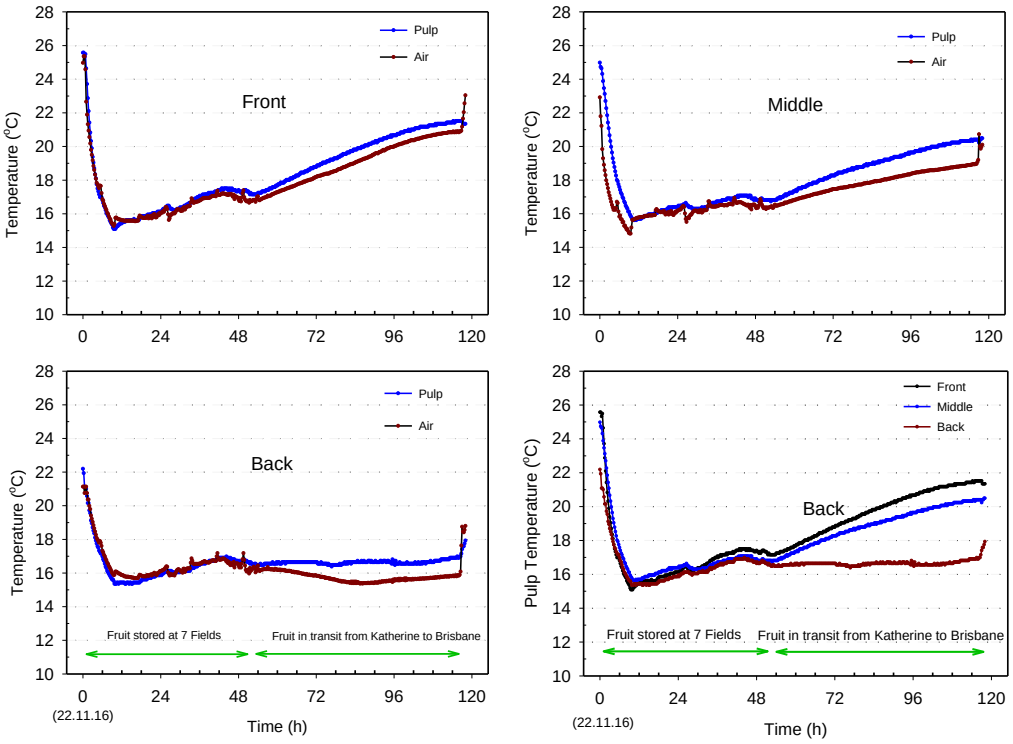


Figure 3. Air and pulp temperature during storage at 7 Fields and road freight journey of 'Honey Gold' mango fruit in control trailer 1.

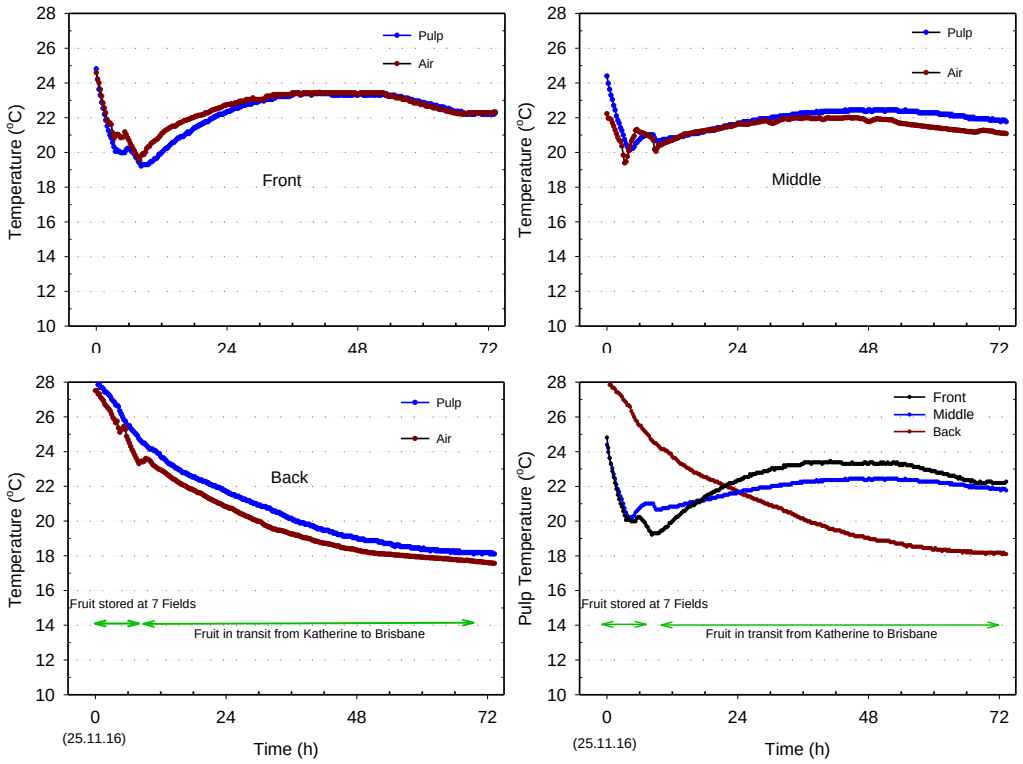


Figure 4. Air and pulp temperature during storage at 7 Fields and road freight journey of 'Honey Gold' mango fruit in control trailer 2.

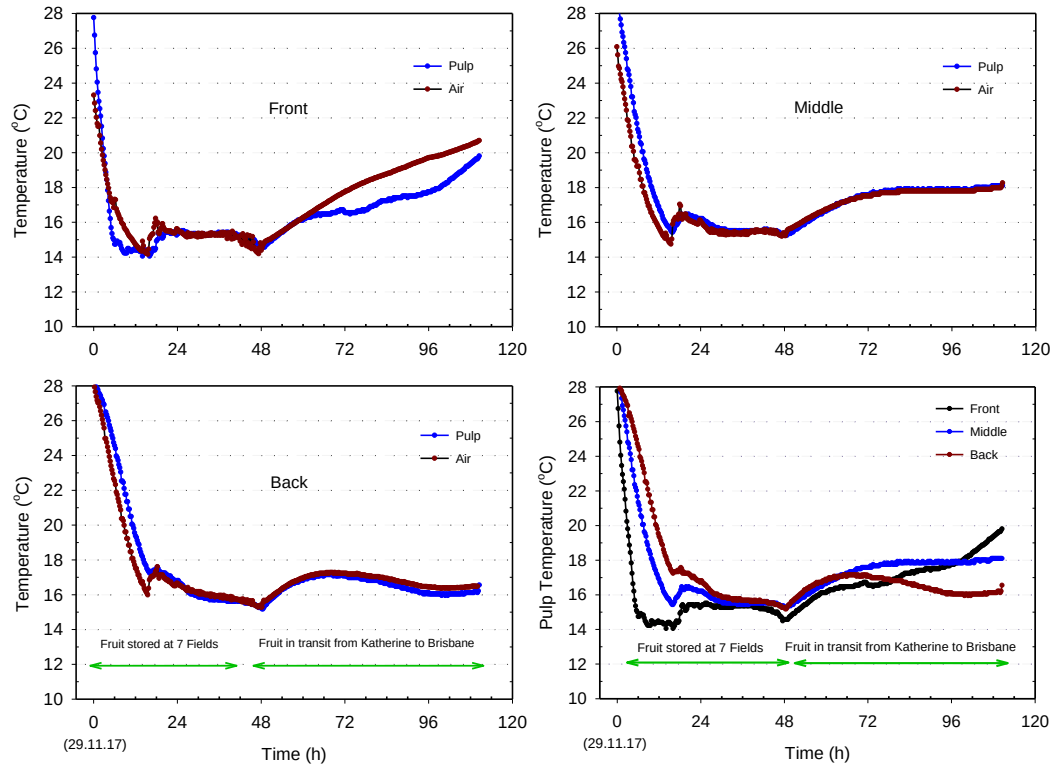


Figure 5. Air and pulp temperature during storage at 7 Fields and road freight journey of 'Honey Gold' mango fruit in control trailer 3.

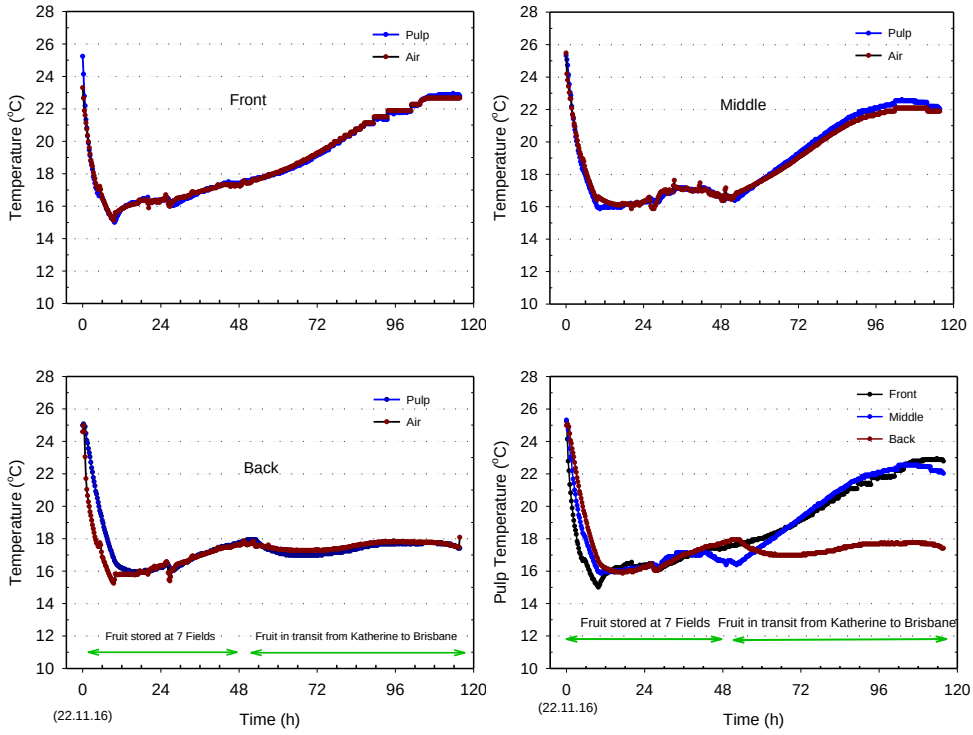


Figure 6. Air and pulp temperature during storage at 7 Fields and road freight journey of 'Honey Gold' mango fruit in treatment trailer 1.

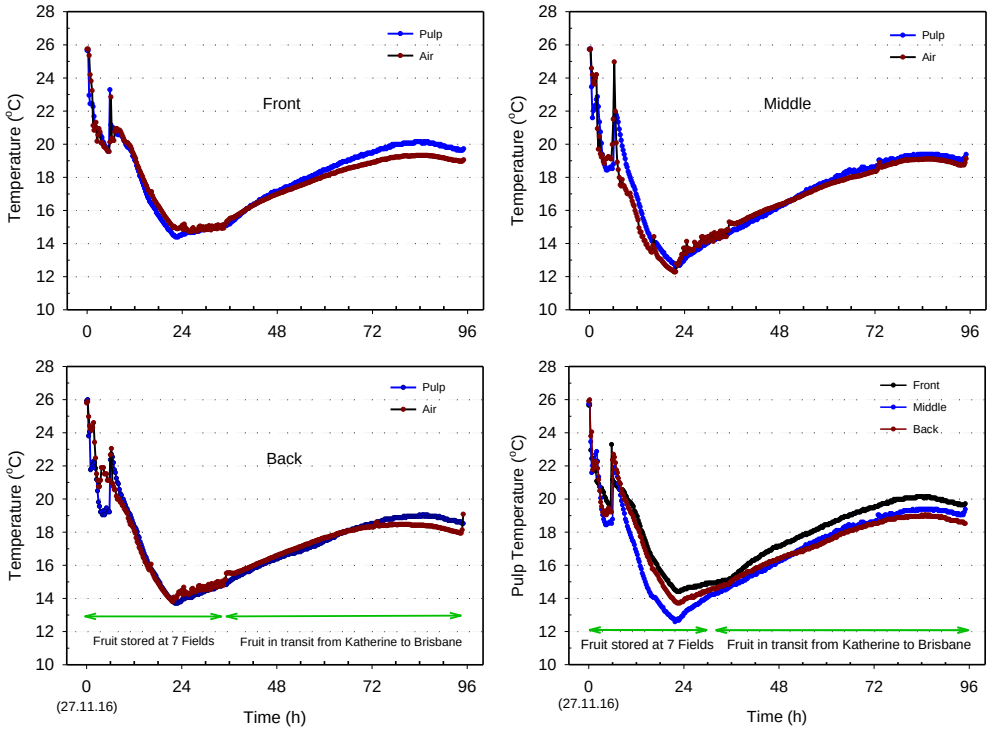


Figure 7. Air and pulp temperature during storage at 7 Fields and road freight journey of 'Honey Gold' mango fruit in treatment trailer 2.

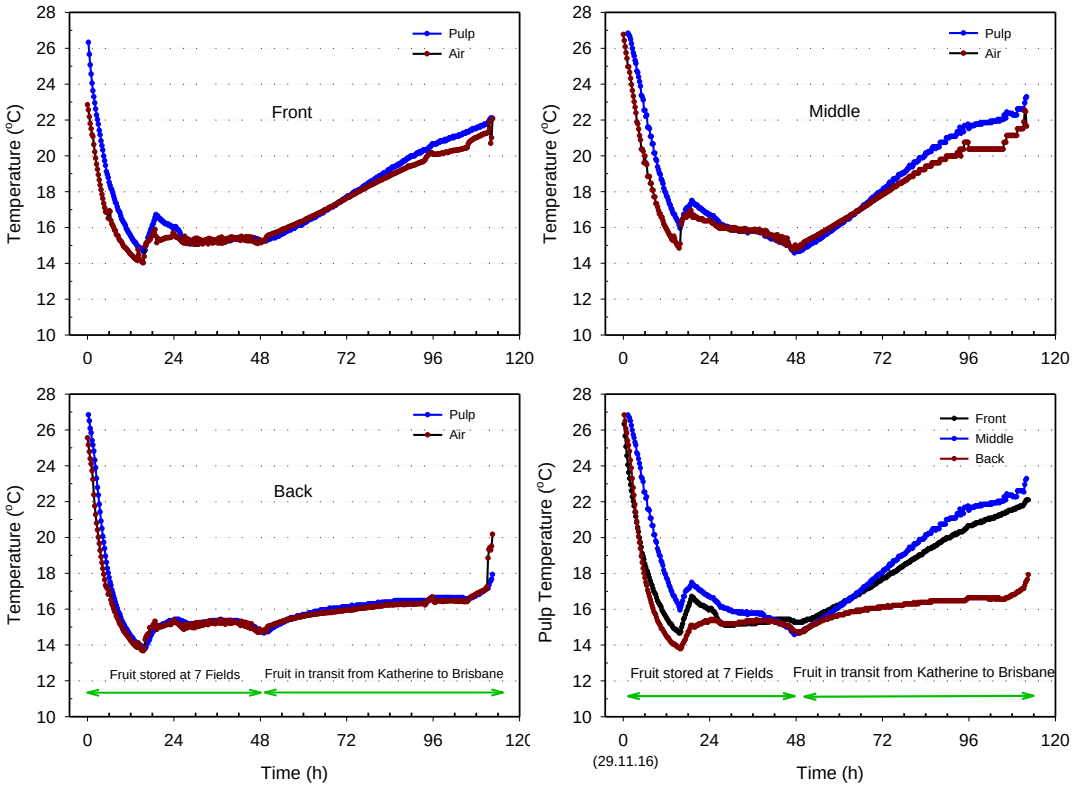


Figure 8. Air and pulp temperature during storage at 7 Fields and road freight journey of 'Honey Gold' mango fruit in treatment trailer 3.

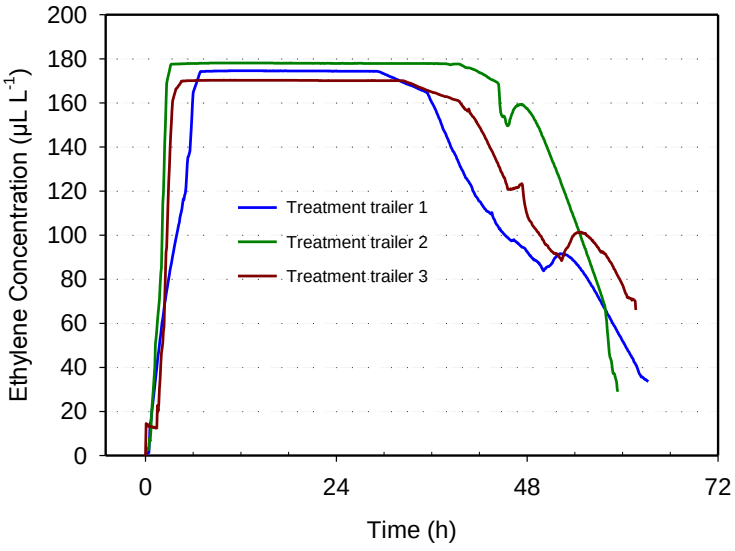


Figure 9. Ethylene concentration (ppm) recorded during refrigerated road freight of 'Honey Gold' mango fruit from Katherine (Northern Territory) to Wamuran (South East Queensland).

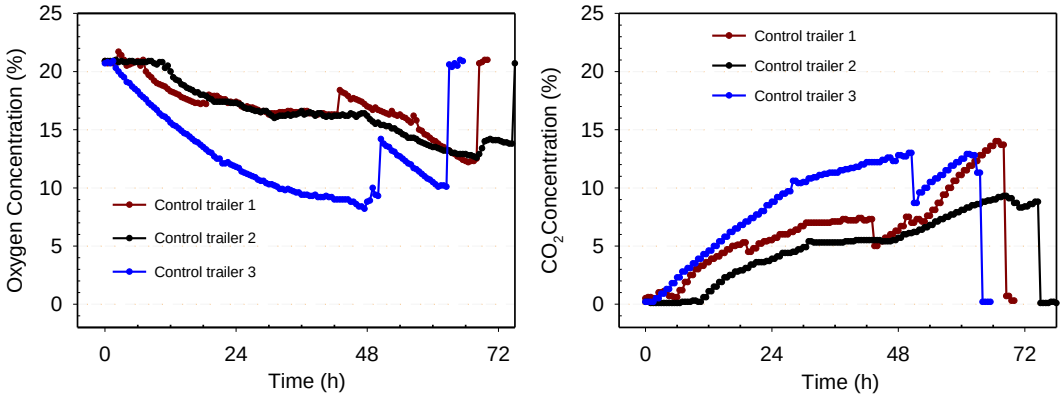


Figure 10. Oxygen and carbon dioxide concentration in refrigerated control trailers with 'Honey Gold' mango fruit from Katherine (Northern Territory) to Wamuran (South East Queensland).

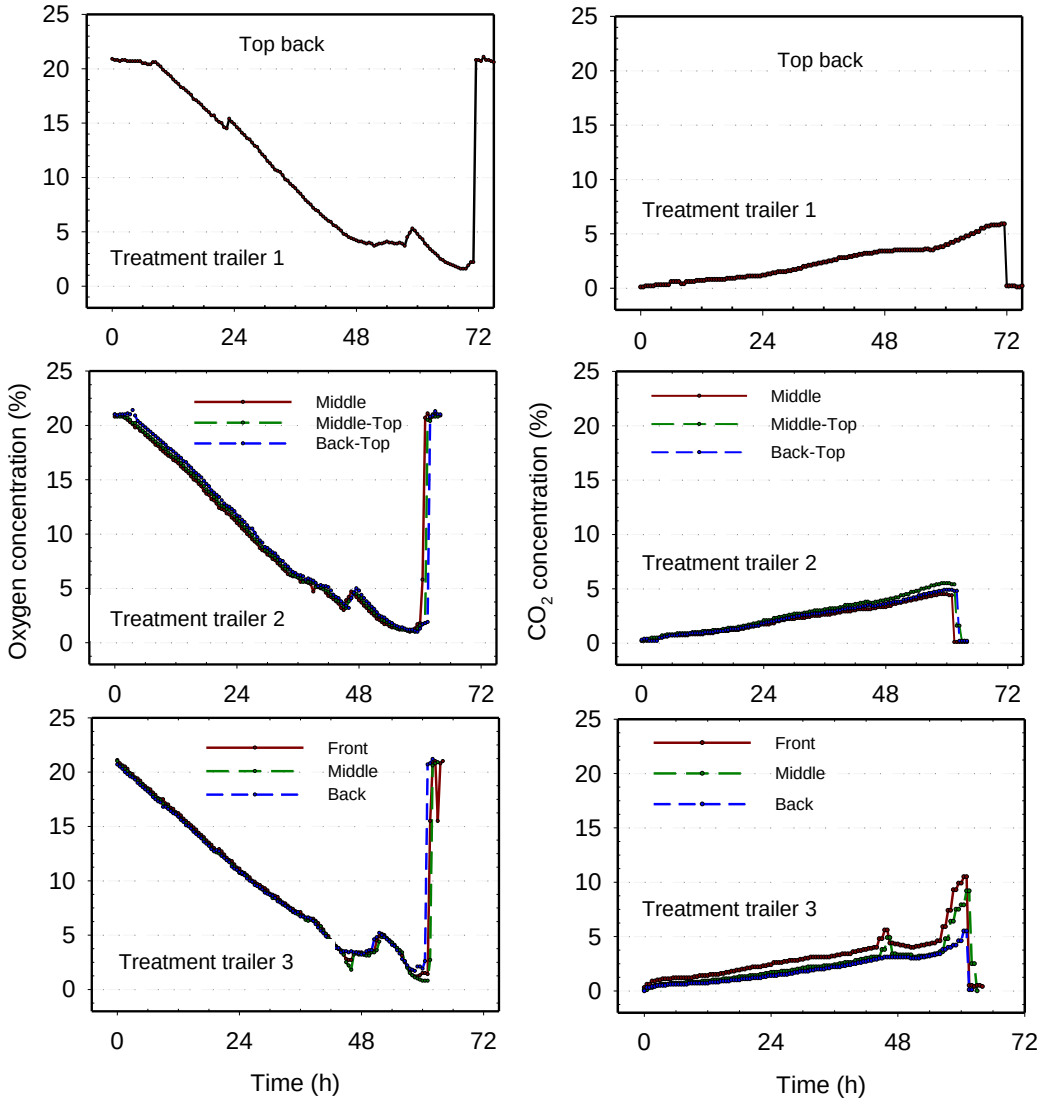


Figure 11. Oxygen and carbon dioxide concentration in refrigerated treatment trailers with 'Honey Gold' mango fruit from Katherine (Northern Territory) to Wamuran (South East Queensland).

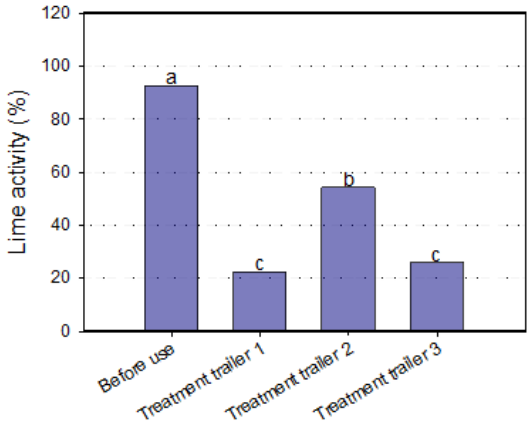


Figure 12. Lime activity before and after use in three treatment trailer. Means with same letter are not significantly different at the *P* =0.05 level. (n=6)

## Fruit ripening and quality

At arrival at the ripener, the Ripestuff-treated fruit were slightly softer, and with less % green colour, compared with the control fruit (**Table 6**). Treatment of fruit with ethylene during transit (via Ripestuff) or post–arrival (reflecting standard commercial practice) reduced the days to ripen by about 3 days, as compared to non-treated fruit (**Table 5**). However, there was no difference in days to ripen between Ripestuff and post-arrival ethylene. This may be because:

- The post-arrival ethylene fruit were treated with ethylene at 20°C, while Ripestuff treatment was at an average of about 18.5°C.
- The significantly lower $O_2$ concentrations in the Ripestuff trailers would have retarded ripening, as confirmed by Duong et al (2016a).
- The short transit time of 2-3 days (**Table 2**) was not sufficient to reduce the ripening time with the Ripestuff treatment.
- The in-transit ripening concept is to transport at about 18°C rather than the recommended 13°C with no in transit ripening. This higher transport temperature with in transit ripening would significantly reduce ripening time, even without ethylene application. However, 'Honey Gold' mango from the Northern Territory is transported at 16°C to reduce the risk of under skin browning (Hofman et al., 2015), but this also reduces the potential benefits of in transit ripening.

Ripestuff-treated fruit had slightly higher TSS content and TA at ripe than control fruit, which may marginally improve flavour. There was no significant difference in TSS and TA between Ripestuff and at arrival ethylene-treated fruit. Ethylene treated fruit had less green skin colour at ripe compared with no ethylene treatment (**Table 6**). There was little treatment effect on brightness, Chroma and Hue. The higher skin red colour (blush) with Ripestuff treatment requires further investigation.

# Conclusions

The results from this study suggest that the Ripestuff and hydrated lime treatments provided acceptable management of ethylene and $CO_2$ concentrations, but that further development is required in the following areas:

- Further development and testing in relation to using less Ripestuff, with adjusted release characteristics, to maintain concentrations between 10-100 µL $L^{-1}$ for 2-3 days.
- Investigating less labour-intensive hydrated lime application systems.

- Using laboratory trials, determine the effect of the temperature difference between control and in transit consignments, and transit duration, on days to ripen. This will indicate the commercial conditions required to realise the benefits of reduced ripening time on arrival using in transit ripening conditions.
- Developing systems to maintain $O_2$ concentrations above at least 10%.

**Table 5**. Effect of Ripestuff during the transport and post-arrival ethylene treatment on 'Honey Gold' mango ripening and fruit quality.

| Treatment | Day to Eating Ripe | TSS (Brix) | Titratable Acid (%) |
|---|---|---|---|
| Control | 12.1 a | 16.0 b | 0.3 b |
| Ripestuff | 9.2 b | 16.5 a | 0.4 a |
| Ethylene | 8.8 b | 16.2 ab | 0.5 a |

Means with same letter in each column are not significantly different at the P = 0.05 level. (n= 126 for TSS and titratable acid, and n= 252 for day to eating ripe)

**Table 6**. Effect of Ripestuff during the transport and post-arrival ethylene treatment on 'Honey Gold' mango fruit colour and firmness.

| Stage | Treatment | Blush (%) | Yellow (%) | Green (%) | Lightness | Chroma | Hue | Firmness (N) |
|---|---|---|---|---|---|---|---|---|
| Initial | Control | 12.6 b | 21.3 c | 66.1 ab | 67.7 b | 45.5 c | 107.1 a | 26.2 a |
| | Ripestuff | 18.0 a | 20.9 c | 61.2 b | 67.2 b | 44.6 d | 108.6 a | 19.4 b |
| | Ethylene | 11.9b | 19.7 c | 68.4 a | 67.3 b | 45.2 cd | 107.3 a | 28.0 a |
| Eating ripe | Control | 12.3 b | 74.0 b | 13.6 c | 68.8 a | 57.2 a | 75.9 b | 4.0 c |
| | Ripestuff | 17.9 a | 78.6 a | 3.5 d | 69.4 a | 55.6 b | 77.4 b | 4.2 c |
| | Ethylene | 11.9 b | 87.7 a | 0.4 d | 69.4 a | 55.1 b | 77.3 b | 4.1 c |

Means with same letter in each column are not significantly different at the P =0.05 level. (n=252)

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# Effect of simulated in-transit gas conditions on ripe fruit quality of ‘Honey Gold’ mango

2015-16 mango season results

In-transit ripening and prediction of outturn quality for mango HIA project MG12016

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This publication has been compiled by Peter Hofman of AgriSciences Qld, Department of Agriculture and Fisheries.

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# Summary

In-transit ripening of mangoes during refrigerated road-freighting from northern production areas of Australia to southern markets can provide significant savings in relation to down-stream energy and infrastructure costs associated with ripening. However, commercial trials over the last 6 years have confirmed the risk of excessive accumulation of carbon dioxide ($CO_2$) levels in freight containers due to fruit respiration, and the associated challenges of maintaining the concentrations below the desired 2-4% level. High $CO_2$ concentrations can potentially affect fruit ripening rates, as well as overall quality after removal and at a ripe stage. The present trial evaluated the potential effects on 'Honey Gold' mango under elevated $CO_2$ and reduced $O_2$ levels over 3-5 days at 18°C under simulated commercial in-transit conditions.

As expected, ethylene significantly enhanced the fruit ripening process compared with untreated fruit, as evidenced by a greater loss of green colour and firmness upon removal from the gas treatments, and a fewer number of days to reach eating soft (ES). All $CO_2$/$O_2$ treatments delayed the loss of green colour and firmness at 3 and 5 days compared to ethylene treatment alone, and required an additional 1-2 days to reach ES. However, at ES all fruit reached 90-100% yellow, with only very minor evidence of green 'blotchiness' occurring at an ES stage. There was no treatment effect on Brix (total soluble solids) levels at ES. Ethylene treated fruit had higher TA (titrable acidity) content than control fruit. No effect of high $CO_2$ and low $CO_2$ on TA was found at ES.

These results indicate that high $CO_2$/low $O_2$ concentrations during in-transit ripening for 3-5 days delayed ripening, thus partly negating the benefits of ethylene treatment during transit. Therefore, ongoing efforts to reduce $CO_2$ accumulation and $O_2$ reduction, would maximise the benefits of an ethylene treatment during transit. Despite this, there was no evidence of any detrimental effects on ripe fruit quality under any of the gas concentrations tested, when compared with no ethylene treatment.

# Introduction

The in-transit ripening of mangoes has the potential to deliver significant benefits in cost and time to the mango industry. Mango fruit are typically harvested at the hard green mature stage, transported under refrigerated conditions, and subsequently ripened at market with the application of ethylene. By integrating two separate phases of the postharvest chain, in-transit ripening would allow fruit to move more rapidly from harvest to market, reducing overall costs, and potentially allowing industry to capitalise on higher prices at the start of the season. The use of in-market ripening rooms could be eliminated, with associated benefits in floor space availability, reduced handling and lower energy costs. The higher set temperatures, in both on-farm pre-cooling and refrigerated transport would also significantly reduce the energy costs associated with temperature management. A well-designed in-transit ripening system would therefore deliver a ready-to-market product with substantial savings in time and costs.

The development of an in-transit ripening system requires the controlled release of ethylene to trigger ripening, combined with the careful management of temperature, $CO_2$ and $O_2$ levels to maintain fruit quality. Several systems have been developed for ethylene release in cartons or containers which may have application to transport containers. These systems are based on very small release canisters (Sharrock et al., 2010 ; Sharrock and Henzell, 2010 ) and encapsulated ethylene (in a sugar matrix) (Ho et al., 2013). Hydrated lime with at least 85-90% lime activity index can effectively absorb $CO_2$ (Bartsch, 2004) and is used in controlled atmosphere rooms to minimise $CO_2$ accumulation. The combination of these ethylene and $CO_2$ regulation systems are being explored in developing an in-transit ripening regime for 'Honey Gold' mangoes.

Our recent studies on in-transit ripening of 'Honey Gold' mangoes showed that $CO_2$ concentrations reached 15-20% after 3.5-4 days in a 12 metre refrigerated road truck. High $CO_2$ concentrations can result in 'Kensington Pride' mango fruit retaining more green skin colour when ripe, and showing greater susceptibility to disease (Nguyen, 2003). However, it is not known whether similar detrimental effects occur in 'Honey Gold'. The aim of the current trial was to determine acceptable carbon dioxide concentration limits for 'Honey Gold' in order to develop in-transit ripening guidelines during road freighting.

# Materials and Methods

## Fruit

Mature hard green 'Honey Gold' mangoes were harvested from Pershouse, Tyas and Piñata Farms in Wamuran, south-east Queensland (SEQ) on approximately 8th February (Pershouse) and 10th February, 2016 and transported to the Piñata Farms packaging shed for sorting. Fruit were then packed in trays of single layer fibre cardboard with a dimension of 43 cm length x 36 cm width x 13 cm height and carefully transported by car to the Maroochy Research Facility, Nambour in SEQ. Mangoes were stored at 20°C prior to treatment.

## Treatment chambers

The treatment system was designed to mimic conditions inside a transport container. Four sealed chambers (90 x 90 x 120 cm) were placed within each of four cold-rooms, with gas inlets and outlets to allow the injection of required gases and air sampling. The chambers were comprised of aluminium box tubing covered with 1.6 mm aluminium sides and a high density polyethylene lid. Air circulation was achieved by using two 15 cm muffle fans (Model YX2514 Sleeve Bearing, Sirocco,Taiwan) inside each chamber, placed over a "chimney" in the centre of the rows of trays as illustrated in Figure 1. A temperature probe was inserted into a fruit within each chamber to control fruit pulp temperatures inside the chambers with a Freshview EC12 Ripening Controller (Pacific Data Systems, Brisbane). To mimic commercial truck conditions, the chambers were filled to about 80% capacity with fruit. The containers were well sealed to allow accumulation of $CO_2$ and other volatiles, and $CO_2$ was controlled using a MRS CA (RoomControl) system.

Additional $CO_2$, nitrogen, ethylene and ambient air were injected from pressurised cylinders when concentrations fell below or exceeded the target values, based on regular automated measurements through a carbon dioxide transmitter (Vaisala GMT 221, Finland) measuring range 0-20% $CO_2$, oxygen analyser (Model 570A, Servomex Ltd., England) and ethylene sensor (Alphasense Photoionisation detector; PID). Relative humidity in the chambers was not controlled but was measured at more than 85% using Vaisala HMP50 humidity probes. The composition of the atmosphere in each chamber was adjusted manually to the desired starting concentrations immediately after fruit were placed in the chamber. The set gas concentrations (Table 1, and detailed in the Appendix) were adjusted daily to mimic truck conditions. In each chamber $CO_2$, $O_2$, ethylene, pulp temperature and RH were monitored, with readings taken at 10 minute intervals at a flow rate of 200 mL $min^{-1}$ using the RoomControl program.

In each chamber, a pallet was stacked with 4 layers of trays, with 6 trays in each layer and a central chimney for air flow (Figure 1). Six trays of experimental fruit, containing 14 fruit per tray, were placed in the third layer on the pallet, arranged as shown in Figure 2. The remaining 18 trays of mangoes were filled with commercially rejected fruit (fruit with defects, out of range size or damage).

## Treatments

The treatments were applied in a factorial design using fruit from 3 farms x 6 storage treatments (as shown in Table 1), and with two different durations of storage (3 or 5 days; 72 and 96 hours, respectively). However, the control treatments were removed only at the end of day 3 due to ripening. Treatments 4-6 aimed to replicate the conditions expected within a commercial refrigerated container based on in-transit observations of 'B74' over several years and of 'Honey Gold' in the current season. Treatments 5 and 6 replicated conditions that had been observed in trucks in previous trials, with $CO_2$ gradually increasing and $O_2$ gradually decreasing during transport. Treatment 3 was included to test the effects of reducing $O_2$ concentrations. Fruit were removed at the end of day 3, and the atmosphere conditions re-established within 2 hours. All fruit were removed from the chambers at day 5 and placed at 18°C until ES.

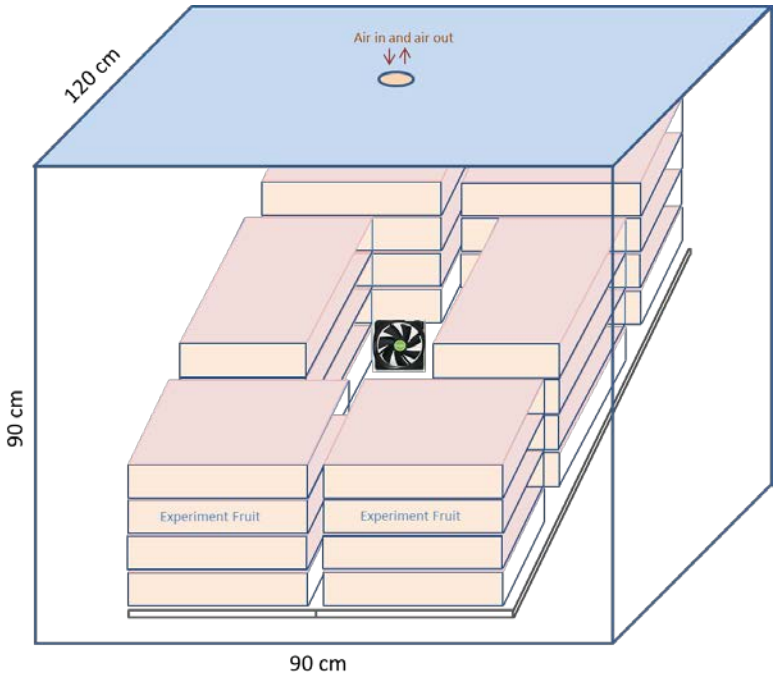


Figure 1. Layout of fruit trays within each chamber.

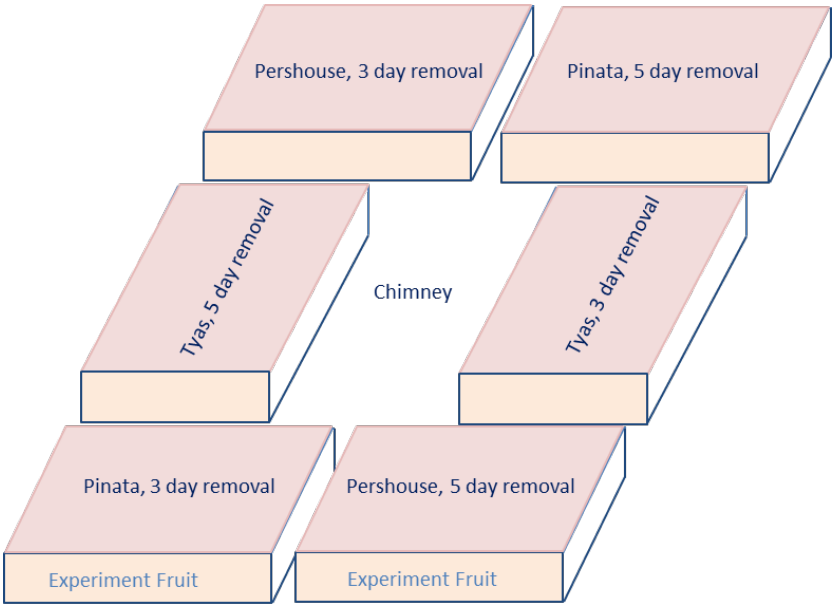


Figure 2. Layout of experimental fruit in the third layer of each chamber

Table 1: $CO_2$ and $O_2$ treatments applied to 'Honey Gold' mangoes at 20°C.

| Treatment | Storage conditions | Target gas concentrations | | | Conditions established over: | Duration of exposure |
|---|---|---|---|---|---|---|
| | | Ethylene (µL $L^{-1}$) | $CO_2$ (%) | $O_2$ (%) | | |
| 1. Control without ethylene | In cold room (no chamber) | 0 | ~0.04 | ~21 | - | 3 days |
| 2. Ethylene control | In cold room (no chamber) | 20 | ~0.04 | ~21 | - | 3 days |
| 3. $CO_2$ 8% / $O_2$ 20% | Sealed chamber (within cold room) | 20 | 8 | 20 | ~4 hours, then constant | 3 / 5 days |
| 4. $CO_2$ 8% / $O_2$ 12% | Sealed chamber (within cold room) | 20 | 8 | 12 | ~4 hours, then constant | 3 / 5 days |
| 5. $CO_2$ 13% / $O_2$ 7% | Sealed chamber (within cold room) | 20 | 13 | 7 | Gradually over ~4 days | 3 / 5 days |
| 6. $CO_2$ 20% / $O_2$ 2% | Sealed chamber (within cold room) | 20 | 20 | 2 | Gradually over ~4 days | 3 / 5 days |

# Assessment

For each farm, 2 trays of fruit were assessed for skin colour and fruit firmness prior to the gas treatment. An experimental tray of fruit from each farm and treatment was removed at the end of day 3 and of day 5, and was stored overnight at 20°C prior to assessment the next morning.

## Fruit dry matter content

Prior to treatment application, five fruit were used to determine % dry matter (DM). A cheek from each mango was cut, peeled and grated. The mango flesh was weighed then placed into a dehydrator at about 65°C until a steady weight was reached. The % DM was calculated as:

(Dry Weight (g)/Fresh Weight (g)) x 100

## Skin colour and appearance

Visual skin colour was rated using the scale of 1-6 (1: 0-10% yellow, 2: 10-30% yellow, 3: 30-50% yellow, 4: 50-70% yellow, 5: 70-90% yellow, 6: 90-100% yellow on the fruit skin) as per the Mango Quality Assessment Manual (Hofman et al., 2010).

The skin colour was also assessed using a Minolta Chroma Meter (Minolta Corp, Ramsey, NJ) calibrated to a white standard plate (L* 97.31, a* -0.18, b* 2.45), in the L*a*b* colour system as L* value (lightness), a* value (redness or greenness) and b* value (yellowness or blueness). Three locations that were free of blush, blemish and sunburn were marked on each fruit and measurements were taken on the same locations upon arrival at the laboratory, at removal and at eating soft (ES; hand firmness of 4 as described below).

Green blotchiness was rated on a scale of 0-4, where 0 = no blotchy green colour, 1 = 10%, 2 = 25%, 3 = 50% and 4 = up to 100% of the skin area with blotchy green colour, the lenticel damage was estimated on a percentage scale.

## Fruit firmness

Individual fruit firmness was measured using an EZ Test Shimadzu, EZ-SX, 100 N (Kyoto, Japan). Firmness was assessed as the Newtons (N) force required to push a 12 mm spherical probe to a depth of 2 mm into the non-peeled fruit at a rate of 10 mm $min^{-1}$. The probe was applied to the cheek area located at the middle to lower stem end of each fruit. Subsequent tests at removal from the chamber and at ES were done on different locations on the fruit.

In addition, individual fruit were rated for hand firmness using the scale of 0-4 (0 = hard, no 'give' in the fruit; 1 = rubbery, slight 'give' in the fruit; 2 = sprung, flesh deforms by 2-3 mm with extreme thumb pressure; 3 = firm soft, whole fruit deforms with moderate hand pressure; and 4 = soft, whole fruit deforms with slight hand pressure (Hofman et al*.,* 2010).

### Total soluble solids, titratable acidity and ethanol content

At eating soft, the peel of each fruit was removed, and a portion of the flesh from each fruit used for total soluble solids (TSS) content using an ATAGO hand refractometer (PAL – 1). The remaining flesh was frozen and the titratable acidity (TA) of the thawed sample determined by titrating the puree to a pH endpoint of 8.2 using a Mettler Toledo G20 compact titrator with 0.1M sodium hydroxide (NaOH). Results are presented as the % citric acid equivalents. Ethanol levels in the thawed samples were tested using a Megazyme Ethanol Kit (Megazyme International Ireland Limited, Bray, Ireland).

### Sensory evaluation

Sensory evaluation was carried out on the treatments removed on day 5, and the ethylene control. The staggered ripening of fruit across treatments prevented sensory evaluation of fresh samples. When each individual fruit reached eating ripe, sensory samples were taken from the cheek, and were frozen. Samples were thawed and evaluated within 3 weeks of freezing. For sensory evaluation, the samples of all fruit from each treatment were combined. After thawing at room temperature, each combined sample was blended to a puree. Each tasting sample contained approximately 30 g of pureed mango cheek flesh, served at room temperature. Samples were evaluated by simple descriptive analysis using line scales. The treatments were evaluated in 3 separate sittings, grouped by farm. A sensory standard was taken using 14 fruit from the bulk fruit in treatment 3, and this standard was evaluated at each sitting. The sensory standard was also evaluated for °Brix (each fruit) and TA (a pooled sample).

At each tasting, treatment groups were randomly assigned 3 digit blinding codes, and were presented in randomly allocated orders of tasting using a Latin Square design. Each set of samples was assessed by 36-38 untrained panellists, recruited from MRF staff. The same panellists were used for all 3 sets of samples where possible, but due to occasional unavailability of tasters, there was some variability in panellists between sets. The panellists assessed each treatment sample for sensory attributes and preference using continuous line scales, as shown in Table 2. Samples were presented in small lidded containers, and panellists were instructed to assess aroma immediately after removing the lid, then to taste the sample. The 10 cm line scales were analysed by assigning continuous measured values of 0-10, where 0 values were assigned to the left scale end, and 10 to the right scale end.

Table 2: Sensory attributes assessed in the 'Honey Gold' $CO_2$ / $O_2$ trial, with line scale labels.

| Sensory attributes or preference | Line scale label at left end (0cm) | Line scale label at right end (10cm) |
|---|---|---|
| Aroma preference | Dislike very much | Like very much |
| Sweetness | Low intensity sweetness | Extremely sweet |
| Sourness | Not at all sour | Very sour |
| Starchiness | Not at all starchy | Very starchy |
| Presence of off-flavours | No off-flavours | Strong off-flavour |
| Overall liking | Dislike very much | Like very much |

## Analysis

Statistical analyses were performed by Genstat® 14 for Windows™ (VSN International Ltd., UK), with variables analysed by two-way ANOVA using individual fruit as the experimental unit (with 'treatment' and 'farm' as factors, or 'combined treatment/duration group' and 'farm' as factors). The least significant difference (LSD) procedure at P = 0.05 was used to test for differences between means.

# Results and discussion

## Chamber conditions

Figure 3 shows gas conditions ($CO_2$, $O_2$ and ethylene) maintained in the chambers during the trial. Carbon dioxide and $O_2$ concentrations were at desired levels, despite being variable. The targeted 2% $O_2$ concentration for treatment 6 did not drop below about 5%.

Ethylene concentration was maintained above 20 µL $L^{-1}$. Air temperatures were maintained at approximately 19˚C during the experiment. Relative humidity was between 80-85% (data not shown).

## Fruit quality

The DM of fruit from Piñata Farm was 16.2% which was significantly lower than that of fruit harvested from Pershouse and Tyas farms, at 18.8% and 18.5%, respectively.

At the end of days 3 and 5, the ethylene treated control fruit were more yellow and softer than the non-ethylene treated control fruit (Table 4). All $CO_2$/$O_2$ treatments retarded the loss of green colour and firmness compared with the ethylene treated control fruit, being generally more noticeable at 5 days after removal from the chamber. 8% $CO_2$+ 12% $O_2$ resulted in a slower loss of green colour and firmness compared with 20% $O_2$. However, the differences between treatments 4-6 were relatively less significant compared with between treatment 3 and 4, and all fruit reached full yellow colour (rating 6) at ES.

Ethylene treatment more than halved the days to reaching ES compared with no treatment (Table 4). The $CO_2$/$O_2$ treatments (with ethylene) all resulted in more days to ES compared with the no ethylene control treatment. The days to ES increased by 1-2 days with higher $CO_2$ concentrations and lower $O_2$ concentrations (Treatments 3-6). However, the days to ES was still less under these conditions compared with the no ethylene control treatment.

There was no treatment effect on Brix of the ripe flesh between the treatments (Table 4). TA content from ethylene treated fruit was higher than that from control fruit. There was no significant difference in TA among all $CO_2$/$O_2$ treatments.

Blotchiness (uneven retention of green colour) was significantly greater in treatments 4-6 (Table 4), although this response was relatively minor since all fruit attained a colour rating of 6 (90-100% yellow colour) at ES (Table 3) and there was little to no differences between treatments in skin L-values, chroma and hue at ES.

Lenticel damage (LD) at an ES stage was higher with an ethylene treatment (Table 4), which is in line with previous observations. Higher $CO_2$/low $O_2$ concentrations generally increased LD compared with an 8% $CO_2$/20% $O_2$ treatment LD under these conditions was also similar to the no-ethylene control treatment.

Ethanol content tended to be higher in ethylene treated fruit, but did not vary consistently under the various $CO_2$ and $O_2$ treatments (Table 5). These results suggest that $CO_2$ and $O_2$ at the levels tested did not induce the formation of off-flavours in the fruit.

### Sensory evaluation

The sensory properties of fruit from the 3 different farms differed significantly (analysis not shown), and significant interaction was observed between treatment and farm effects. The data for each farm is therefore presented separately (Table 6, Table 7, Table 8).

The CA treatments trialled did not cause any detrimental sensory changes in the fruit at eating ripe. Across the 3 farms, the ethylene treated control fruit were the lowest rated for overall acceptability and were perceived as the most sour, as was reflected in the higher TA measured in these fruit. The ethylene control fruit also tended to be perceived as the least sweet (significant in Pinata and Tya's fruit) and having the most off-flavours (significant in Pinata and Tya's fruit). These results align with the significantly higher levels of ethanol detected in the ethylene control fruit. There was little variation in sensory properties between the different levels of CA storage. While some significant differences between the CA treatments were observed, the differences were slight, and no consistent patterns emerged.

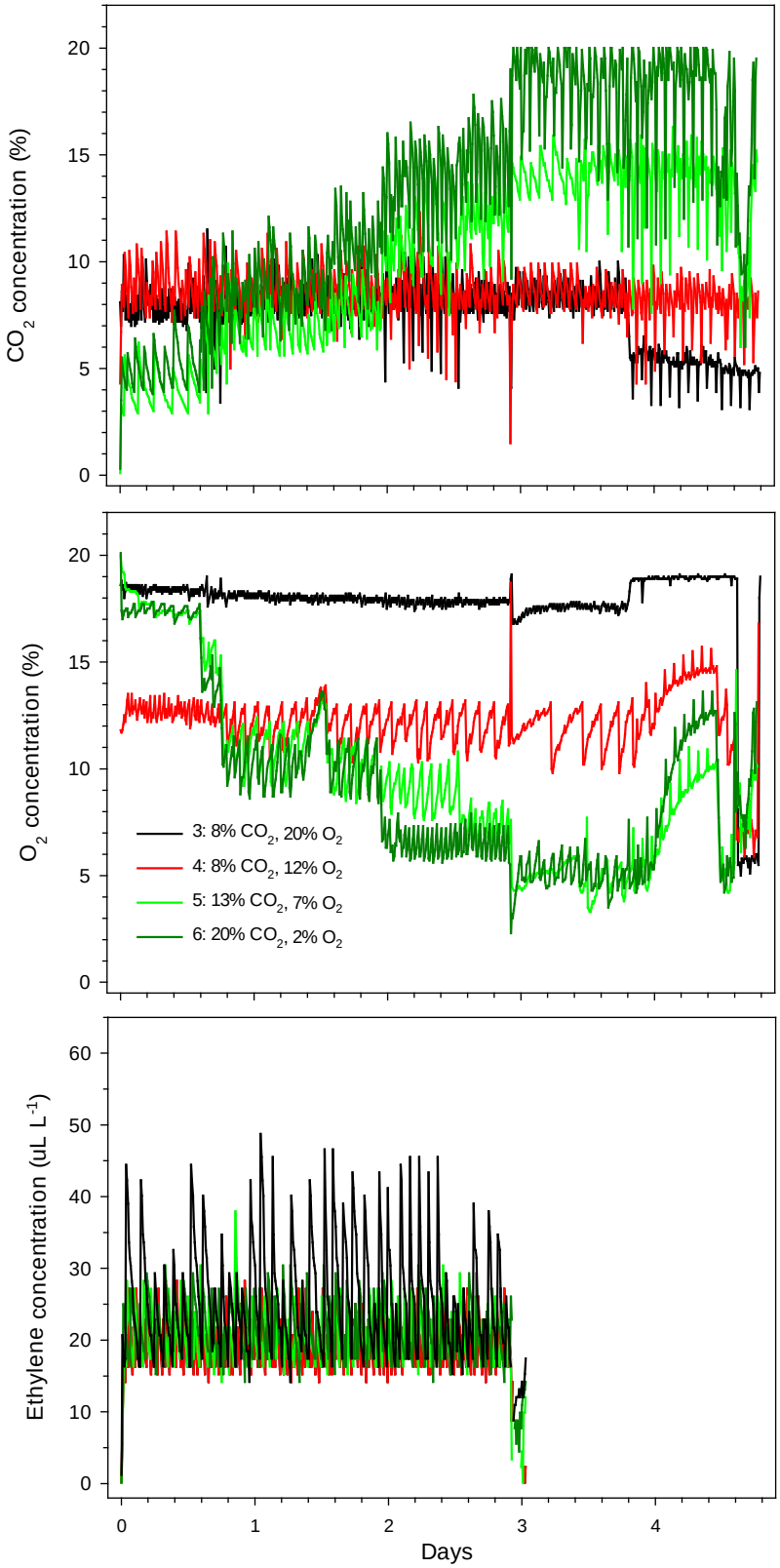


Figure 3. Gas concentrations during treatment of 'Honey Gold' mango in chambers and 5 days. Refer to Table 1 for treatment details.

Table 3. Effect of varying CO2 / $O_2$ gas concentrations on 'Honey Gold' fruit skin colour and flesh firmness after treatment and at an eating soft stage (hand firmness of 4).

| | | Colour Rating (1-6) | | | | Firmness Rating (0-4) | | | | EZ Tester Firmness (N) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Storage duration | Day 0 | Day 3 | Day 5 | At eating ripe | Day 0 | Day 3 | Day 5 | At eating ripe | Day 0 | Day 3 | Day 5 | At eating ripe |
| 1. Control - no ethylene | 3d / 5d | 1.0 | 2.1 d | 3.1 d | 6.0 | 0.0 | 1.1 e | 1.2 d | 4.0 | 43.0 | 25.5 a | 16.9 a | 5.0 c |
| 2. Ethylene control (20ppm) | 3d / 5d | 1.0 | 4.1 a | 5.8 a | 6.0 | 0.0 | 3.5 a | 3.5 a | 4.0 | 44.5 | 9.0 bc | 5.8 c | 5.2 abc |
| 3. 8:20 (8% $CO_2$, 20% $O_2$) | 3 d | | 3.6 b | | 6.0 | | 3.1 b | | 4.0 | | 9.5 bc | | 5.6 a |
| 4. 8:12 (8% $CO_2$, 12% $O_2$) | 3 d | | 3.0 c | | 6.0 | | 2.7 c | | 4.0 | | 8.2 c | | 5.4 ab |
| 5. 13:7 (13% $CO_2$, 7% $O_2$ - changing conditions) | 3 d | | 2.9 c | | 6.0 | | 2.5 d | | 4.0 | | 10.1 b | | 5.2 bc |
| 6. 20:2 (20% $CO_2$, 2% $O_2$ - changing conditions) | 3 d | | 2.6 c | | 6.0 | | 2.5 d | | 4.0 | | 9.3 bc | | 5.1 bc |
| 3. 8:20 (8% $CO_2$, 20% $O_2$) | 5 d | | | 4.8 b | 6.0 | | | 1.7 bc | 4.0 | | | 7.3 b | 4.9 c |
| 4. 8:12 (8% $CO_2$, 12% $O_2$) | 5 d | | | 3.9 c | 6.0 | | | 1.8 b | 4.0 | | | 7.5 b | 5.1 bc |
| 5. 13:7 (13% $CO_2$, 7% $O_2$ - changing conditions) | 5 d | | | 3.4 d | 6.0 | | | 1.9 b | 4.0 | | | 8.2 b | 5.1 bc |
| 6. 20:2 (20% $CO_2$, 2% $O_2$ - changing conditions) | 5 d | | | 3.3 d | 6.0 | | | 1.5 c | 4.0 | | | 7.8 b | 5.0 c |

Data analysed by factorial ANOVA (treatment x farm), with fruit as the experimental unit (n=42). Means with the same letter within each column are not signficantly different (at $p<0.05$) as tested by LSD.

Table 4. Effect of varying $CO_2$ / $O_2$ gas concentrations on 'Honey Gold' fruit quality at an Eating Ripe stage.

| Treatment | Storage duration | Days to eating ripe | | Brix (°) | TA (%) | | Blotchiness rating (0-4) | | Lenticel damage (% of surface) | | L-value | | Chroma | | hue (°) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Control - no ethylene | 3d / 5d | 14.2 | a | 15.1 | 0.5 | c | 0.81 | f | 17.0 | bcd | 67.7 | b | 54.2 | a | 78.1 | e |
| 2. Ethylene control (20ppm) | 3d / 5d | 6.7 | e | 14.6 | 0.9 | a | 0.79 | f | 26.7 | a | 68.9 | a | 51.9 | cd | 79.6 | bc |
| 3. 8:20 (8% $CO_2$, 20% $O_2$) | 3d | 8.1 | d | 14.7 | 0.8 | ab | 0.91 | ef | 11.3 | e | 67.2 | bc | 50.2 | f | 78.1 | e |
| 4. 8:12 (8% $CO_2$, 12% $O_2$) | 3d | 8.2 | d | 14.5 | 0.8 | ab | 1.93 | ab | 13.0 | de | 67.6 | bc | 50.1 | f | 80.9 | a |
| 5. 13:7 (13% $CO_2$, 7% $O_2$ - changing conditions) | 3d | 8.8 | cd | 14.7 | 0.7 | b | 1.74 | abc | 18.2 | bc | 67.7 | b | 50.4 | ef | 80.2 | abc |
| 6. 20:2 (20% $CO_2$, 2% $O_2$ - changing conditions) | 3d | 9.2 | c | 14.8 | 0.7 | b | 1.29 | de | 18.5 | bc | 67.7 | b | 51.6 | cd | 79.4 | bcd |
| 3. 8:20 (8% $CO_2$, 20% $O_2$) | 5d | 9.2 | c | 15.0 | 0.7 | b | 1.45 | cd | 14.9 | cde | 67.8 | b | 51.5 | de | 78.2 | de |
| 4. 8:12 (8% $CO_2$, 12% $O_2$) | 5d | 9.5 | c | 15.0 | 0.7 | b | 2.10 | a | 17.8 | bcd | 66.8 | c | 48.6 | g | 80.4 | ab |
| 5. 13:7 (13% $CO_2$, 7% $O_2$ - changing conditions) | 5d | 11.2 | b | 14.8 | 0.7 | b | 1.62 | bcd | 16.3 | bcde | 67.6 | bc | 53.5 | ab | 80.3 | ab |
| 6. 20:2 (20% $CO_2$, 2% $O_2$ - changing conditions) | 5d | 11.0 | b | 14.8 | 0.7 | b | 1.64 | bcd | 21.0 | b | 67.5 | bc | 52.7 | bc | 79.0 | cde |

Data analysed by factorial ANOVA (treatment group (including duration) x farm), with fruit as the experimental unit (n=42). Means with the same letter within each column are not significantly different (at $p<0.05$) as tested by LSD.

Table 5: Effect of varying $CO_2$ / $O_2$ gas concentrations on the ethanol content at eating soft stage of 'Honey Gold' fruit from 3 southeast Queensland farms.

| Treatment | Storage duration | Ethanol content (% v/v) | | |
|---|---|---|---|---|
| | | Pershouse | Pinata | Tya's |
| Control - no ethylene | 3d / 5d | 0.001 $^{e}$ | 0.003 | 0.001 $^{d}$ |
| Ethylene control (20ppm) | 3d / 5d | 0.034 $^{ab}$ | 0.035 | 0.021 $^{ab}$ |
| 8% $CO_2$, 20% $O_2$ | 3d | 0.014 $^{cde}$ | 0.008 | 0.009 $^{cd}$ |
| 8% $CO_2$, 12% $O_2$ | 3d | 0.009 $^{de}$ | 0.010 | 0.010 $^{bcd}$ |
| 13% $CO_2$, 7% $O_2$ | 3d | 0.013 $^{cde}$ | 0.010 | 0.008 $^{cd}$ |
| 20% $CO_2$, 2% $O_2$ | 3d | 0.021 $^{bcd}$ | 0.012 | 0.003 $^{d}$ |
| 8% $CO_2$, 20% $O_2$ | 5d | 0.016 $^{bcde}$ | 0.011 | 0.018 $^{abc}$ |
| 8% $CO_2$, 12% $O_2$ | 5d | 0.013 $^{cde}$ | 0.008 | 0.025 $^{a}$ |
| 13% $CO_2$, 7% $O_2$ | 5d | 0.040 $^{a}$ | 0.010 | 0.007 $^{d}$ |
| 20% $CO_2$, 2% $O_2$ | 5d | 0.028 $^{abc}$ | 0.003 | 0.000 $^{d}$ |
| LSD | | 0.02 | 0.02 | 0.01 |

Data analysed by general ANOVA of treatment group (including duration) separately within each farm, with fruit as the experimental unit (n=2). Means with the same letter within each column are not significantly different (at $p<0.05$) as tested by LSD.

Table 6: Effect of varying $CO_2$ / $O_2$ gas concentrations on the sensory characteristics at eating soft stage of 'Honey Gold' fruit from Pershouse farm.

| Treatment | Aroma | Sourness | Sweetness | Starchiness | Off-flavours | Acceptability |
|---|---|---|---|---|---|---|
| Sensory standard | 5.6 $^{a}$ | 3.1 $^{c}$ | 6.1 $^{a}$ | 2.6 $^{c}$ | 1.8 | 6.8 $^{a}$ |
| Ethylene control | 4.4 $^{c}$ | 5.5 $^{a}$ | 4.4 $^{c}$ | 4.3 $^{ab}$ | 3.1 | 4.0 $^{c}$ |
| 8% $CO_2$, 20% $O_2$ | 4.3 $^{c}$ | 4.1 $^{b}$ | 4.9 $^{bc}$ | 4.1 $^{b}$ | 2.8 | 5.1 $^{b}$ |
| 8% $CO_2$, 12% $O_2$ | 5.2 $^{ab}$ | 4.5 $^{b}$ | 5.0 $^{bc}$ | 4.9 $^{ab}$ | 2.3 | 5.3 $^{b}$ |
| 13% $CO_2$, 7% $O_2$ | 4.5 $^{bc}$ | 3.9 $^{b}$ | 5.7 $^{ab}$ | 4.6 $^{ab}$ | 2.4 | 5.3 $^{b}$ |
| 20% $CO_2$, 2% $O_2$ | 4.7 $^{bc}$ | 4.4 $^{b}$ | 5.0 $^{bc}$ | 5.1 $^{a}$ | 2.4 | 5.2 $^{b}$ |

Data analysed by ANOVA with panellist as a blocking factor (n=36). Means with the same letter within each column are not significantly different at $P<0.05$ as tested by LSD.

Table 7: Effect of varying $CO_2$ / $O_2$ gas concentrations on the sensory characteristics at eating soft stage of 'Honey Gold' fruit from Piñata farm.

| Treatment | Aroma | Sourness | Sweetness | Starchiness | Off-flavours | Acceptability |
|---|---|---|---|---|---|---|
| Sensory standard | 5.5 $^{b}$ | 3.0 $^{d}$ | 6.3 $^{a}$ | 2.7 $^{b}$ | 1.5 $^{b}$ | 6.6 $^{a}$ |
| Ethylene control | 3.9 $^{c}$ | 6.4 $^{a}$ | 3.7 $^{b}$ | 4.1 $^{a}$ | 3.8 $^{a}$ | 3.3 $^{b}$ |
| 8% $CO_2$, 20% $O_2$ | 5.5 $^{b}$ | 4.6 $^{b}$ | 6.3 $^{a}$ | 3.1 $^{b}$ | 1.4 $^{b}$ | 6.7 $^{a}$ |
| 8% $CO_2$, 12% $O_2$ | 5.9 $^{ab}$ | 4.1 $^{bc}$ | 6.2 $^{a}$ | 2.6 $^{b}$ | 1.7 $^{b}$ | 6.8 $^{a}$ |
| 13% $CO_2$, 7% $O_2$ | 6.0 $^{ab}$ | 4.0 $^{bc}$ | 5.7 $^{a}$ | 3.1 $^{b}$ | 1.4 $^{b}$ | 6.9 $^{a}$ |
| 20% $CO_2$, 2% $O_2$ | 6.3 $^{a}$ | 3.7 $^{cd}$ | 5.8 $^{a}$ | 3.1 $^{b}$ | 1.4 $^{b}$ | 7.0 $^{a}$ |

Data analysed by ANOVA with panellist as a blocking factor (n=37). Means with the same letter within each column are not significantly different at P<0.05 as tested by LSD.

Table 8: Effect of varying $CO_2$ / $O_2$ gas concentrations on the sensory characteristics at eating soft stage of 'Honey Gold' fruit from Tyas's farm.

| Treatment | Aroma | Sourness | Sweetness | Starchiness | Off-flavours | Acceptability |
|---|---|---|---|---|---|---|
| Sensory standard | 4.9 $^{bc}$ | 3.9 $^{b}$ | 4.4 $^{b}$ | 2.2 $^{b}$ | 1.8 $^{b}$ | 5.3 $^{c}$ |
| Ethylene control | 4.6 $^{c}$ | 5.9 $^{a}$ | 4.3 $^{b}$ | 2.5 $^{b}$ | 2.6 $^{a}$ | 4.6 $^{c}$ |
| 8% $CO_2$, 20% $O_2$ | 5.2 $^{abc}$ | 2.3 $^{d}$ | 5.7 $^{a}$ | 3.4 $^{a}$ | 1.5 $^{bc}$ | 6.2 $^{b}$ |
| 8% $CO_2$, 12% $O_2$ | 5.0 $^{bc}$ | 3.8 $^{bc}$ | 5.4 $^{a}$ | 2.7 $^{b}$ | 1.7 $^{b}$ | 6.3 $^{ab}$ |
| 13% $CO_2$, 7% $O_2$ | 5.3 $^{ab}$ | 3.0 $^{cd}$ | 6.0 $^{a}$ | 2.8 $^{ab}$ | 1.5 $^{bc}$ | 6.4 $^{ab}$ |
| 20% $CO_2$, 2% $O_2$ | 5.7 $^{a}$ | 2.5 $^{d}$ | 5.9 $^{a}$ | 2.4 $^{b}$ | 0.9 $^{c}$ | 7.0 $^{a}$ |

Data analysed by ANOVA with panellist as a blocking factor (n=38). Means with the same letter within each column are not significantly different at P<0.05 as tested by LSD.

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# Appendix

| Treatment | Day 1 | | | | | | Day 2 | | | | Day 3 | | | | Day 4 | | | | Day 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | | 16 | | 24 | | 36 | | 48 | | 60 | | 72 | | 84 | | 96 | | 108 | | 120 | |
| | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ |
| 3 (8;20) | 8 | 20 | constant | | | | | | | | | | | | | | | | | | | |
| 4 (8;12) | 8 | 10 | constant | | | | | | | | | | | | | | | | | | | |
| 5 (13;8) | 3 | 17 | 5 | 13 | 6 | 9 | 8 | 8 | 10 | 7 | 12 | 5 | 13 | 3 | 14 | 3 | 13 | 3 | 13 | 3 | 13 | 3 |
| 6 (20;2) | 4 | 16 | 6 | 12 | 8 | 8 | 10 | 8 | 13 | 4 | 15 | 4 | 17 | 3 | 8 | 2 | 20 | 2 | 20 | 2 | 20 | 2 |

**Conditions set in the RoomControl program at specific hours after sealing the tents**

| Treatment | Day 1 | | | | | | Day 2 | | | | Day 3 | | | | Day 4 | | | | Day 5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Thurs 6 pm | | Fri 8 am | | Fri 5 pm | | Sat 8 am | | Sat 6 pm | | Sun 8 am | | Sun 6 pm | | Mon 8 am | | Mon 5 pm | | Tues 8 am | | | |
| | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | $CO_2$ | $O_2$ | | |
| 3 (8;20) | 9 | 19 | Constant | | | | | | | | | | | | | | | | | | | |
| 4 (8;12) | 9 | 9 | Constant | | | | | | | | | | | | | | | | | | | |
| 5 (13;8) | 4 | 16 | 6 | 12 | 7 | 8 | 9 | 7 | 11 | 6 | 13 | 4 | 14 | 2 | 15 | 2 | 14 | 2 | 14 | 2 | | |
| 6 (20;2) | 5 | 15 | 7 | 11 | 9 | 7 | 11 | 7 | 14 | 3 | 16 | 3 | 18 | 2 | 9 | 1 | 21 | 1 | 21 | 1 | | |

# Modelling the responses of 'Honey Gold' mango fruit to in-transit ripening conditions – preliminary results

## 2015/16 and 2016/17 season results

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Note: the preliminary results in this report are part of a research higher degree program, and are therefore confidential until published.

This publication has been compiled by Peter Hofman of Horticulture and Forestry Sciences, the Department of Agriculture and Fisheries.

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# Summary

Mango postharvest handling, transport and ripening in Australia relies on adequate pre-cooling of fruit and maintaining acceptable temperatures and environmental atmospheres throughout the cool chain to ensure high quality mangoes for consumers. In this experiment, repeated over two mango seasons, cv. 'Honey Gold' mangoes from three growing regions were exposed to simulated in-transit ripening temperatures of 18, 22 and 26°C, and 0, 20 and 80 µL $L^{-1}$ ethylene and 1, 4 and 8% carbon dioxide ($CO_2$) in treatment chambers for 4 days, then ripened at 20°C. 'Honey Gold' fruit quality parameters including firmness and skin colour were measured on arrival at the laboratory, on removal from the treatment chambers, and at eating ripe. 'Honey Gold' fruit showed reduced days to eating ripe in response to higher temperatures and ethylene treatments, compared to lower temperature and no ethylene treatment. Carbon dioxide had relatively little effect on fruit quality and ripening. The treatment effects on fruit quality were modelled using regression analysis, which is a practical approach to developing predictive models. The model fitting is currently in progress and will investigate the possibility of establishing a 'best fit' model for all quality parameters. The prediction model will assist mango industry personnel to estimate the effects of ripening conditions (especially during transport) on mango quality, and remaining days required after arrival in market to provide fruit at the correct ripeness stage as specified by retailers and consumers.

# Introduction

Current recommendations for postharvest handling of mangoes includes cooling fruit to 13°C on farm, and transporting at 13°C before increasing fruit temperatures to 20°C for ripening in market. These recommendations are based on the principle of retarding ripening during transit because of the risk of excessively high temperatures and accumulation of gases (specifically carbon dioxide; $CO_2$) in the transport container, and the subsequent risk of quality loss during and after transit.

Previous research and monitoring of commercial consignments of 'B74' mango from the Northern Territory to southern states indicated that the newer transport containers have the capacity to maintain temperatures of ripening fruit, assuming adequate air circulation and stow patterns in the container. However, given variations in commercial practices and container performance, air temperatures can still increase above the desired 18°C during transit, and $CO_2$ concentrations can exceed 10%. The use of hydrated lime to absorb the $CO_2$ could maintain concentrations below 5%.

Having the capability to predict the response of mangoes during transit will help commercial operators to minimise quality loss during the transport/ripening stages, providing a more consistent product for the retailers. Scientifically, it will also provide an understanding of fruit tolerance to less than ideal conditions and thus provide targets for commercial operators in terms of temperature and gas composition in order to minimise the risk of quality loss.

The research in this report aims to provide preliminary data for developing a predictive model of the response of 'Honey Gold' mango to likely temperature and gas conditions during commercial road-freight over a 3-5 day period. The fruit were held under three air temperatures, and three concentrations of ethylene and $CO_2$ for four days, and then held at 20°C to determine effects on ripe quality.

This report outlines preliminary analysis of the data collected during the 2015/16 and 2016/2017 mango seasons as part of a higher research degree program.

# Materials and methods

## Fruit

Over two mango seasons, 2015/2016 and 2016/2017, mature hard green 'Honey Gold' mango fruit were harvested at commercial maturity (>15% dry matter content) from single orchards near Katherine, Northern Territory (NT), Dimbulah, North Queensland (NQ) and Bundaberg, South East Queensland (SEQ). The fruit were picked and packed according to standard commercial practice. This included fruit being de-stemmed in

a mango wash solution to prevent sapburn injury, treatment with a postharvest fungicide and insecticide spray, grading for uniform size and quality, and then packing into cardboard trays.

A total of 22 trays per farm of premium grade, count 14 'Honey Gold' fruit were used for each experiment. Fruit were obtained from each farm's packing line. Fruit history was recorded, including farm location, management practices, picking date, packing date, transport temperatures and times. The fruit from Katherine were transported in an air-conditioned vehicle to Darwin, NT, and by air freight to Brisbane, Qld. The fruit were collected and transported in an air-conditioned vehicle to the Maroochy Research Facility (MRF), Nambour, Qld (~48h in transit). The NQ fruit were road transported to Cairns and by air freight to Brisbane, then by road to Maroochy Research Facility (MRF) (~24h in transit). The SEQ fruit were road transported directly to MRF (~4h in transit). The fruit were numbered 1-300 and allocated to treatments within two hours of arrival.

# Treatments

The fruit from each farm were randomly allocated to the following targeted atmosphere treatments in 30 L chambers in a factorial design: 0, 20 and 80 µL $L^{-1}$; 1, 4 and 8 % $CO_2$, and 18°, 22° and 28°C. A hanging control treatment of no added ethylene or $CO_2$ in the barrel, was included. Ten fruit were used per treatment and the fruit held under these atmospheres for four days, to simulate road transportation time.

The fruit for each treatment were placed in 30L plastic chambers, attached to a flow-through gas delivery system based on the concepts of Smith et al. (1997), and pictured in Figure 1. The $CO_2$ was supplied directly from a pressurised cylinder, the ethylene was diluted from 3.9% ethylene in nitrogen ($N_2$; supplied by BOC), to about 2000 µL $L^{-1}$ before being diluted to the desired concentration, and air was provided from low pressure air pumps. The required concentration and flow rates for each gas were obtained by using glass capillaries of a specific internal diameter and length. Targeted flow rates for each gas concentration combination are presented in Table 1. The maximum flow rate of about 1000 ml $min^{-1}$ was sufficient to prevent $CO_2$ accumulation in the chambers derived from fruit respiration (about one barrel volume exchange every 30 min). The relative humidity (RH) in the barrels was above 80%, and required no additional control. A separate gas flow board was used for each temperature (cold room), which controlled 10 chambers in each room.

The outlet from each barrel was connected to a gas sampling system that channelled the outlet from each barrel at predetermined intervals into a single outlet. The single outlet was connected to a flow meter (Honeywell or AALBORG DFM) and an infrared gas analyser (Horiba, Vaisala) for $CO_2$ analysis. Ethylene was measured with an electrochemical ethylene detector (Gas Alarms, Sydney), or a photo ionisation detector cell (Alphasense, UK). Ethylene concentrations were also measured with a Shimadzu gas chromatograph (GC-2010 Plus) fitted with a macro capillary column and a flame ionization detector (FID). Standard gases (BOC) of 5, 20 and 97 µL $L^{-1}$ in nitrogen were used as standards.

Each chamber contained an i-Button™ temperature logger to monitor chamber air temperature, and one chamber per temperature (cold room) contained an i-Button™ RH logger to monitor chamber relative humidity.

The fruit were removed from the chambers after four days and held in a cold room set at 20°C and 85-95% RH until eating soft (hand firmness rating of 4 as described below).

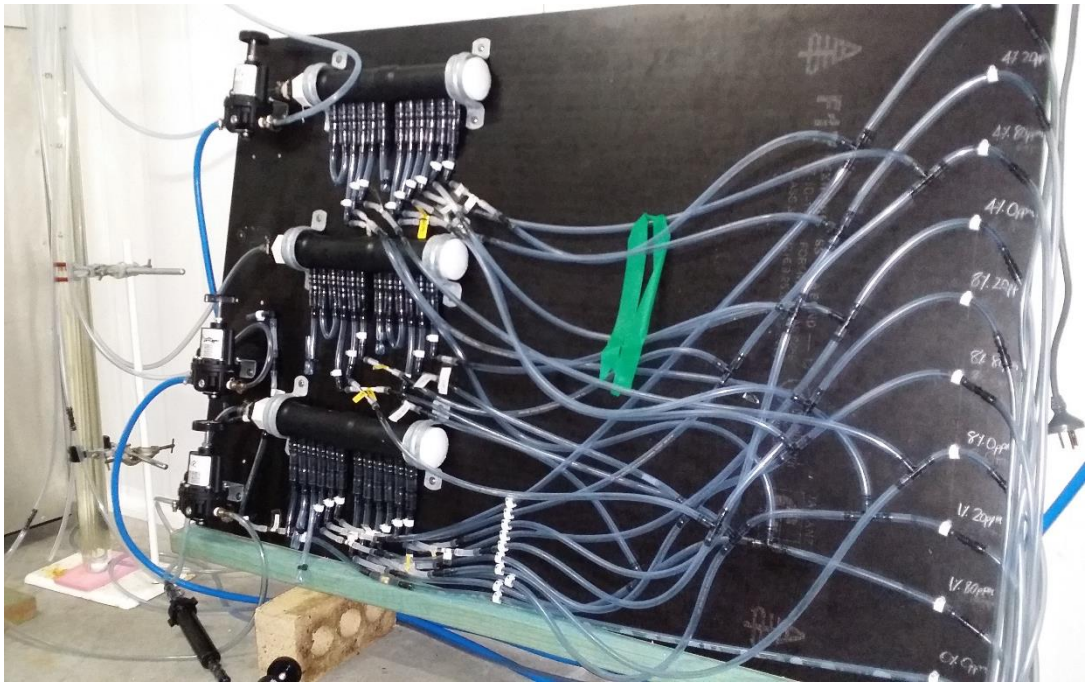


Figure 1: Flow through gas delivery system, or flow board, mixing $C_2H_4$ and $CO_2$ gas and air prior to entering chambers.

Table 1: Indicative flow rates for each of carbon dioxide (CO2) ethylene (C2H2) and air combination for each of the 10 (three for CO2, three for ethylene and a hanging control) atmospheres. The total targeted chamber flow rate was 1000 mL $L^{-1}$.

| Treatment | Target $CO_2$ (%) | Target $C_2H_4$ (µl $L^{-1}$) | Flow rate (mL $L^{-1}$) | | |
|---|---|---|---|---|---|
| | | | $CO_2$ | $C_2H_4$ | Air |
| 1 | 1 | 0 | 10 | 0 | 990 |
| 2 | 1 | 20 | 10 | 8 | 982 |
| 3 | 1 | 80 | 10 | 32 | 958 |
| 4 | 4 | 0 | 40 | 0 | 960 |
| 5 | 4 | 20 | 40 | 8 | 952 |
| 6 | 4 | 80 | 40 | 32 | 928 |
| 7 | 8 | 0 | 80 | 0 | 920 |
| 8 | 8 | 20 | 80 | 8 | 912 |
| 9 | 8 | 80 | 80 | 32 | 888 |
| 10 | 0 | 0 | 0 | 0 | 1000 |

## Assessments

Upon arrival at MRF, the ten fruit per treatment were assessed for weight, skin colour, firmness and presence of external blemishes. After four days, fruit were removed from the chambers and assessed again for weight, skin colour, firmness and external blemishes. Half of the fruit were destructively sampled for total soluble sugars (TSS) and titratable acidity (TA). Fruit firmness was then assessed daily by hand until reaching the eating soft (ES) stage (hand firmness of 4) when they were assessed as above, and then destructively sampled for TSS and TA.

## Fruit dry matter (% DM) content and weight loss

Prior to treatment application, five fruit were used to determine percent dry matter (DM). A cheek from each mango was cut, peeled and grated. A subsample of the mango flesh was weighed then placed into a dehydrator at 65°C until a steady weight was reached. The % DM was calculated as:

Dry Weight (g)/Fresh Weight (g) x 100

Fruit dry matter for both seasons and all regions were above commercial maturity (>15%) (Table 2).

The physiological weight loss was expressed as the % loss of individual fruit based on the original fruit weight.

## Skin colour

Visual skin colour was rated using a scale from 1-6 (1: 0-10% yellow, 2: 10-30% yellow, 3: 30-50% yellow, 4: 50-70% yellow, 5: 70-90% yellow, 6: 90-100% yellow on the fruit skin) as per the Mango Quality Assessment Manual (Hofman et al., 2010).

The skin colour was also assessed using a Minolta Chroma Meter (Minolta Corp, Ramsey, NJ) calibrated to a white standard plate (L* 97.31, a* -0.18, b* 2.45 or X=91.34, Y=93.24 and Z=106.2), in the L*a*b* colour system as L* value (lightness), a* value (redness or greenness) and b* value (yellowness or blueness) and in the X, Y, Z value. Three locations that were free of blush, blemish and sunburn were marked on each fruit and measurements were taken on these locations upon arrival at the MRF, after removal from the chambers and at an ES stage. The L*, a*, b* and X, Y, Z measurements were converted to hue (H°) and chroma (C) with the following calculation:

$L^* = 116(Y/Y_n)^{1/3} – 16$; $a^* = 500\ ((X/X_n)^{1/3} - (Y/Y_n)^{1/3})$; $b^* = 200\ ((Y/Y_n)^{1/3} - (X/Z_n)^{1/3})$ where $X_n$, $Y_n$ and $X_n$ is calibration standard value.

$C^* = (a^{*2} + b^{*2})^{1/2}$;

$H^o = \arctan(b^*/a^*)$.

## Fruit firmness

Individual fruit firmness was measured using an EZ Test Shimadzu, EZ-SX, 100 N (Kyoto, Japan). Firmness was assessed based on the Newtons (N) force required to push a 12 mm spherical probe to a depth of 2 mm into the fruit surface (peel attached) at a rate of 10 mm $min^{-1}$. The probe was applied to the cheek area located at the middle to lower stem-end of each fruit. Subsequent tests after removal from the barrel and at ES were done on different locations on the fruit.

In addition, individual fruit were rated for hand firmness using the scale of 0-4 (0 = hard, no ‘give’ in the fruit; 1 = rubbery, slight ‘give’ in the fruit; 2 = sprung, flesh deforms by 2-3 mm with extreme thumb pressure; 3 = firm soft, whole fruit deforms with moderate hand pressure; and 4 = soft, whole fruit deforms with slight hand pressure) as per the Mango Quality Assessment Manual (Hofman et al*.,* 2010).

## Total soluble solids and titratable acidity

At removal from the chambers and at ES, the peel of each fruit was removed, and a portion of the flesh from each fruit was used for total soluble solids (TSS) content using an ATAGO hand refractometer (PAL – 1). The remaining flesh was frozen and titratable acidity (TA) of the thawed sample was determined by titrating the puree to a pH endpoint of 8.2 using a Mettler Toledo G20 compact titrator with 0.1M sodium hydroxide (NaOH). Results are presented as % citric acid equivalents.

# Experimental design and statistical analyses

A 3 x 3 factorial design was used consisting of three $CO_2$ and three $C_2H_4$ concentrations with three temperatures. A single hanging control was included with each temperature. Ten individual fruit were used per treatment. Prior to each experiment, chambers were randomised on shelving in each of the three cold rooms using a Latin-square design. Cold room temperatures were also randomised for each of the experiments for each production location. Statistical analyses were conducted using Genstat® statistical

software and results are presented using the averaged data per chamber for each harvest region analysed. Each chamber is considered an experimental unit. Three-way ANOVA and regression analysis were conducted using the same data set, which allows for comparison of fitted models.

# Results and discussion

## % dry matter

The % DM of the fruit harvested from NT and NQ stayed unchanged over the season while DM of SEQ fruit harvested in 2016 was much lower than that in 2017. (Table 2).

Table 2: Average % dry matter of 'Honey Gold' mango fruit obtained from the three growing regions for trials in the 2015/16 and 216/7 seasons.

| Region | % dry matter | |
|---|---|---|
| | 2015/2016 | 2016/2017 |
| **Northern Territory** | 19.2 b | 18.7 b |
| **North Queensland** | 15.9 cd | 16.7 c |
| **South East Queensland** | 15.5 d | 21.2 a |

Means with same letter in the column are not significantly different at the P = 0.05 level. (n= 5)

### *2015/2016 season*

### *Katherine*

The desired ethylene gas concentrations were not achieved for the Katherine experiment, mostly due to ethylene contamination in the air used to provide the targeted gas concentrations. Also, the time from harvest to the start of the atmosphere treatments was about 2.5 days, by which time the ripening process had progressed sufficiently for the atmosphere treatments to have minimal effect. Hence, fruit from all treatments had similar firmness and skin colour on removal and all fruit ripened within about nine days from harvest.

Design improvements (including the initial 20 times dilution of ethylene performed outside the laboratory rather than in the cold rooms to reduce air contamination) and reduced time between harvest and the start of treatment were implemented for the NQ and SEQ experiments. Typical gas concentrations for the NQ an SEQ trials are presented in Figure 2.

### *Fruit responses*

Similar results were obtained for both the NQ and the SEQ fruit; selected results for SEQ only are presented below.

Treatments of 20 and 80 µL $L^{-1}$ of ethylene resulted in significantly softer fruit at removal from the chambers (Figure 3), compared with no ethylene. There was little difference between 20 and 80 µL $L^{-1}$. Fruit held at 26°C were softer at removal, for all ethylene concentrations. There was no interaction between ethylene and $CO_2$ effects. 4% $CO_2$ resulted in firmer fruit compared with 1% $CO_2$, but there was little difference between 4 and 8% $CO_2$ (Figure 4).

Following removal from each chamber, fruit held at 26°C were more yellow than those held at 18°C (Plate 1). 20 and 80 µL $L^{-1}$ ethylene resulted in similar, and significantly more yellow skin colour at removal, compared with no ethylene. At 18°C, 4 and 8% $CO_2$ resulted in less yellow colour compared with 1% $CO_2$,

and this effect was more noticeable at 20 and 80 µL $L^{-1}$ ethylene. These effects were less obvious at 22° and 26°C.

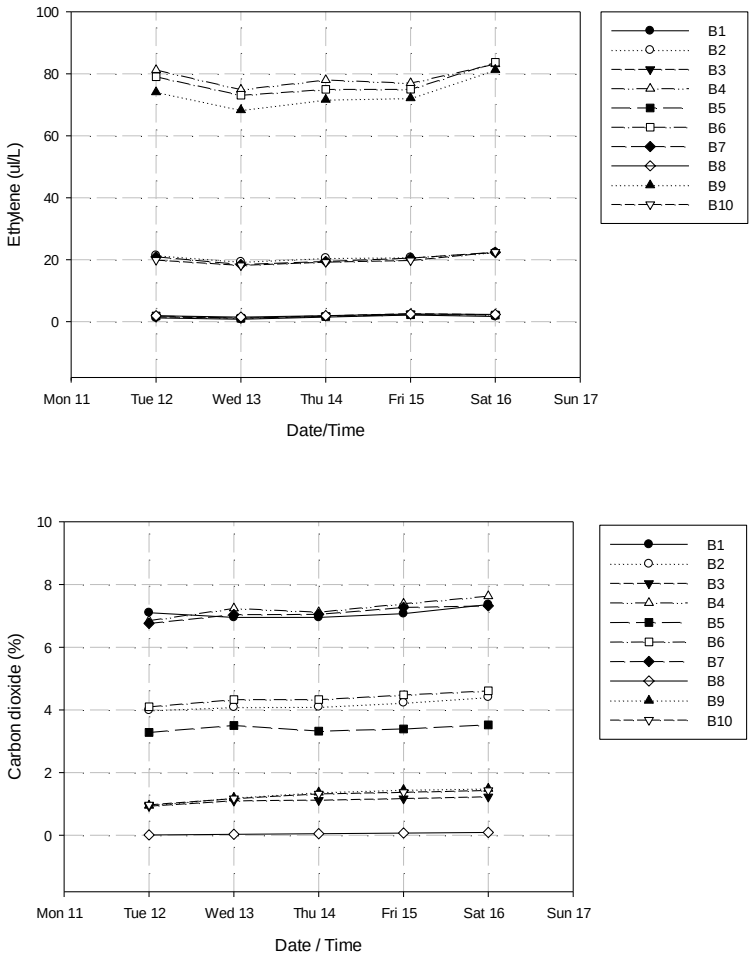



Figure 2: Ethylene and carbon dioxide concentrations in the trial with north Queensland fruit held at 18°C. B refers to barrel number. The target concentrations were 0, 20 and 80 µL L-1 ethylene, and 1, 4 and 8% CO2. A hanging control at ambient CO2 and ethylene.

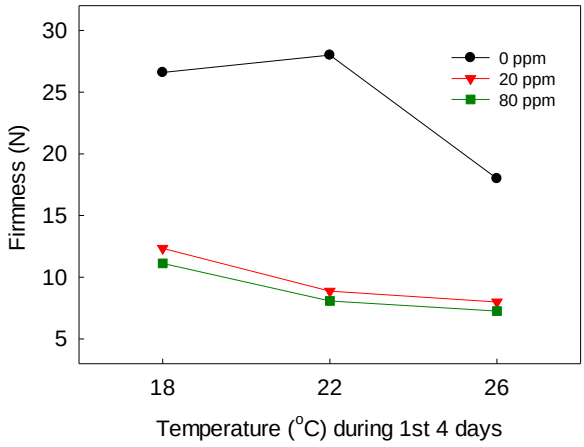



Figure 3: The firmness (N) of 'Honey Gold' mango fruit from south-east Queensland following four days treatment at different temperatures and ethylene concentrations (µL $L^{-1}$). The results are averaged across the three carbon dioxide concentrations.

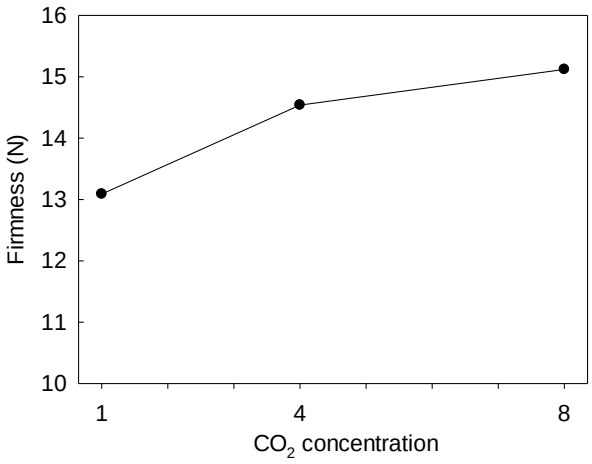



Figure 4: The firmness (N) of 'Honey Gold' mango fruit from Southeast Queensland following four days treatment at different carbon dioxide concentrations (%). The results are averaged across the three ethylene concentrations and temperatures.

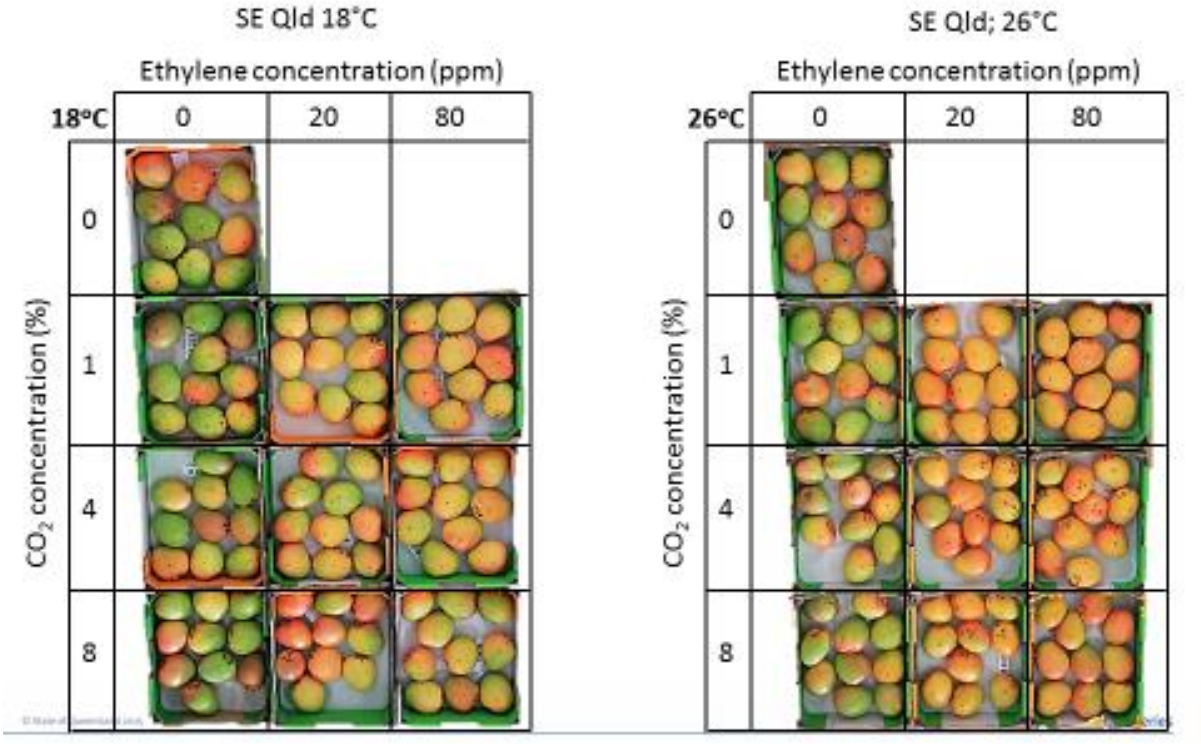



Plate 1: Visible effects on 'Honey Gold' mango fruit from Southeast Queensland, following exposure to several ethylene and carbon dioxide concentrations for four days at 18° and 26°C.

## *Preliminary analysis and modelling 2015/2016 and 2016/2017 seasons*

Fruit quality assessment data collected from the 2015/2016 and 2016/2017 mango seasons were initially analysed using a general 3-way ANOVA. The ANOVA analysis used region (NT, NQ and SEQ) + a full factorial model:

Region + Temperature * $C_2H_4$ * $CO_2$

This model included all possible interactions, and used target gas concentrations (1, 4 and 8% $CO_2$ and 0, 20 and 80 µL $L^{-1}$ $C_2H_4$). The table of significance for the ANOVA models for the 2015/2016 and 2016/2017 seasons at removal from the treatment chambers (Exiting) and at eating soft (Final) indicates that temperature and $C_2H_4$ are important for most quality parameters, with $CO_2$ having a lesser effect on firmness and brix, but significant effect of $CO_2$ on colour (Hue and chroma) was found at the removal for both seasons (Table 3 and Table 4). Both tables of significance show the coefficient of variation (CV%) and adjusted $R^2$. The CV% is used to measure the spread of variation from the mean. In both tables of significance it is used instead of the standard deviation to compare the spread of data sets that have different units. For example, firmness is measured in Newtons (N) and Hue is measured in degrees (°). Generally expressed as a percentage, the higher the CV%, the greater the dispersion around the mean (National Institute of Statistics and Economic Studies, 2016). The adjusted $R^2$ (adj $R^2$) is a modified version of $R^2$ that has been adjusted for the number of predictors in the model. An increase in the adj $R^2$ is only seen if the new term improves the model than would be expected by chance.

The ANOVA model assumes that the treatment levels (1, 4 and 8% $CO_2$ and 0, 20 and 80 µL $L^{-1}$ $C_2H_4$) are accurate, however the actual measured levels sampled were different from the set levels. For example, the 2016/2017 NT experiment recorded a sample average of 23.67 µL $L^{-1}$ of ethylene over 4 days treatment, with a range of 19.6 – 34.73 µL $L^{-1}$ for a 20 µL $L^{-1}$ target concentration (Table 5). The target concentration of 4% $CO_2$ measured a sample average of 4.92% over 4 days (Table 6). The minimum and maximum range shows the variability in response from supposed stable concentrations. Due to this variability and range in sample characteristics between target concentrations and actual measured values, the data was then analysed using a full regression model. Two terms were introduced to better understand the differences between regions; Territory and North. The full regression model is:

Territory/North+Temperature.$C_2H_4$.$CO_2$

Using the averaged actual measured values of $C_2H_4$ and $CO_2$, the regression models produced the best fit for each measured parameter by removing the least significant terms. An example of the 2015/2016 season Chroma and Hue at 'Exit' ANOVA and regression models are compared in Table 7. As the regression models only use the significant terms, the adjusted $R^2$ increases, which gives the best fit for both the Chroma and Hue parameters compared to the ANOVA models (77 and 85 adj $R^2$ compared to 25 and 76 adj $R^2$ ANOVA). Table 8 compares regression models of fruit parameters over the two mango seasons. There are similarities between model terms, with $C_2H_4$ and temperature influencing the majority of models.

Table 3: Table of significance for 2015/2016 'Honey Gold' mango quality parameters at 'Exiting' the treatment chambers and at eating soft ('Final').

| | Fruit characteristic | | | | | |
|---|---|---|---|---|---|---|
| | **Firmness** | **Hue** | **Chroma** | **TA** | **Brix** | **Water loss** |
| **2015_2016 Quality parameters at removal from the chambers (Exiting)** | | | | | | |
| Region | NA | NA | NA | NA | NA | NA |
| Temp | *** | *** | *** | *** | *** | *** |
| C2H4 | *** | *** | *** | *** | *** | *** |
| CO2 | * | *** | *** | ns | * | ns |
| Temp*C2H4 | ** | *** | *** | ns | *** | ns |
| Temp*CO2 | ns | ns | ns | ns | * | ns |
| C2H4*CO2 | ns | ** | * | ns | ns | ns |
| Temp*C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| CV % | 137 | 19 | 12 | 81 | 50 | 23.2 |
| adj $R^2$ % | 61 | 76 | 75 | 20 | 77 | 85 |
| **2015_2016 Quality parameters at ripe (Final)** | | | | | | |
| Region | NA | NA | NA | NA | NA | NA |
| Temp | ns | ** | *** | ns | ns | *** |
| C2H4 | ns | *** | *** | ns | ns | *** |
| CO2 | * | ns | ns | ns | ns | * |
| Temp*C2H4 | ns | ns | ns | ns | ns | ns |
| Temp*CO2 | ns | ns | ns | ns | ns | ns |
| C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| Temp*C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| CV % | 7 | 13 | 31 | 4 | 258 | 46 |
| adj $R^2$ % | 33 | 65 | 61 | 79 | 5 | 67 |

Table 4: Table of significance for 2016/2017 'Honey Gold' mango quality parameters at 'Exiting' the treatment chambers and at eating soft ('Final').

| | Fruit characteristic | | | | | |
|---|---|---|---|---|---|---|
| | **Firmness** | **Hue** | **Chroma** | **TA** | **Brix** | **Water loss** |
| **2016_2017 Quality parameters at Exiting** | | | | | | |
| Region | *** | *** | ** | *** | *** | *** |
| Temp | *** | *** | *** | *** | *** | *** |
| C2H4 | *** | *** | *** | ns | *** | *** |
| CO2 | ns | *** | ** | ns | ns | ns |
| Temp*C2H4 | ns | ** | ns | ns | ns | ns |
| Temp*CO2 | ns | ns | ns | ns | ns | ns |
| C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| Temp*C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| CV % | 79 | 12 | 13 | 49 | 22 | 41 |
| adj R2 % | 78 | 82 | 70 | 43 | 61 | 85 |
| **2016_2017 Quality parameters at Final** | | | | | | |
| Region | *** | *** | ns | *** | *** | *** |
| Temp | ns | *** | * | ns | ns | *** |
| C2H4 | *** | *** | *** | *** | *** | *** |
| CO2 | ns | ns | ns | ns | ns | ns |
| Temp*C2H4 | ns | ns | * | ns | ns | ns |
| Temp*CO2 | ns | ns | ns | ns | ns | ns |
| C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| Temp*C2H4*CO2 | ns | ns | ns | ns | ns | ns |
| CV % | 16 | 11 | 63 | 16 | 71 | 32 |
| adj R2 % | 79 | 76 | 38 | 73 | 70 | 84 |

Table 5: The average and minimum and maximum of actual measured $C_2H_4$ (µL $L^{-1}$) concentrations from three regions. Average of each chamber over 4 days, (treatment duration), for the 2016/2017 mango season. Averaged actual measured values differ from target concentrations, which may have a physiological effect on fruit treated with the target concentrations of 0 µL $L^{-1}$ that actually received $C_2H_4$ treatment (SD is standard deviation and SE is standard error)..

| | Average | Min | Max | SD | SE |
|---|---|---|---|---|---|
| **NT** | | | | | |
| **0 µL $L^{-1}$** | 0.17 | 0 | 0.44 | 0.14 | 0.04 |
| **20 µL $L^{-1}$** | 23.67 | 19.60 | 34.73 | 4.56 | 1.52 |
| **80 µL $L^{-1}$** | 86.27 | 72.34 | 94.62 | 7.57 | 2.52 |
| **NQ** | | | | | |
| **0 µL $L^{-1}$** | 0.67 | 0 | 7.64 | 2.20 | 0.63 |
| **20 µL $L^{-1}$** | 21.62 | 18.44 | 32.95 | 4.37 | 1.46 |
| **80 µL $L^{-1}$** | 78.72 | 70.53 | 95.20 | 7.25 | 2.42 |
| **SEQ** | | | | | |
| **0 µL $L^{-1}$** | 0.30 | -0.40 | 1.14 | 0.40 | 0.12 |
| **20 µL $L^{-1}$** | 21.95 | 17.36 | 31.72 | 4.37 | 1.46 |
| **80 µL $L^{-1}$** | 79.32 | 69.01 | 99.23 | 8.42 | 2.81 |

Table 6: The average and minimum and maximum range of actual measured $CO_2$ (%) concentrations compared to target concentrations for all regions from the 2016/2017 mango season. $CO_2$ concentrations averaged from each chamber over 4 days (SD is standard deviation and SE is standard error).

| | Average | Min | Max | SD | SE |
|---|---|---|---|---|---|
| **NT** | | | | | |
| **0 %** | 0.23 | 0.11 | 0.31 | 0.11 | 0.06 |
| **1 %** | 1.67 | 1.28 | 2.05 | 0.28 | 0.09 |
| **4 %** | 4.92 | 3.52 | 8.02 | 1.31 | 0.44 |
| **8 %** | 8.02 | 7.29 | 8.68 | 0.53 | 0.18 |
| **NQ** | | | | | |
| **0 %** | 0.14 | 0.07 | 0.25 | 0.09 | 0.05 |
| **1 %** | 1.58 | 1.19 | 2.99 | 0.55 | 0.18 |
| **4 %** | 4.50 | 3.51 | 7.56 | 1.25 | 0.42 |
| **8 %** | 7.65 | 7.06 | 8.44 | 0.50 | 0.17 |
| **SEQ** | | | | | |
| **0 %** | 0.18 | 0.07 | 0.28 | 0.10 | 0.06 |
| **1 %** | 1.53 | 1.36 | 1.75 | 0.13 | 0.04 |
| **4 %** | 4.55 | 3.51 | 7.75 | 1.31 | 0.44 |

| | | | | | |
|---|---|---|---|---|---|
| **8 %** | 7.64 | 7.12 | 8.45 | 0.56 | 0.19 |

Table 7: Model comparison table, comparing two colour parameters at 'Exit' and their ANOVA and regression models from the 2015/2016 mango season. The higher the adj $R^2$, the better the fit for the model. Model comparison is possible due to using the same data set.

| Model No. | Parameter | Model Terms | df | adj $R^2$ | p value | Comments |
|---|---|---|---|---|---|---|
| **1 (ANOVA)** | Exit Chroma | Region*Temp*C2H4*CO2 *Temp_C2H4*Temp_CO2 *C2H4_CO2*Temp_C2H4_ CO2 | 52 | 75 | | Region + full factorial model |
| **2 (Regression)** | Exit Chroma | Territory*North*C2H4*C 2H4Sq*TempCO2*TempS q | 74 | 77 | <0.001 | Removed CO2, CO2Sq, Temp, CO2_C2H4, Temp_C2H4 interactions |
| **1 (ANOVA)** | Exit Hue | Region*Temp*C2H4*CO2 *Temp_C2H4*Temp_CO2 *C2H4_CO2*Temp_C2H4_ CO2 | 52 | 76 | | Region+full factorial model |
| **2 (Regression)** | Exit Hue | Territory*North*C2H4*T emp*C2H4Sq*Temp_CO2 *TempSq | 72 | 85 | | Removed CO2, CO2Sq, Temp_C2H4 interactions |

Table 8: Comparisons between regression models for 2015/2016 and 2016/2017 season. Potential model terms are listed across the top of the table. Highlighted cells indicate significant model terms. Each parameter has an individual model, with some terms similar to other models. Further investigations will identify if a 'common' model for all parameters is possible.

| Parameter | df | adj $R^2$ | Territory | North | $B_1$ Averaged $C_2H_4$ | $B_2$ Temp | $B_4 C_2H_4^2$ | $B_5$ Temp$^2$ | $B_3$ Averaged $CO_2$ | $B_6 CO_2^2$ | $B_7$ Temp.$C_2H_4$ | $B_8$ Temp.$CO_2$ | $B_9$ $C_2H_4$.$CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2015/2016** | | | | | | | | | | | | | |
| Exit Hue | 72 | 85 | | | | | | | | | | | |
| Exit Chroma | 74 | 77 | | | | | | | | | | | |
| Exit Brix | 74 | 87 | | | | | | | | | | | |
| Exit Firmness | 76 | 80 | | | | | | | | | | | |
| Exit WL% | 79 | 87 | | | | | | | | | | | |
| Exit TA | 78 | 27 | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| **2016/2017** | | | | | | | | | | | | | |
| Exit WL% | 74 | 87 | | | | | | | | | | | |
| Exit Hue | 74 | 75 | | | | | | | | | | | |
| Exit Chroma | 75 | 73 | | | | | | | | | | | |
| Exit TA | 67 | 48 | | | | | | | | | | | |
| Exit Firmness | 75 | 73 | | | | | | | | | | | |
| Exit Brix | 76 | 64 | | | | | | | | | | | |

The regression models have the potential to be used as predictive models, by using the following formula:

$$Y = B_0 + B_1 E + B_2 T + B_3 C + B_4 E^2 + B_5 T^2 + B_6 C^2 + B_7 T.E + B_8 T.C + B_9 E.C$$

Where:

Y = dependent variable (Brix, Ta etc.)

E = average level of Ethylene

C = average level of $CO_2$

T = average temperature

E, C and T = independent (predictor) variables

$B_0$ to $B_9$ = estimated regression parameters

The formula can be used to change the terms specific to the model, i.e. $C_2H_4$ or $CO_2$ gas concentrations or temperature, to predict the outcome of the fruit quality parameter. An example is shown in Figure 5. The potential model terms are highlighted in red on the left hand side. The Brix model has significant terms of: Constant, Territory, North, Av_$C_2H_4$, Temperature, $C_2H_4^2$ and Temperature$^2$ (highlighted in green). The TA model has the same significant terms (highlighted in blue). By using the above formula, the $C_2H_4$ and temperature concentrations can be increased using the slider bar, this increase in turn changes the predicted outcome of the Brix and TA. Changing the $CO_2$ level will not affect the predicted outcome for Brix or TA in this example, as $CO_2$ is not a significant term in either model. The current settings of 0 µL $L^{-1}$ and 18°C gives a predicted outcome of 9.5° Brix and 1.2 TA (Figure 5). The graph on the right hand side is for predicted Brix and reflects the predicted Brix outcome.

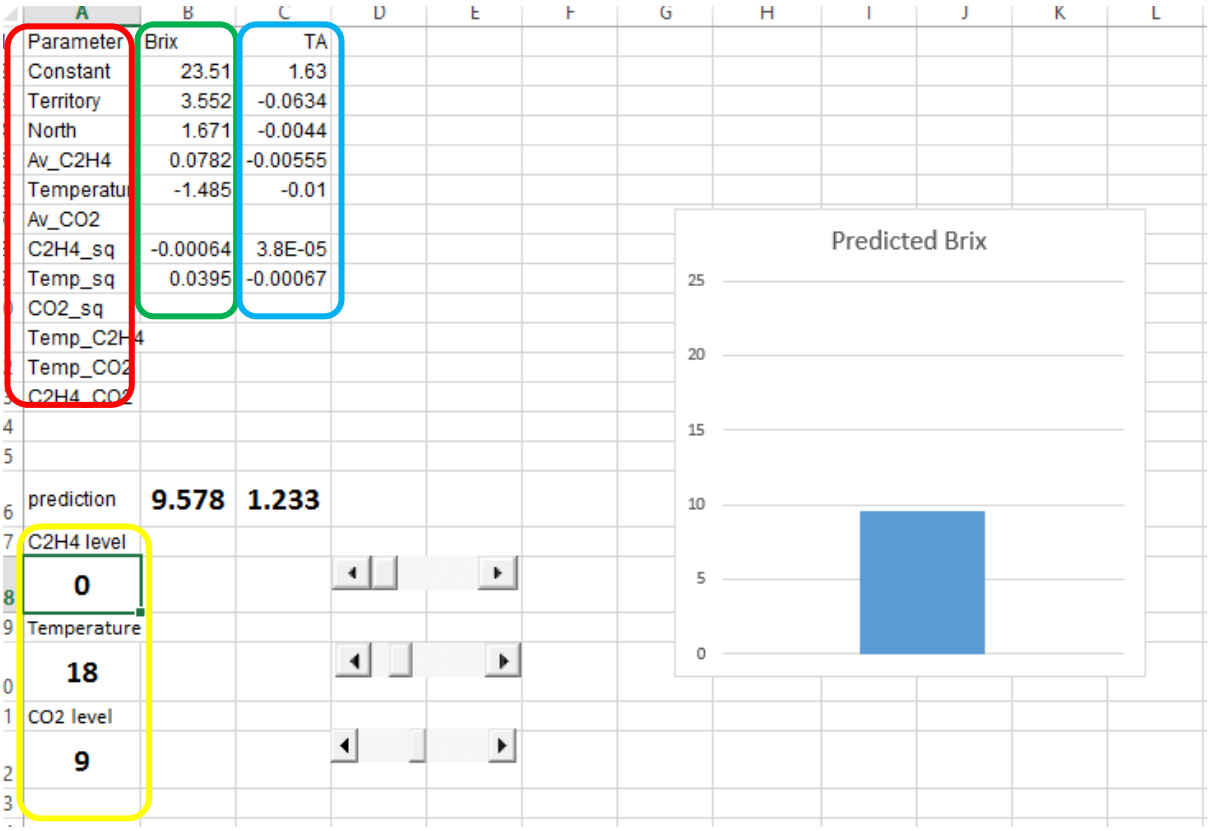
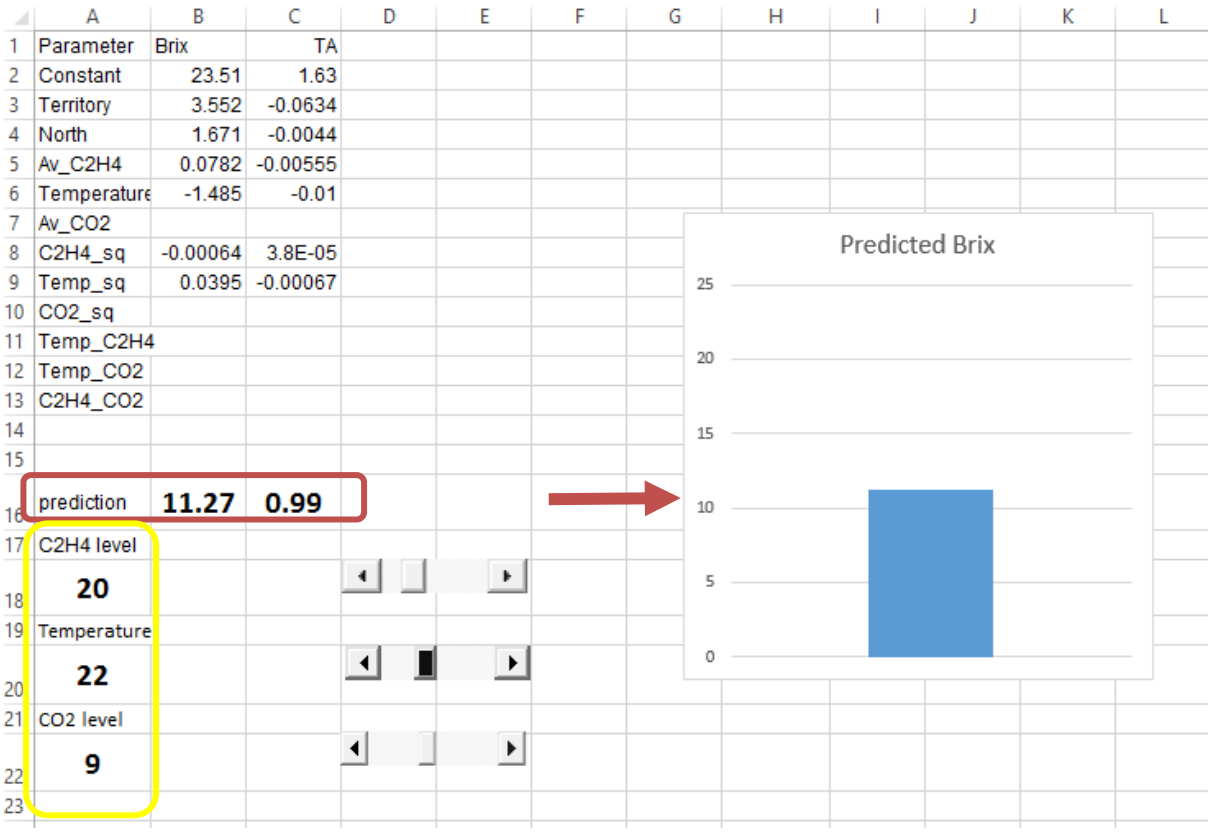



Figure 5: Example of predictive models for 2015/2016 'Honey Gold' Brix and TA at 'Exit' (on removal from the treatment chambers). The model potential terms are shown on the left hand side (highlighted in red). The significant terms for each model are highlighted: Brix (green) and TA (blue). The predicted outcome for each parameter (in bold, Brix and titratable acidity) can change as the $C_2H_4$ concentration (currently set on 0 µL $L^{-1}$) and temperature level (currently set on 18°C) changes. Changing the $CO_2$ level for both of these models will not affect the predicted outcome, as $CO_2$ is not considered a significant term for either the Brix or TA model. All levels are highlighted in yellow. As the predicted outcome for Brix changes, the change is reflected in the graph on the right hand side.




As the $C_2H_4$ and temperature numbers (highlighted in yellow) are increased to 20 µL $L^{-1}$ and 22°C (Figure 6), the predicted outcome for Brix increases 11.8° and the TA is 0.99 (highlighted in orange). The Brix graph has also changed to reflect the new predicted outcome (orange arrow).

Figure 6: Example of predictive models for 2015/2016 'Honey Gold' Brix and TA at 'Exit' (on removal from the treatment chambers). The $C_2H_4$ concentration has increased to 20 µL $L^{-1}$ and the temperature has increased to 22°C (highlighted in yellow). The predicted outcome for Brix is now 11.27° and 0.99 for TA (highlighted in orange). The graph on the right hand side has also changed to reflect the new predicted outcome (orange arrow).

Increasing the $C_2H_4$ and temperature (highlighted in yellow) to 80 µL $L^{-1}$ and 26°C (Figure 7), changes the predicted outcome for Brix to 13.77° and the TA is 0.717 (highlighted in orange). The Brix graph has also changed to reflect the new predicted outcome (orange arrow).

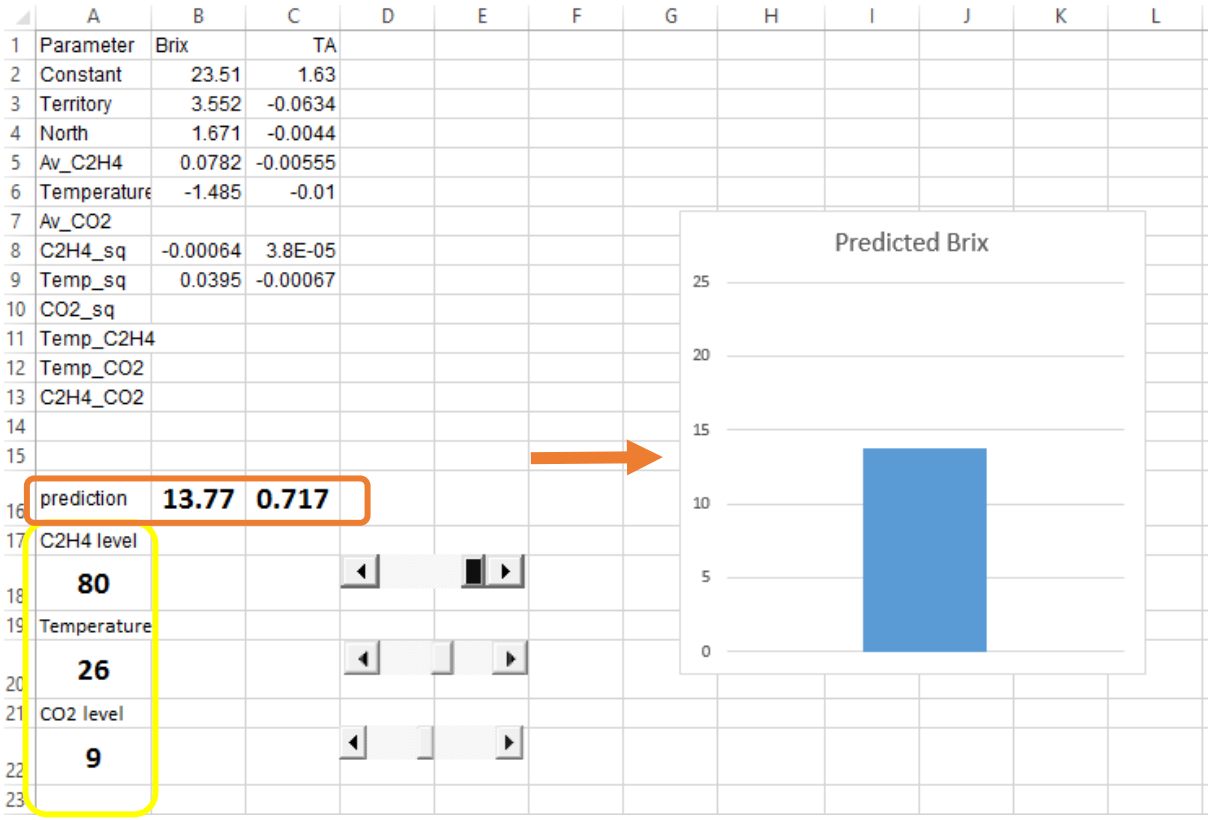



Figure 7: Example of predictive models for 2015/2016 'Honey Gold' Brix and TA at 'Exit' (on removal from the treatment chambers). The $C_2H_4$ concentrations has increased to 80 $\mu L\ L^{-1}$ and the temperature has increased to 26°C (highlighted in yellow). The predicted outcome for Brix is now 13.77° and 0.717 for TA (highlighted in orange). The Predicted Brix graph also reflects the new predicted Brix outcome (orange arrow).

There is potential to continue developing this predictive model further, by introducing an outcome per region. Figure 8 shows three parameters: Brix, TA and Days to ER and their individual model terms. Once again the $C_2H_4$ and temperature settings can be adjusted to predict their outcomes, with $CO_2$ considered a non-significant term for the three models. This predictive model has included the three separate regions: NT, NQ and SEQ. As the predicted outcome changes, so too does the corresponding graph. This predictive model has the ability to indicate differences between regions for individual quality parameters.

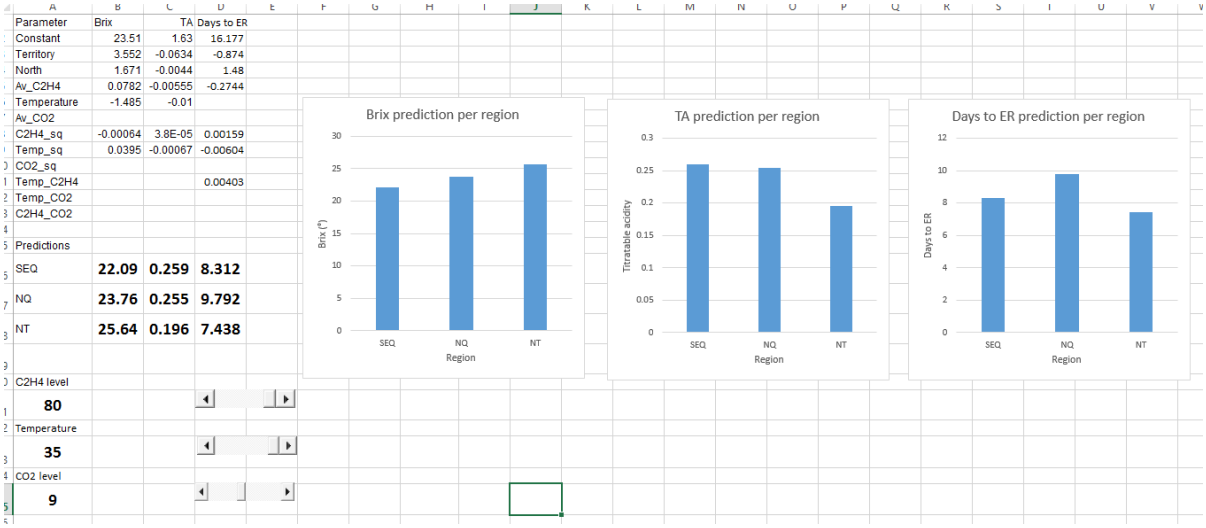



Figure 8: An example of predictive models for 2015/2016 'Honey Gold' Brix, TA and days to eating ripe at 'Exit' (on removal from the treatment chambers) per region (NT, NQ and SEQ). The predicted outcome for each parameter (in bold, Brix, TA and days to eating ripe) can change as the $C_2H_4$ concentration (currently set on 80 µL $L^{-1}$) and temperature level (currently set on 35°C) changes. Changing the $CO_2$ level for these models will not affect the predicted outcome, as $CO_2$ is not considered a significant term for either the Brix, TA or days to eating ripe model. As the predicted outcome for Brix, TA and days to eating ripe changes, the change is reflected in the graphs on the right hand side. The bars on the graph show the difference between regions for each predicted outcome.

Another example of predictive modelling is presented in Figure 9, with the regression model of firmness at Exit. The Firmness model terms are used to calculate predictions which are displayed in a heat map (the table with coloured cells). As the $C_2H_4$ concentration (µL $L^{-1}$) (across the top of the heat map) and the temperature (°C) (left hand side of the heat map) increases, the firmness (N) predictions change, along with the corresponding colour. For example, 50 µL $L^{-1}$ $C_2H_4$ and 27°C is the darkest red cell and is the softest firmness prediction, which predicts a firmness of 3.6445 N. Firmness predictions can then be graphed, showing the relationship between the treatment temperatures (18, 22 and 26°C), $C_2H_4$ levels (0-80 µL $L^{-1}$) and firmness predictions. The graph in Figure 8 shows the difference between temperatures is greater at 0 µL $L^{-1}$ $C_2H_4$. This difference between temperatures decreases as $C_2H_4$ concentrations increase and predicted firmness levels decrease.

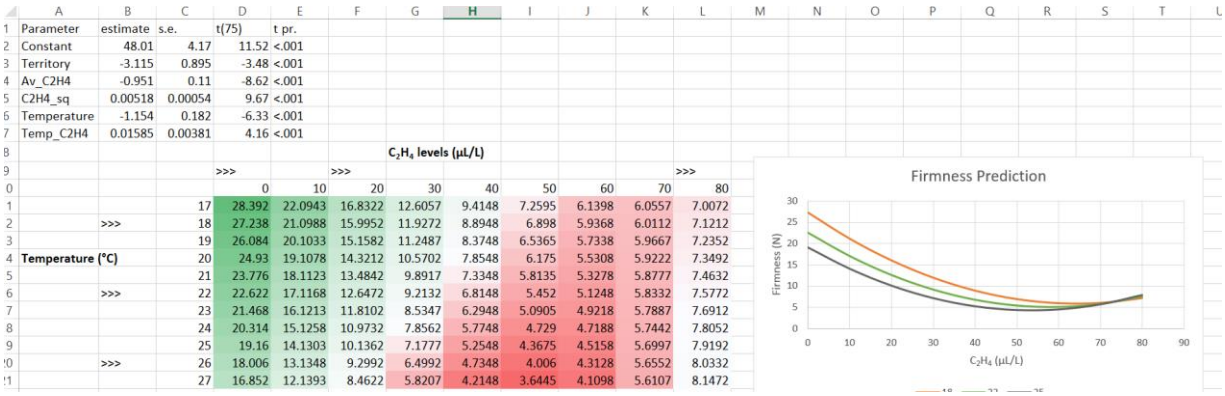



Figure 9: Example of firmness prediction using a heat map of 2015/2016 'Honey Gold' mangoes at 'Exit' (on removal from the treatment chambers). As temperature and $C_2H_4$ concentrations increase, the fruit firmness (N) decreases. The softer the mango, the darker the colored red cell. For example, at 50 µL $L^{-1}$ $C_2H_4$ and 27°C, the fruit firmness is 3.6445 (N).

Using the average $C_2H_4$ concentrations recorded for the 2015/2016 experiment, a polynomial response surface that is a function of E (ethylene), T (temperature) and C ($CO_2$) can be fitted. An example for 'Honey Gold' Brix is shown in Figure 10, where averaged $C_2H_4$ concentrations recorded ranged from 0 – 107 µL $L^{-1}$. The graphs show the relationship between the predicted curve (solid line) for Brix (°) and the actual measured values at different $C_2H_4$ concentrations and temperatures (a mark represents a data point). It also shows there is a difference between regions at each temperature, with 26°C fruit producing the highest Brix readings.

The preliminary modelling between the actual and predicted days to ER at different C2H4 concentrations and temperatures is presented in **Error! Reference source not found.** . The preliminary model suggests decreasing days to ER with increasing ethylene concentrations up to about 60 µL L-1, an increasing days to ER thereafter. Further analysis may indicate a better fitting model based on decreasing days to ER between 0-20 µL L-1 then little effect of increasing ethylene concentration up to 80 µL L-1. The long delay (>48 hours) in transporting 'Honey Gold' fruit from the NT to the laboratory and higher than targeted C2H4 concentrations reduced the treatment effects on NT fruit, and the fruit ripened at 8 days after harvest irrespective of ripening temperature.

# Conclusions

The preliminary statistical analysis suggests $CO_2$ within the concentrations tested had relatively little effect on quality at simulated ripening arrival, and when ripe. The major determinants of ripening time were ethylene concentration, and to a lesser extent, ripening temperature. Ethylene could override any negative effects of high $CO_2$ concentrations on skin colour, when transport temperatures are about 22°C. More detailed statistical analysis will provide more insights into 'Honey Gold' mango treatment responses.

The regression analysis and model fitting is a viable approach, and by including only the significant terms in the model a better fit for the model is obtained compared to the ANOVA model. To continue the modelling process with the simulated in-transit ripening data, consistencies across seasons and across parameters will be investigated. These investigations may identify a possible common model with useful terms that can be used to predict outturn and ripe 'Honey Gold' mango fruit quality based on the measured parameters of ethylene and $CO_2$ concentrations, and transit temperature. Additional laboratory trials may also help predict the effect of the duration of in transit ripening conditions. For example, the four day treatment used in these trials reflect longer journey times from the Northern Territory to southern markets, rather than the shorter, 2-3 day journey from the Northern Territory to Brisbane.

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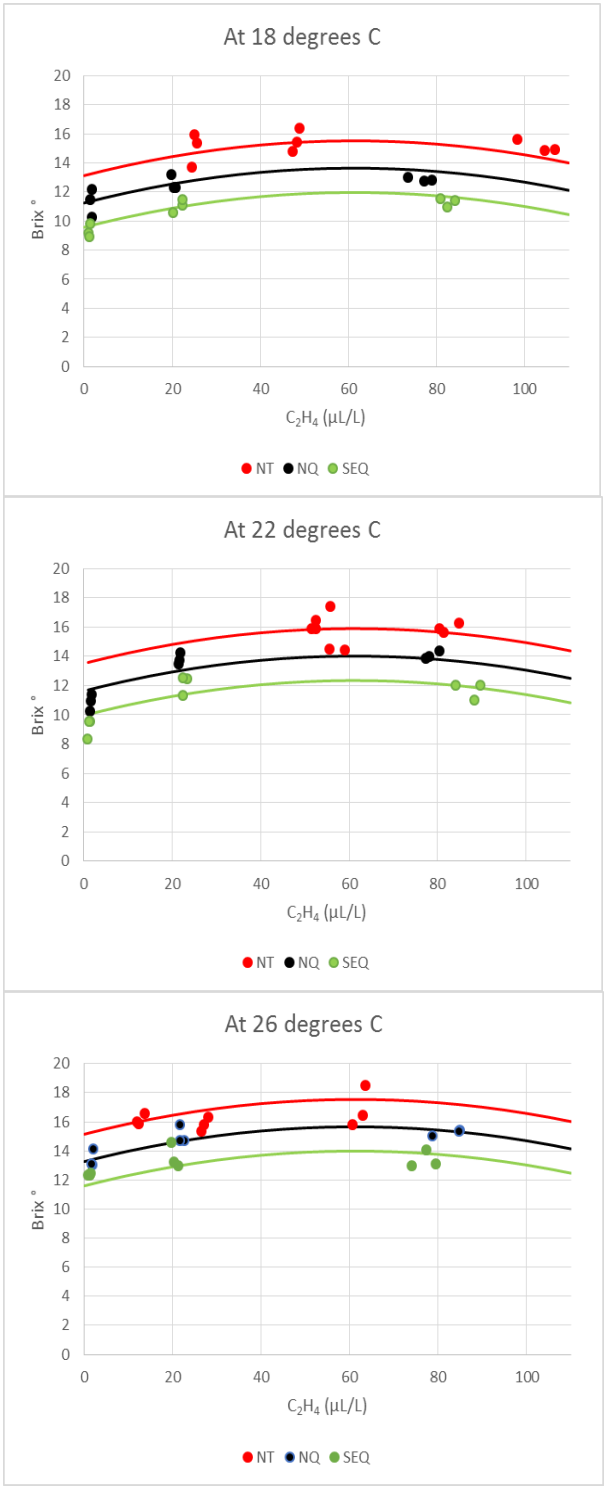


Figure 10: Preliminary modelling of the effect of $C_2H_4$ (µL $L^{-1}$) concentration and temperature on the 'Honey Gold' mango Brix from three regions (NT, NQ and SEQ) in 2015/2016. Fruit were held at differing temperatures (18, 22 and 26°) for four days, then ripened. The solid lines represent the predicted results based on averaged data and the marks represent the actual measured data.

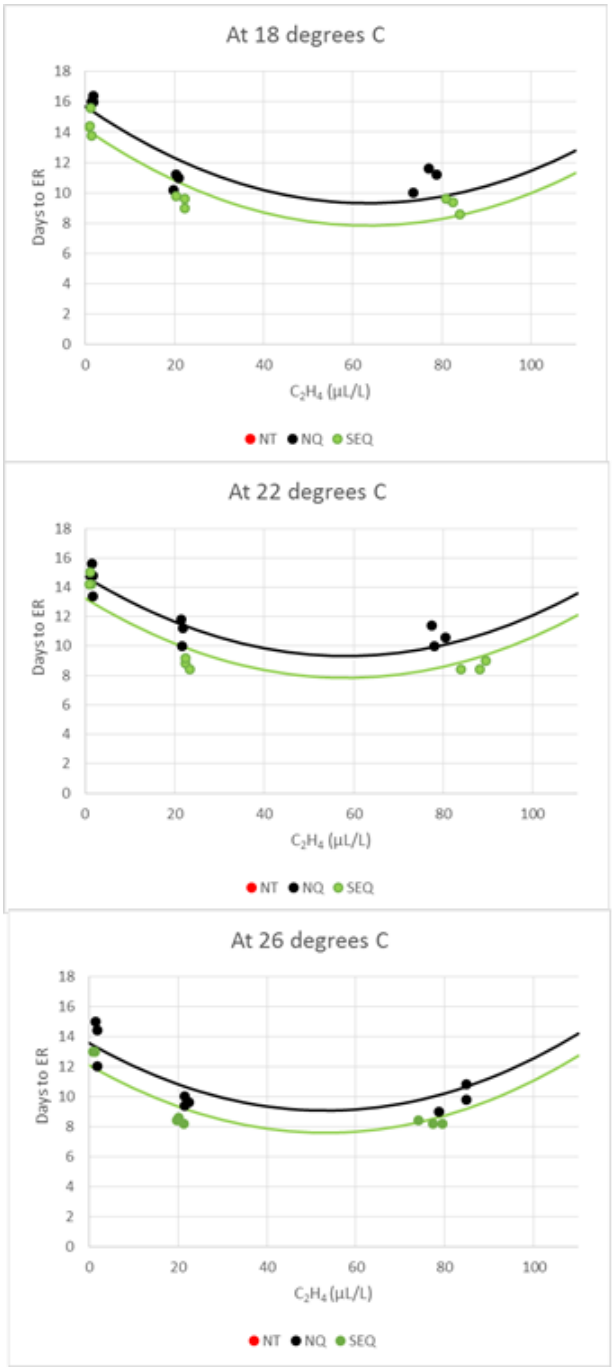


Figure 11: Preliminary modelling of the effect of $C_2H_4$ (µL $L^{-1}$) concentration and temperature on 'Honey Gold' mango fruit days to eating ripe (ER) from two regions (NQ and SEQ) in 2015/2016. Fruit were held at differing temperatures (18, 22 and 26°) for four days, then ripened. The solid lines represent the predicted results based on averaged data and the symbols represent the actual measured data. Further refinement of the model may indicate a better fit based on reduction in days to ER between 0 and 20 µL $L^{-1}$ ethylene, and little change between 20-80 µL $L^{-1}$.

# In-transit ripening of mango fruit: Concepts and considerations.

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**Abstract**

**Refrigerated containers are used to transport mangoes over long distances. In Australia, mangoes grown in northern tropical production areas for the domestic market are transported by road and / or rail for up to 4000 km to southern ripening centres. There is no other viable option for grower's to ripen their fruit. A current project is investigating sustained ethylene release systems for ripening mango fruit in road transport containers over up to 4 days in-transit. This approach could potentially provide an alternative cost-effective ripening strategy for the mango industry. Studies have been and will be performed using shipments of 'B74' and 'Honey Gold' mango fruit, respectively, in refrigerated road containers. In-container environment monitoring, fruit shelf life and quality evaluation at outturn are carried out. Corresponding laboratory experiments that simulate transit are to be conducted to with a view to model mango fruit responses to in-transit conditions.**

**Keywords:** mango, temperature, ethylene, carbon dioxide

## INTRODUCTION

Mango production within Australia occurs in the Northern Territory, Queensland, Western Australia, New South Wales, Victoria and South Australia. The mango cultivars grown for domestic and export markets include Kensington Pride, B74, R2E2, Honey Gold and Keitt (AMIA, n.d.). Mango production in Australia for the last 5 years has been ~53,000 tonnes, with a gross farm gate value ~$140 million per annum (AMIA, n.d.).

About half of Australian mangoes are produced in tropical areas (Figure 1). The climacteric fruit are harvested at mature firm green. They are generally pre-cooled to 12°C within 48 hours of harvest. Such practices prolong their storage life and maximise ripe fruit quality, including final fruit skin colour development (Meurant et al., 1999).

In Australia, refrigerated containers are used to transport fruit by road and / or rail to ripening facilities located up to 3000-4000 km away in metropolitan centres. Controlled ripening techniques applied in Australia involve exposing mangoes to ethylene for 2-3 days (Ledger et al., 2014). The set concentrations are 10µl/L continuous ethylene in trickle systems and 100µl/L ethylene every 8 to 12 hours in shot systems (Ledger et al., 2014). After gas ripening, most mangoes are sold through wholesale agents and/or supermarket chains.

Transporting fresh mango fruit within Australia poses challenges in association with maintaining uniform product with high end quality from farm gate to consumer. The many causes of postharvest loss can be classified into the two

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categories (Wills et al., 2007) of physical loss and quality loss. Physical loss can be from mechanical, pest and disease damage and also from transpiration leading to loss in saleable weight. Loss of quality can be from physiological and compositional changes that alter the appearance, texture and / or taste, making produce less desirable aesthetically for consumers. Normal senescence processes and abnormal events like chilling injury are other effectors of quality loss (Wills et al., 2007).

To minimise or avoid physical and quality losses when transporting mangoes by refrigerated containers, it is important to establish and maintain desirable environmental conditions in terms of temperature, relative humidity (RH%), ethylene concentration, and carbon dioxide concentration. These conditions should determine the success or failure of the in-transit ripening strategy.

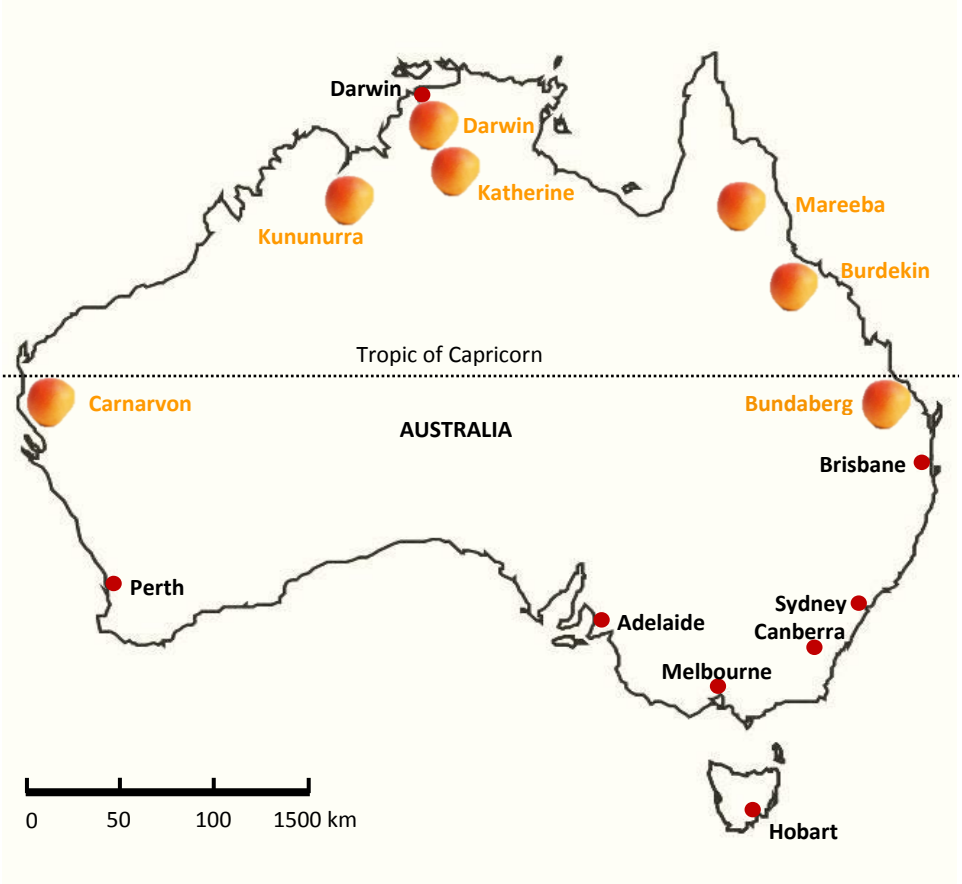


Figure 1. Map of Australia highlighting the main mango production areas and major city markets. Harvested mango fruit are transported up to 4000 km from farm to consumer (after Quotes 2014).

## THE CONCEPT

Logistically, ripening in-transit represents a currently unrealised opportunity for the Australian mango industry. It represents potential to reduce the time that mango fruit spend in the postharvest system prior to their sale. It should prove a more cost effective option for fruit ripening in decreasing requirements for expensive infrastructure for cooling, storage and ripening on-farm and / or in-market.

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## CONSIDERATIONS

### Temperature

Maintaining optimum ripening temperature and relative humidity at recommended 18°C and 90-95% (Ledger et al., 2014), respectively, is important during postharvest for mangoes. Temperature is the single most important environmental factor controlling physiological changes associated with optimum ripening (Crisosto and Mitcham, 2015). Refrigerated containers are insulated boxes fitted with modular mechanical refrigeration units driven by diesel or electric motors (Wills et al., 2007). They are designed to maintain product at a desired temperature. Rarely do they have capacity to uniformly reduce the temperature of the mass of cargo to a desired level in a timely way (Anon, 1989). Pre-cooling on farm is typically the first step in the cool chain process. Rapidly removing field heat from crops is essential (Burdon, 1997). Meurant et al. (1999) recommend that close stacked pallets of mango fruit in trays be pre-cooled to 18-22°C in a forced air system. Pre-cooling and transporting mangoes at a maintained storage or holding temperature of 18-22°C is intended to avoid premature ripening (Meurant et al., 1999) and attendant loss of quality and shelf life (Brecht et al., 2009). Failure to do so can lead to an increase in water loss and pathogen development (Burdon, 1997). In-transit, fruit temperature is determined by relationships between the container set temperature, refrigeration capacity, airflow around and over the product, heat influx from outside, and product heat of respiration, which is greater at higher temperatures.

In monitoring refrigerated commercial mango consignments of 18-20 pallet road and rail shipments, Hofman et al. (2014) found that air temperatures within the stow can be well above recommended levels. Marked temperature variation was recorded within a container with a set temperature of 18°C (Figure 2). The temperature had increased to ~35°C in the course of the 3-day journey. The temperature near the rear of the truck was 20°C at loading. Throughout the 3-day journey it fell slightly and then increased to ~22°C. The increase in temperatures in the face of ambient and product heat sources was attributed to poor air circulation throughout the stow. In another 3 day duration interstate truck shipment, higher temperatures were recorded to the rear of the truck and lower temperatures to the front (Figure 3). The fruit temperature at the front was ~14°C at loading. It increased steadily over the 3 days of transport to ~19°C. The logger toward the rear recorded higher temperatures of ~25°C at loading, decreasing to 22°C before increasing again to ~25°C.

Elevated and variable temperature deviations from recommended industry standards can lead to poor and uneven ripening throughout a load. In turn, irregular ripening can reduce product quality, increase waste, and decrease profits.

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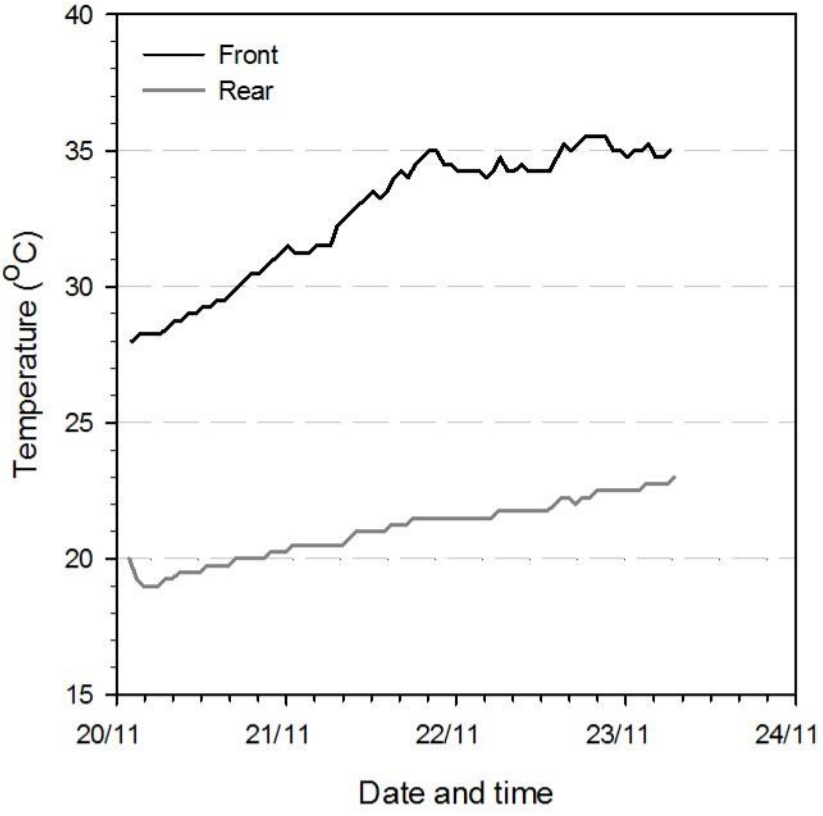


Figure 2. Air temperatures during a 3-day shipment as measured at two locations throughout the stow. The first logger was placed centrally in the 2nd row of pallets from the front of the truck, four trays from the top of the pallet. The second logger was placed centrally in the 2nd row from the rear of the truck, four trays from the top of the pallet. The temperature at the front of the truck was 28°C at outturn. The truck container was set at 18°C and contained cv. B74 mango fruit. The temperature in the front of the truck exceeded 35°C (from Hofman et al., 2014).

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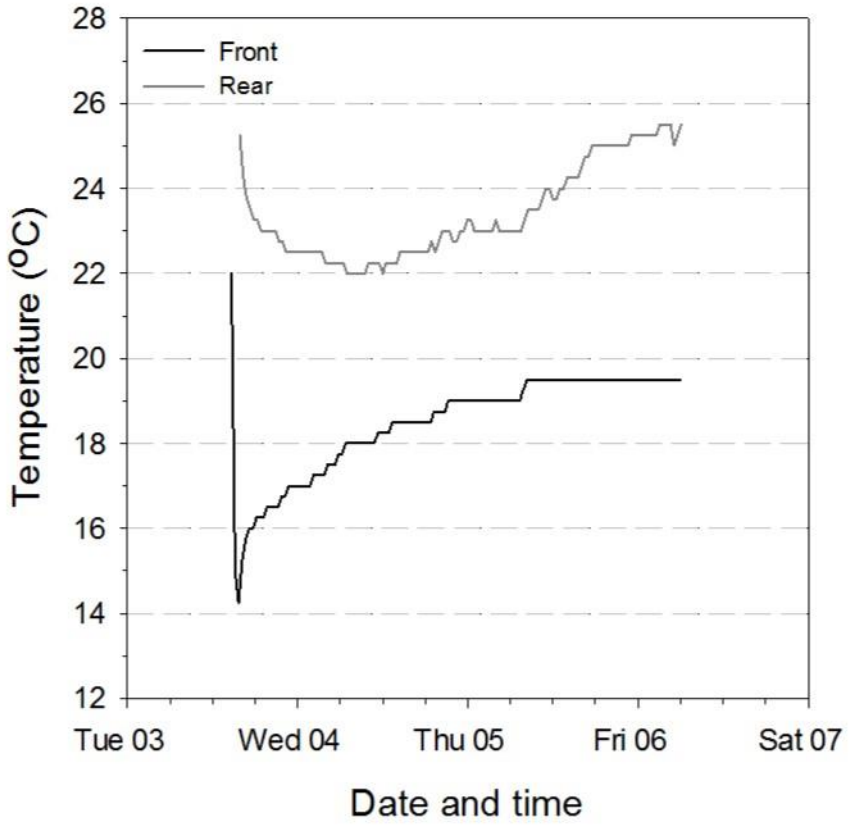


**Figure 3:** Air temperatures at the front and rear of another truck container of cv. B74 mango fruit. The first logger was placed centrally in the 2nd row of pallets from the front of the truck, four trays from the top of the pallet. The second logger was placed centrally in the 2nd row from the rear of the truck, four trays from the top of the pallet. The temperature at the rear of the truck reached 25°C despite a set point of 18°C (Hofman et al., 2014).

**Ethylene release**

Ethylene ($C_2H_4$) concentration in a container is determined by its release from abiotic sources, such as Ethylene Release Canister™ (ERC™) to condition pallets of pears (Sharrock, et al., 2010), from biotic sources, particularly its production by the product, and by container leakage rates. Ethylene production by mango fruit ripened at 20°C is typically moderate, peaking at 1-3µl/kg/h (Brecht and Yahia, 2009). In regard to concentration effects, Nguyen et al. (2002) found there was little differential effect on ripening and quality of 'Kensington Pride' mango fruit with between 10-100 µl/L ethylene.

Initial trialling ethylene release from an ethylene powder formulation, Ripestuff™, (Ho et al., 2013) (Figure 4) and from a semi-permeable plastic sleeve membrane (Hofman et al., 2014) (Figure 5) established their potential to maintain ethylene in container atmospheres over 2-3 days. However, undesirably faster release can occur in sub-optimal circumstances such as higher permeability of plastic sleeve release systems. Ethylene leakage rates from transport containers are a major factor in maintaining the required ethylene concentrations in-transit.

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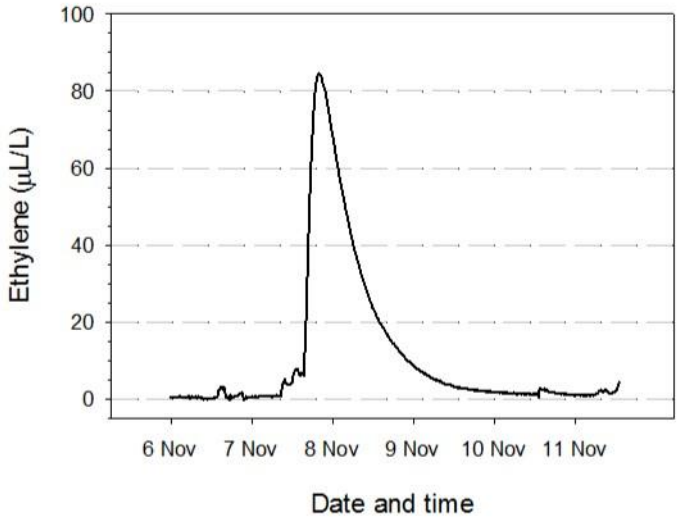


Figure 4. Rapid release of ethylene from bags of Ripestuff™ into a road container containing cv. B74 in-transit for 5 days. This method is similar to the shot ethylene treatment method, but without re-injection of ethylene into the system. The ethylene concentration peaked over 80µL/L (Hofman et al., 2014).

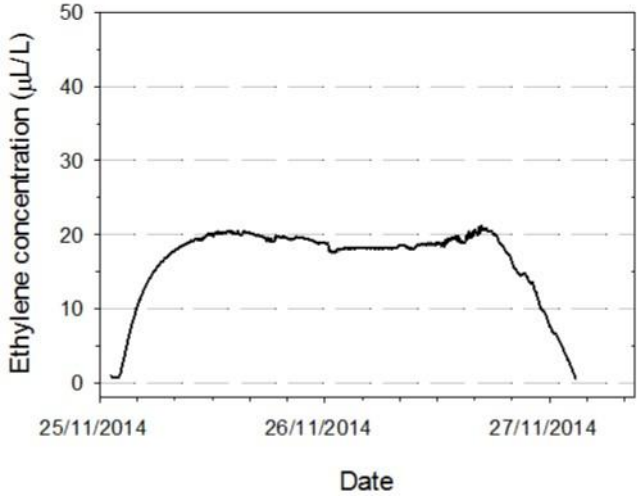


Figure 5. Slow release of ethylene from ethylene in nitrogen (4%) filled semi-permeable plastic sleeves of ~60cm circumference, of two thicknesses of 50µm and 75 µm into a truck container cv. B74 in-transit for 3 days. The ethylene concentration remained steady at ~ 20µL/L (Hofman et al., 2014).

**Carbon dioxide ($CO_2$)**

Mangoes are climacteric fruit that produce 70-160 mg/$CO_2$/kg-hr at 20-22°C (Thompson, 2015). Elevated $CO_2$ can suppress the effect of ethylene in initiating and

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co-ordinating ripening processes (Thompson, 2015). Keeping $CO_2$ levels below 0.5% assist in maintaining ripening times (Thompson, 2015). It is also important to limit worker exposure by adhering to Occupational Health and Safety guidelines (Thompson, 2015).

The container $CO_2$ concentration is determined by product respiration in and leakage rates from the container. Controlled ventilation is currently not technically possible in-transit. Absorption of $CO_2$ can be achieved by using hydrated lime powder scrubbers.

Monitoring $CO_2$ production from fruit and utilising $CO_2$ absorption methods in-transit will minimise exposure risks to fruit in-transit and humans at outturn.

**Air circulation**

Efficient airflow within the truck container is critical in managing temperature and ethylene distribution when transporting fruit. Factors such as pallet stacking pattern and placement within the truck, void space around the refrigeration unit and bulkhead, air circulation aids like air delivery roof chutes and floor channels, and the placement and type of any load stabilising boards can all affect air flow (Figure 6). Currently there are marked differences between refrigerated trucking containers in terms of their fan capacity, air circulation aids, engineering design, stow dimensions, and general condition and age (Brecht et al., 2009). There are also differences between transportation mode and whether the container is suitable for road, rail, or a combination of both. The majority of mango consignments in Australia are transported by road; however a combination of road and rail transport can be used, where generic containers are suitable for both forms of transport.

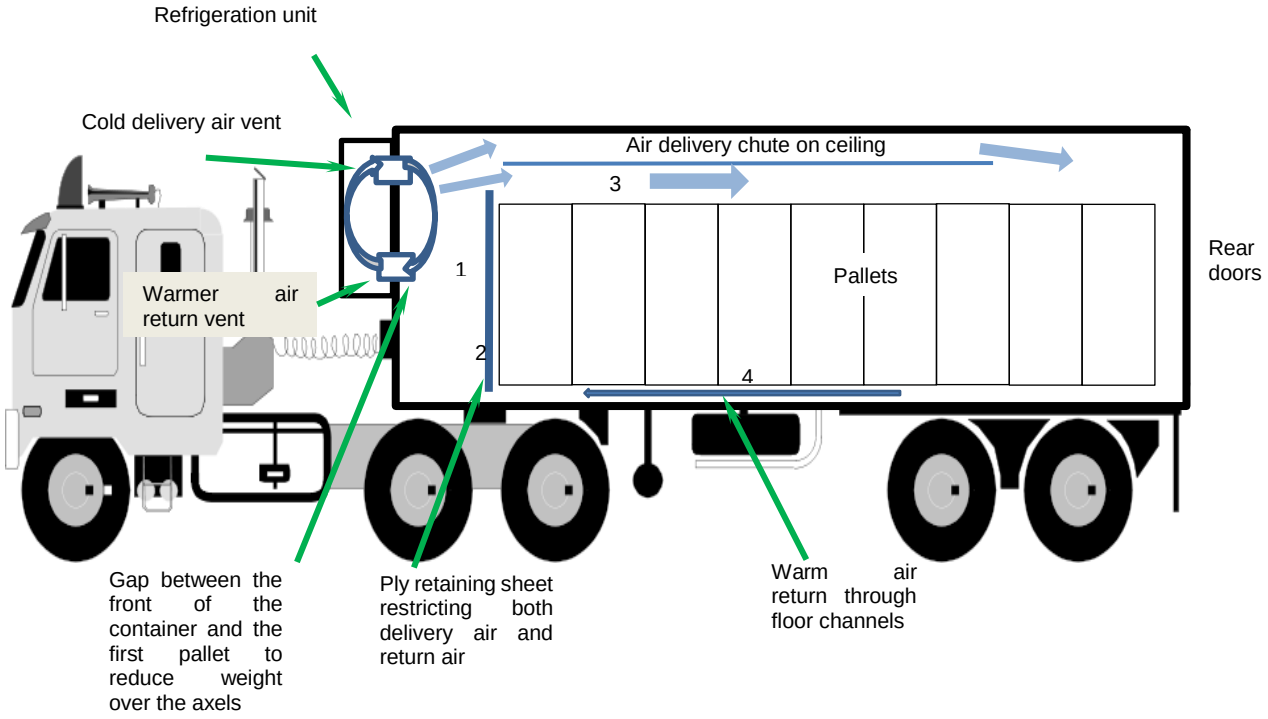


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Figure 6. Schematic diagram of the stowage in a road shipment container (after Hofman et al., 2014, p. 10). The factors impacting adversely on air circulation throughout the stow include 1: Gap where cold air is short circuiting directly to the return vent, with no cold air going to the rest of the container; 2: Ply sheet obstructing cold air delivery to front pallets and obstructing return of warm air to return vent; 3: Restricted cold air to front of pallets with interference from ceiling delivery chute, and 4: Restricted warm return air through pallet boards and/or floor vents.

**CONCLUSION**

Initiating and managing mango fruit ripening in-transit would reduce the capacity demand for cold rooms on farm and the attendant cooling costs. At market, holding and ripening infrastructure costs and their attendant operating costs would be reduced by mangoes being ripened in-transit. Controlled ripening must be done well in order to realise good fruit quality, reduced product losses, increased sales, and greater profits (Crisosto and Mitcham, 2015). In this context, in-transit ripening should provide a useful alternative method for ripening mangoes without the need for specialised facilities. Moreover, it may provide for greater flexibility in managing bottle necks in the supply chain. Thereby, producers, wholesalers, retailers and consumers in the supply chain might all stand to benefit from in-transit ripening.

Research and development is continuing on cost-effective sustained ethylene release systems for up to 4-day in-transit treatment of mango fruit. Additionally, the modelling of 'Honey Gold' fruit quality and shelf life under a range of ethylene and $CO_2$ concentrations at several temperatures is also underway.

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# Uses of an innovative ethylene-α-cyclodextrin inclusion complex powder for ripening of mango fruit

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ABSTRACT

A novel ethylene-α-cyclodextrin (α-CD) inclusion complex (IC) powder was investigated to ripen Calypso mango fruit. Modulated release of ethylene gas from the IC powder was achieved by admixture with deliquescent salt $CaCl_2$ at RHs of 75.5% and 93.6%. The IC powder was tested in the laboratory and for in-transit ripening of mango fruit over two seasons. In the laboratory experiment, ethylene gas started to release from the IC powder in 2 h and complete release was achieved in 24 h. Assessments of fruit colour and firmness showed that encapsulated ethylene and commercial grade ethylene from pressurised cylinder similarly shortened the ripening time to 9–10 days (after harvest) for treated fruit as compared with 15 days for untreated mango. Mango fruit treated in both ways with ethylene showed more uniform ripening than the control. For the in-transit ripening using the IC powder, ethylene was found to be between 4.9 and 10.5 $\mu L\,L^{-1}$ in the headspace of the truck containers over 48 h. Mango fruit from the treated containers shortened the ripening time by 3–6 days as compared to the untreated control fruit. Thus, the safe and convenient IC powder has demonstrated promise for in-transit fruit ripening.



## 1. Introduction

Mango (*Mangifera indica*Linn.) is a popular tropical fruit with good flavour and also a source of antioxidants (e.g. beta-carotene) and vitamins (Lizada, 1993; Rocha Ribeiro et al., 2007). Global production of mango fruit is >40 million tonnes annually, mainly by Bangladesh, Brazil, China, Guinea, India, Indonesia, Nigeria, Mexico, Pakistan, Philippines, Thailand and Vietnam (FAO, 2010, 2013). The prevalent commercial mango varieties in Australia are Kensington Pride, Calypso, Honey Gold and R2E2 with total annual production of 40,000 tonnes (FAO, 2013). As a climacteric fruit, mangoes are usually harvested green mature and ripened with ethylene gas to achieve more uniform and predictable ripening (Hofman et al., 2001; Montalvo et al., 2007). Mango fruit ripening is a complex physio-chemical process characterised by marked skin and flesh colour, firmness, taste and biochemical changes (viz. enzymes, sugars and acids) (Abeles et al., 1992; Ibarra-Garza et al., 2015; Prasanna et al., 2007; Tovar et al., 2011). Fruit ripening in Australia is commonly performed in a controlled temperature room, sometimes with relative humidity (RH) control and often with ethylene injection systems (Abeles et al., 1992; Wills et al., 2007). Recommended ripening conditions encompass 10–100 μL/L ethylene (Saltveit, 1999) at 18–20 °C for 24–72 h (Hofman et al., 2001; Lalel et al., 2004; Sivakumar et al., 2011; Wills et al., 2007; Yahia, 2011). A RH of 85–90% is generally recommended for ripened fruits to minimise weight loss (Kader, 2008).

Ethylene ($C_2H_4$) is a gaseous natural plant hormone that can have both beneficial and detrimental effects on fruit ripening (Barry and Giovannoni, 2007; Saltveit, 1999). Mature climacteric fruit ripen gradually after harvest but typically ripen at differing rates. This variability results in fruit at differing stages of ripening in the same tray, which is unattractive and reduces value. Ethylene treatment can virtually remove the temporal variability and also reduce the ripening time. Ethylene analogues can trigger the same response. Calcium carbide ($CaC_2$) releases acetylene and is being used in various circumstances because of its availability and low cost. However, due to its toxicity and human health concerns (Asif, 2012; ScienceLab.com, 2013), $CaC_2$ is recently banned in certain jurisdictions. Ethrel® or ethephon (2-chloroethylphosphonic acid) releases ethylene gas when the pH is raised above 4.0, as occurs in fruit cells, and has been used for decades as a dip to ripen fruit (Bondad and Pantastico, 1972; Singh and Dwivedi, 2008). However,

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dipping fruit to trigger fruit is often commercially impractical when fruit have to be held for a time before retail sale. Ethylene can also be produced by converting ethanol into ethylene in ethylene generator systems (Blankenship and Sisler, 1991; Saltveit, 1999). These systems are used commercially in various countries, such as in the USA. However, this method requires additional cost over capital investment and has reduced practical flexibility. The fruit ripening industry in Australia mostly uses ethylene gas from a pressurised cylinder (Saltveit, 1999; Wills et al., 2007). However, this approach is impractical for occasional ripening of small batches and for starting the ripening process while fruit are in transit from the farm to the retailer. The issues include significant equipment costs and the risk of explosion at ethylene concentrations greater than 2.7% in air (lower explosive level) (Sundaram et al., 1991).

An ethylene-α-cyclodextrin (α-CD) inclusion complex (IC) powder is a potential alternative source of ethylene for fruit ripening. Methodologies to produce the IC powder for release of ethylene gas have been reported (Bhandari and Ho, 2014; Ho et al., 2011a,b). The IC powder is easy and safe to handle provided that ethylene concentrations do not rise to explosive concentrations. Ethylene gas can be fully released from the IC powder within 14 days at 93.6% RH. Moreover, there is potential to use deliquescent salts such as $CaCl_2$ in admixture to absorb water from the atmosphere to affect solubilisation of the IC powder as in the case of 1-MCP (1-methylcylopropene) release. Kostansek (2002) used $CaCl_2$ to release 1-MCP from an 1-MCP-α-CD IC powder. Mixing the IC with deliquescent salt has been reported to provide sustained ethylene release (Ho et al., 2015). The ideal scenario would be a relatively constant concentration of the gas over 2–4 days as a convenient, cost-effective and safe means of ethylene treatment during transport.

Seasonal fruit are often in high demand, and particularly at the start of the season. They are often transported long distances from farm to market. In Australia, this transport can take 2–5 days. Initiating mango fruit ripening in transit allows fruit to reach a marketable ripeness stage earlier and also reduces the ripening infrastructure requirements. Therefore, there is potential for in-transit ripening to shorten the supply chain from harvest to market and reduce the costs of handling and storage. In-transit ripening using ethylene gas from a standard pressurised commercial cylinder has been used with pears to achieve uniform and quality fruit for consumption (Mitcham et al., 2000). In-transit ripening for the transport of Anjou pears has also been achieved using Ethylene Release Canisters™ (ERC™) (Sharrock and Henzell, 2010). An IC powder offers an alternative potentially more convenient, environmental-friendly and less hazardous system for in-transit ethylene treatment to trigger the ripening of climacteric fruit. The current study investigated the efficacy of an IC powder for ripening of mango fruit at laboratory scale and during commercial rail transport.

## 2. Materials and methods

### 2.1. Ethylene-α-CD IC and other chemicals

The IC powder was made at The University of Queensland (Brisbane, Australia) using the method of Ho et al. (2011a). The crystalline powder was collected under a 355 μm sieve (Endecotts Ltd., London, UK). The concentration of ethylene gas in the IC powder was 0.92 mol ethylene/mole CD and the moisture content was 8.00 ± 0.34% (Ho et al., 2011a). All chemicals were analytical grade. Mili-Q water was used throughout the experiment.

### 2.2. Ethylene release

#### 2.2.1. Ethylene gas release by dissolving the IC powder in water

20 mg IC was weighed into a 15 mL test tubes and water was added at IC:water ratios of 1:5, 1:10, 1:30 and 1:50 and then the mixtures were quickly placed in a 2000 mL desiccator pre-equilibrated with saturated solution NaCl (220 mL) for 75.5% RH at 18 °C (Rahman, 2009). Equilibrium time in the dessicator after adding sample was 45 min. 500 μL of headspace was removed at 1, 2, 3, 5, 7, 12, 24, 48, 72 and 96 h for ethylene concentration determination. Ethylene gas release was expressed as the percentage of released ethylene to the total amount of ethylene gas entrapped in the IC powder.

#### 2.2.2. Ethylene gas release with calcium chloride

The ethylene release rate in the admixture with deliquescent salt calcium chloride ($CaCl_2$) was determined by placing 10 mg IC into a plastic dish (35 × 10 mm) and adding 50 mg of $CaCl_2$ spread evenly over the IC (ratio 1:5). One dish was then put into a 2000 mL desiccator equilibrated with 220 mL saturated solutions of either NaCl for 75.5% RH or $KNO_3$ for 93.6% RH (Rahman, 2009). Equilibrium time in the dessicator after adding sample was 45 min. The RH range used was chosen to reflect the RH typically measured in mango shipments within the same transport containers used in the in transit ripening trials. The headspaces in the desiccators were analysed for ethylene concentrations at 1, 2, 3, 5, 7, 12, 24, 48, 72, 96 and 168 h following the method as described below.

**Table 1**
Different methods of ethylene treatments for Calypso mango ripening in laboratory.

| Treatments | Description |
|---|---|
| A | Untreated with ethylene gas (control): no ethylene gas added |
| B | Ethylene released from the IC powder[a] by deliquescent $CaCl_2$: insertion of (42 mg IC powder + 210 mg $CaCl_2$) |
| C | Ethylene shot from a commercial cylinder (BOC, North Ryde, NSW, Australia): injection of 660 μL ethylene |
| D | Combination of ethylene shot (treatment C) and ethylene released from IC powder with $CaCl_2$: injection of 330 μL ethylene gas from cylinder and insertion of (21 mg IC powder + 105 mg $CaCl_2$) |
| E | Ethylene releasing from IC powder by water: insertion of 42 mg IC powder + 15 mL $H_2O$ (ratio complex to water is 1:15) |
| F | Combination of the ethylene releasing from the IC powder by water and by deliquescent $CaCl_2$: insertion of (21 mg IC + 7.5 mL $H_2O$) and (21 mg IC + 105 mg $CaCl_2$) |

[a] Ethylene α-cyclodextrin inclusion complex powder.

### 2.3. IC powder and Calypso responses

#### 2.3.1. Laboratory experiments

Calypso mango fruit were obtained from commercial orchards at Dimbulah (17.15°S, 145.12°E) and Childers (24.85°S, 152.35°E) during December 2011 and January 2012. Medium-sized fruit (320–410 g/fruit) were commercially harvested and packed, and then air transported (Dimbullah) or road transported (Childers) to the laboratory at The University of Queensland, St. Lucia Campus (Brisbane, Australia) within 24 h of harvest. On arrival at the laboratory, non-uniform fruit and fruit with full red blush, physical damage or softening fruit were rejected. The remaining fruit were assessed for firmness, CIELAB colour, weight, colour and hand firmness as described below. Ethylene treatments were performed in 18 L high density polyethylene plastic containers (10 fruit per container). The container headspace was maintained at 93.6% RH using saturated $KNO_3$ solution. Each container had a 12-mm hole fitted with a rubber septum for gas sampling to measure ethylene and $CO_2$ concentrations. $O_2$ was not analysed as $CO_2$ could be used to express the respiration rate of fruit during ripening (Singh et al., 2007; Wills et al., 2007).

Treatments of mango fruits with the IC powder or commercial grade ethylene were described in Table 1. Treatments B–F were prepared to achieve combined ethylene concentration of 50 $\mu L\,L^{-1}$ in the headspace. The combination treatments D and F were prepared with a 1:1 ratio to give 25 $\mu L\,L^{-1}$:25 $\mu L\,L^{-1}$ from each source. A paper bag containing 100 g of calcium hydroxide (hydrated lime; Cement Australia Pty., Ltd., Darra, QLD, Australia) was placed into each container to reduce carbon dioxide ($CO_2$) accumulation during ripening (Wills et al., 2007). The ethylene treatments were performed at 18 °C for 72 h. For the release treatments using $CaCl_2$ (B, D and F), a 1:5 (w/w) IC:salt ratio was used with the salt spread evenly over the IC. An 1:50 (w/w) IC: water ratio was used for the water treatment. The IC:$CaCl_2$ powder was placed into a plastic dish (35 × 10 mm) and the water treatment placed in a 10 mL glass vial. After treatment, fruit were transferred into paper fruit trays and ripened as described below.

#### 2.3.2. Commercial in-transit trials

In-transit ripening trials were performed over two seasons in November 2011 and 2012 using typical commercial conditions except for the ethylene and $CO_2$ treatments. Mature, non-ripe Calypso mangoes were harvested from a commercial orchard in Katherine (Northern Territory, Australia; warm tropics). Fruit were commercially packed on the same day of picking or on the day after into either 7 kg or 10 kg corrugated cardboard trays with perforated plastic liners and stacked either 8 or 6 per layer, respectively, onto pallets at 16 layers high. Within about 4 h of packing the pallets were placed onto forced air-cooling tunnels overnight and cooled to 14–18 °C before being loaded into containers for the 2–4 day rail journey to Adelaide (South Australia, Australia).

The truck containers were loaded with 20 pallets each comprising a total of about 20 tonnes of fruit. Each container was 103 $m^3$ volume (length, width and height of 14.2, 2.5 and 2.9 m), with a calculated free air spaced after loading of 51 $m^3$. They were less than about 3 years old, in very good condition with well-sealing doors. They were fitted with Carrier refrigeration units (Carrier Pty., Ltd., Acacia Ridge, QLD, Australia) with top cold air delivery and a bottom air return temperature controller set at 18 °C. There was no free air venting capacity in either the containers or refrigeration units. The humidity in the truck containers was not controlled but other containers carrying Calypso mango had RH of 80–90%.

The mass of IC powder required per container was calculated based on the estimated free air volume in the loaded container, an ethylene treatment duration of at least 2 days, and 100% release of ethylene from the IC powder. About 30 $\mu L\,L^{-1}$ was targeted to provide a margin of error above the recommended 10 $\mu L\,L^{-1}$, but was within an acceptable range of about 10–50 $\mu L\,L^{-1}$ (Bhandari and Ho, 2014). For each container, two 5 L plastic trays (L, W, H of 26, 23, 11 cm) were utilised for releasing the ethylene gas.

The tray with IC powder and $CaCl_2$ was placed on the top of a pallet at the front of the container against the plenum. Before placement in the container, the tray was spread evenly with the IC powder and the $CaCl_2$ then spread evenly on top of the IC powder to give an IC:$CaCl_2$ ratio of 1:5. The other release tray with IC powder was placed on the top of a pallet at the back of the container near the doors. To this tray, water was added at an ICs: water ratio of 1:50 just before closing the container doors. In 2012, two containers of IC/$CaCl_2$ were used (as described above) because of fairly similar ethylene release rates (96% of ethylene from the IC released in 24 h), and in order to simplify the procedures.

On arrival in Adelaide, two trays of medium size mango fruit were sampled from each truck container. The sample trays were then air freighted to the laboratory at The University of Queensland, St. Lucia Campus (Brisbane, Australia) within 24 h of sampling for further ripening as described below. Two trays from the same farm, harvest and pack day in Katherine were sampled before loading into the container and air freighted to Brisbane (The University of Queensland) for ripening at 20 °C without ethylene as described below.

Ethylene loggers were used to monitor the ethylene gas concentration in the in-transit fruit containers. In 2011, the ethylene loggers (CO2Meter, Ormond Beach, USA) gave unreliable results. In 2012, an MSR ethylene gas logger (MSR electronics, Nuremberg, Germany) (±1.0 ppm accuracy) was placed in the middle of a pallet placed in about the middle of the container. The logger employs chemical cell sensors with optimum measuring ranges of 0–200 and 0–100 $\mu L\,L^{-1}$, respectively. One ethylene logger was used for each truck container. The Type T thermocouple probe of a Hobo temperature logger (Onset Corporation, Cape Cod, MA, USA) (±0.6 °C accuracy) was inserted into randomly selected fruit in the fourth tray layer from the top in pallets located in the second, middle and second last rows of each container. There were 3 temperature loggers per container. The fruit were adjacent to the other pallet in the same row and so were away from the wall of the container.

### 2.4. Fruit ripening and quality assessment

#### 2.4.1. External characteristics

Mango fruit from the laboratory and commercial in-transit treatments were ripened at 20 °C and 80–90% RH in a continuously venting environmental chamber (Steridium Pty., Ltd., Brisbane, Australia). Ripening fruit were assessed daily for fruit firmness, skin colour, fruit hand firmness and weight loss. The CIELAB colour ($L^*$, $a^*$ and $b^*$) of the non-blushed area of the skin was measured at two locations on the equatorial plane of the fruit using a Chroma Meter (Konica Minolta Sensing, Inc., Pullman, USA). Sensory skin colour assessment of the non-blushed area of the skin was based on a rating scale of 1 (0–10% of the non-blush area with yellow skin colour) to 6 (90–100% yellow) (Hofman et al., 2009).

The Hue angle ($h^\circ$) and Chroma ($C^*$) values were calculated using the following formulas (McGuire, 1992):

$$C* = \left[(a*^2 \times b*^2)\right]^{1/2} \text{ and } h^\circ = \left(\frac{\text{arctangent}(b*/a*)}{6.2832}\right) \times 360$$

Where, $h^\circ$ is in between the hypotenuse and 0° on the bluish-green and red–purple ($a^*$) axis (McGuire, 1992).

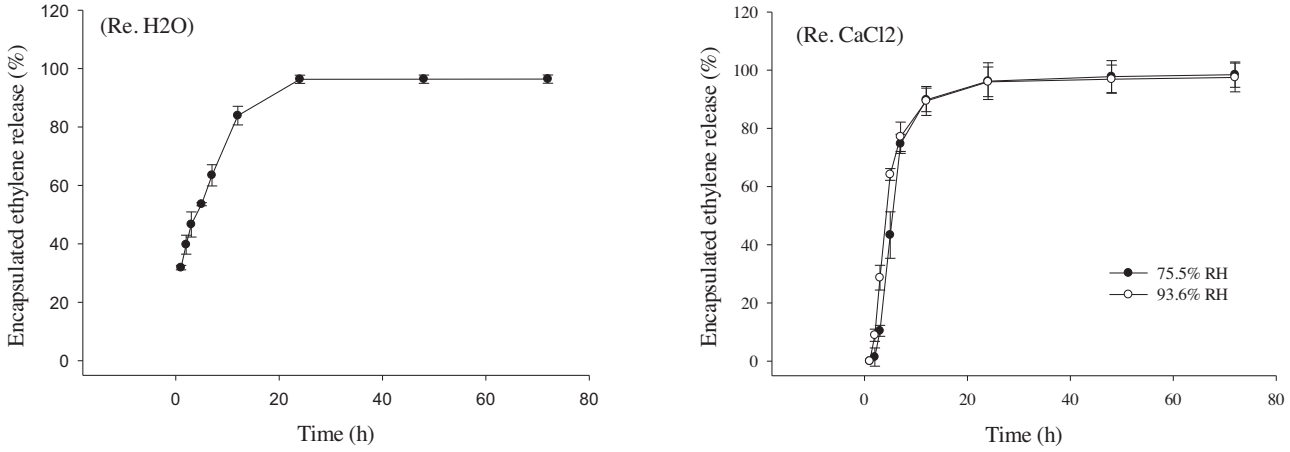


**Fig. 1.** Release of ethylene gas from the ethylene-α-cyclodextrin inclusion complexes (IC) powder by the addition of water (IC powder to water ratio of 1:50) (Re.H2O) and by deliquescent $CaCl_2$ (IC:$CaCl_2$ ratio of 1:5) (Re.CaCl2) at 75.5% RH and 93.6% RH (18 °C). Data were expressed with standard deviations.

Firmness at two locations on the equatorial plane of the fruit was measured using a Texture Analyser TA-XT Plus (Stable Micro Systems Ltd., Godalming, UK), as the force (N) required to push a 6 mm diameter hemispherical probe to 2 mm deep into the fruit. Fruit hand firmness was evaluated on a scale from 0 (hard) to 4 (soft). Days to full colour (DTFC) was recorded as the days for individual fruit to reach full colour according to the colour scale. Weight loss of individual fruit was calculated as the proportional (%) weight loss per day. For undertaking fruit quality data, the commencement of the fruit ripening was considered day 0 when the treatments started for the laboratory scale or when the loading of fruit finished at packing house (Katherine, Australia) for the in-transit treatment.

*2.4.2. Flesh characteristics*

At skin colour rating 6 and firmness ≤ 6 N, a 5 mm slice was removed from each fruit and the flesh was measured for firmness (N), CIELAB colour and total solids (°Brix). The flesh firmness was measured as above, but using a cylindrical probe and 5 mm penetration into the flesh.

*2.5. Ethylene and carbon dioxide ($CO_2$) measurement*

Ethylene concentrations in the headspace of the experimental containers was determined using a gas chromatograph (GC) (PerkinElmer 600 series) and TurboChrom 4 software (The PerkinElmer Corporation, Norwalk CT, USA). The GC was fitted with a stainless steel column (3 m × 1.2 mm) packed with 100–120 Mesh Porapak N (Waters, Milford, MA, USA). Helium at 40 mL/min was used as the carrier gas. Temperatures for oven, injector and flame ionisation detector (FID) were 90 °C, 105 °C and 150 °C, respectively. Headspace samples (500 μL) were manually injected into the GC. $CO_2$ concentrations were determined using the same GC, but with a TCD detector running that 100 °C. The ethylene concentrations were monitored after 1, 2, 3, 5, 7, 12, 24, 48 and 72 h and the $CO_2$ concentration was checked daily at 24, 48 and 72 h.

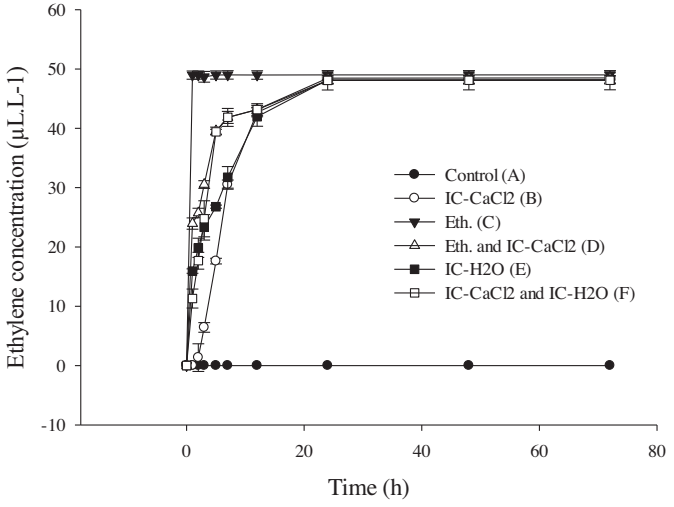


**Fig. 2.** Ethylene concentrations ($\mu L L^{-1}$) in the laboratory treatment containers in containers for Calypso mango ripening of untreated control (A), ethylene release from the ethylene-α-cyclodextrin inclusion complex (IC) powder released by $CaCl_2$ (IC-CaCl2) (B), pure ethylene shot (Eth.) (C), pure ethylene shot and ethylene release from the IC by $CaCl_2$ (Eth. and IC-CaCl2) (D), ethylene release from the IC by water (IC-H2O) (E) and ethylene release from the IC with water and $CaCl_2$ (IC-CaCl2 and IC-H2O) (F) at18 °C and 93.3% RH. Data were expressed with standard deviations.

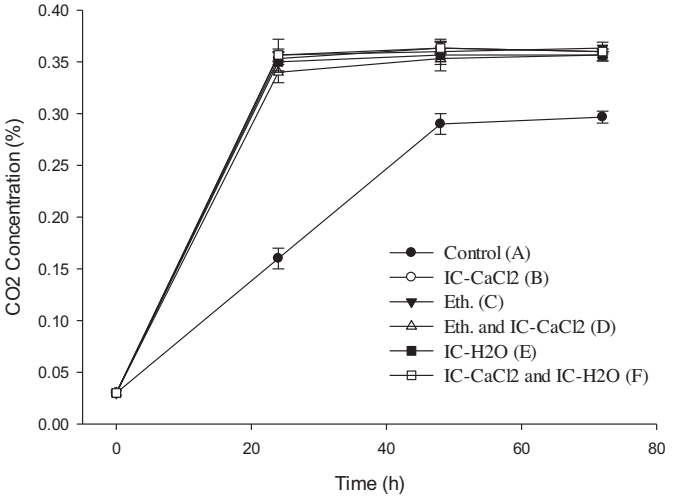


**Fig. 3.** $CO_2$ concentrations (%) in the containers of laboratory ripening treatments of Calypso mango fruit. Untreated control (A), ethylene release from the ethylene-α-cyclodextrin inclusion complex (IC) powder released by $CaCl_2$ (IC-CaCl2) (B), pure ethylene shot (Eth.) (C), pure ethylene shot and ethylene release from the IC by $CaCl_2$ (Eth. and IC-CaCl2) (D), ethylene release from the IC by water (IC-H2O) (E) and ethylene release from the IC with water and $CaCl_2$ (IC-CaCl2 and IC-H2O) (F) at18 °C and 93.3% RH. Data were expressed with standard deviations.

### 2.6. Experimental design and data analysis

For ethylene release from the IC powder by addition of water or $CaCl_2$, completely randomised designs with 3 replicates were performed. For the laboratory scale, experiments were randomised complete block designs (RCBD) with 6 treatments including an untreated control without ethylene gas (A) and 5 ethylene treated treatments with different supply methods (B–F). 10 uniform fruit were used as samples for each treatment. Each replicate included above 6 treatments and 3 replicates were performed. For the in-transit ripening trials, there were two replicates (truck containers) for the IC powder treatment and one replicate for the untreated control container. Replication was constrained by commercial costs and volumes and logistics. On arrival at Adelaide city, two fruit trays were sampled from each truck container. 10 fruit from a sampled tray were chosen randomly for ripening assessment. Data were analysed with Minitab 16.0. Differences in fruit quality parameters were evaluated by analysis of variance (ANOVA) at $P < 0.05$. Graphs were prepared using SigmaPlot 12.0.

## 3. Results and discussion

### 3.1. Ethylene release from the IC powder

#### 3.1.1. Addition of water

All ratios of IC powder to water from 1:5 to 1:50 showed tiny gas bubbles upon the addition of water. With no stirring, some IC crystals were visibly evident in the bottom of the 1:5 ratio treatment tube after 96 h. At 1:10, some IC crystals were still seen after 24 h, but not after 48 h. The remaining IC crystals were decanted and dried by silica gel for 24 h. The ethylene gas content in the dried crystals as analysed by GC (Ho et al., 2011a) was 0.37 mole ethylene/mole CD. Mixing the IC powder with water in higher ratios of 1:30 and 1:50 gave no visible powder after 12 h. At 1:10, some IC crystals were still seen after 24 h, but not after 48 h. Complete dissolution of the IC powder in water resulted in full release of ethylene gas from the IC under the experimental conditions (Ho et al., 2011a). Szejtli (1996) reported that saturation of the α-CD IC crystal in water occurs at 9 g per 100 mL at 20 °C. Ethylene gas release from the IC by addition of water (ratio 1:50) was found to be 33% after only 1 h (Fig. 1 (Re.H2O)) and 96% after

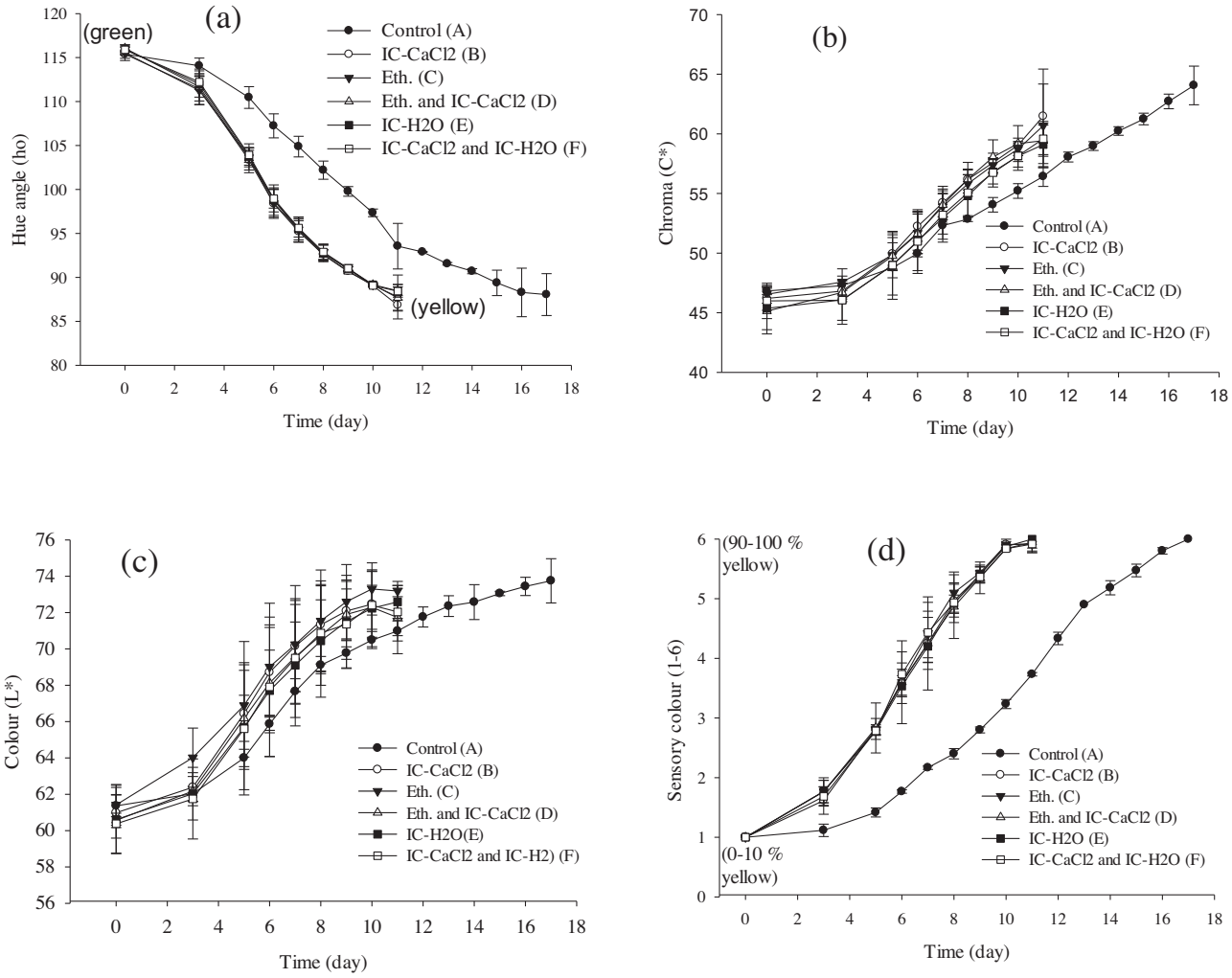


**Fig. 4.** Skin colour of Calypso mango in the laboratory ripening treatments. Untreated control (A), ethylene release from the ethylene-α-cyclodextrin inclusion complex (IC) powder released by $CaCl_2$ (IC-CaCl2) (B), pure ethylene shot (Eth.) (C), pure ethylene shot and ethylene release from the IC by $CaCl_2$ (Eth. and IC-CaCl2) (D), ethylene release from the IC by water (IC-H2O) (E) and ethylene release from the IC with water and $CaCl_2$ (IC-CaCl2 and IC-H2O) (F) at 18 °C and 93.3% RH. (a), Hue angle ($h°$); (b), Chroma ($C^*$); (c), colour light $L^*$ and (d), sensory colour. Data were expressed with standard deviations.

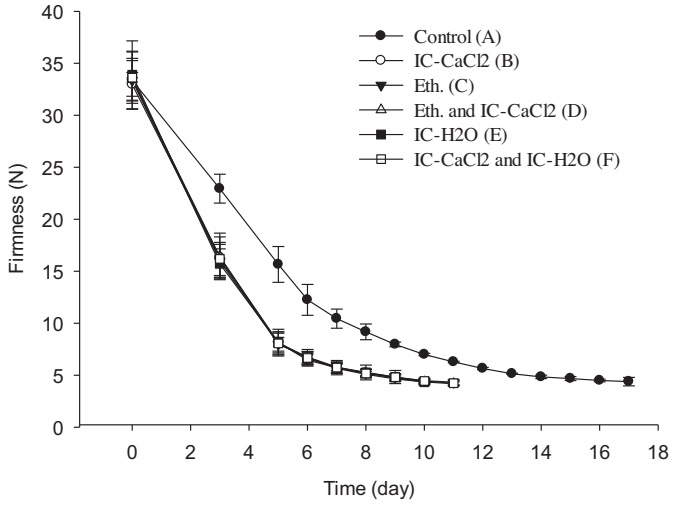


**Fig. 5.** Fruit firmness (N) of Calypso mango during the Laboratory ripening treatments: Untreated control (A), ethylene release from the ethylene-α-cyclodextrin inclusion complex (IC) powder released by $CaCl_2$ (IC-CaCl2) (B), pure ethylene shot (Eth.) (C), pure ethylene shot and ethylene release from the IC by $CaCl_2$ (Eth. and IC-CaCl2) (D), ethylene release from the IC by water (IC-H2O) (E) and ethylene release from the IC with water and $CaCl_2$ (IC-CaCl2 and IC-H2O) (F) at18 °C and 93.3% RH. Data were expressed with standard deviations.

24 h. The 1:50 ratio was used throughout the experiment where water was used to release ethylene gas from the IC powder.

### 3.1.2. Admixture with $CaCl_2$

At 75.5% and 93.6% RH ethylene gas started to release after 2 h from the IC/$CaCl_2$ mixture and reached ≥10 μL $L^{-1}$ after 5 h. More than 50% of the ethylene was released in 7 h and 90% and 96% of ethylene was released after 12 h and 24 h, respectively (Fig. 1 (Re. CaCl2)). As a deliquescent salt, $CaCl_2$ absorbs moisture from the air which dissolves the salt. The solution then dissolves the IC crystals and releases the ethylene. In a study on the release of 1-MCP from a 1-MCP-α-CD IC powder, an IC:$CaCl_2$ ratio of 1:5 at high RH (value not reported) released 93% of 1-MCP within 24 h (Kostansek, 2002), which is similar to the results reported here.

Based on these findings, subsequent trials used a 1:50 ratio of IC: water, and 1:5 ratio of IC:$CaCl_2$.

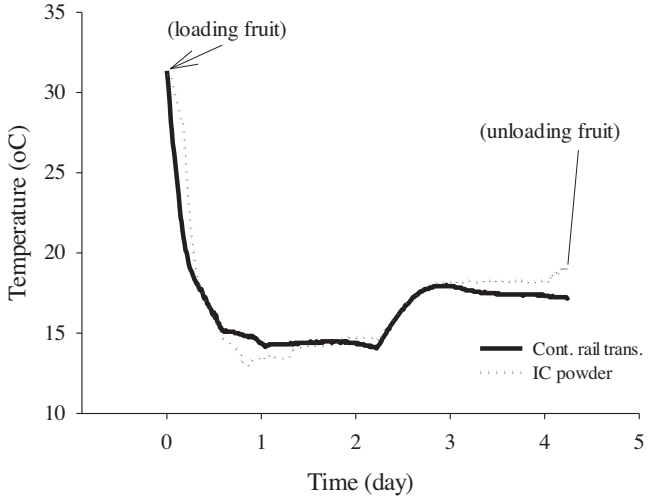


**Fig. 6.** Temperature of fruit pulp of the IC powder treated (IC powder) and the untreated control fruit (Cont. rail trans.) during the in-transit ripening of Calypso mango (2011 season).

## 3.2. Laboratory trial—ethylene release systems and mango fruit responses

### 3.2.1. Gas concentration

Ethylene gas concentration was 0 μL $L^{-1}$ in the control container (A) and it was detected at 1.35 μL $L^{-1}$ in the container with the IC powder and $CaCl_2$ (B) after 2 h (Fig. 2). Ethylene remained stable at 49 μL $L^{-1}$ for the ethylene shot treatment (C). After 1 h the ethylene concentration was 15.9 μL $L^{-1}$ in the container using the IC with water (E) and 11.3 μL $L^{-1}$ in the combined treatment of the IC with water along with $CaCl_2$ (F). After 24 h, ethylene gas concentrations in all IC powder treated containers reached between 48.2 and 49.0 μL $L^{-1}$ ($P > 0.001$). Ethylene supplied from a pressurised gas cylinder was immediately available after injection (C and D). In contrast, the IC powder treatments required 12–24 h for full ethylene release. Both water and $CaCl_2$ in combination with the IC powder allowed 50% ethylene release after 5–7 h, which is adequate for commercial in-transit ripening.

The $CO_2$ concentrations in all treatments including the untreated control reached only 0.35–0.36% after 72 h (Fig. 3), at least in part of the hydrated lime absorbing the $CO_2$ produced from fruit respiration. The control container had significantly lower $CO_2$ concentrations than the ethylene treatment containers at 24, 48 and 72 h ($P < 0.001$). This difference suggests that fruit in the plus ethylene containers were respiring more quickly because of ethylene enhanced ripening processes such that the fruit reached their respiration climacteric earlier (Wills et al., 2007). As Calypso mango can tolerate as high as 10% $CO_2$ during ripening, the concentrations in this trial were considered unlikely to affect ripening physiology.

### 3.2.2. Mango fruit responses

*3.2.2.1. Skin colour.* Despite slightly delayed ethylene gas release from the IC powder especially with $CaCl_2$, mango fruit treated with the IC (B, E and F) exhibited similar visual colour changes to those treated with the shot ethylene (C) and by the combination treatment (D) (Fig. 4a–d. All ethylene treated fruit (B–F) showed faster green colour loss than the non-ethylene treated control.

$h°$ values of the treated fruit (B–F) (Fig. 4a) showed a decrease from 115° to 89° in 10 days after harvest, indicating a change from green to yellow (McGuire, 1992). Untreated control fruit required 15–17 days to reach an $h°$ of 88°. At day 10, the $h°$ value of the control fruit at 97 $h°$ was significantly higher than that of 89° for the ethylene treated mangoes ($P < 0.001$). $h°$ is widely used to assess the colour colours of fresh commodities (Mitcham et al., 1996). For green, freshly harvested fruit which lose green colour during ripening, $h°$ usually decreases from about 100–115° at harvest to about 90° (Agar et al., 2000; McGuire, 1992), which is similar to the results obtained here.

The change in mango skin from green to yellow during ripening is largely due to chlorophyll breakdown and carotenoid formation (Hofman et al., 2009; Wills et al., 2007), and is one measure of the ripening stage. The changes in Chroma ($C^*$) provided further evidence of ethylene effects on mango ripening (Fig. 4b), since treated fruit had higher $C^*$ values of 58–59 than the untreated control mangoes (Fig 4d). $C^*$ is an index of colour saturation proportional to the colour strength (Maskan, 2001), suggesting that the ethylene treated fruit could have greater colour saturation on ripening using ethylene gas.

### 3.2.3. Firmness

Fruit treated with encapsulated versus shot ethylene exhibited similar firmness changes ($P > 0.05$) during ripening. Fruit firmnesses before treatment were between 33.0 and 33.9 N (Fig. 5).

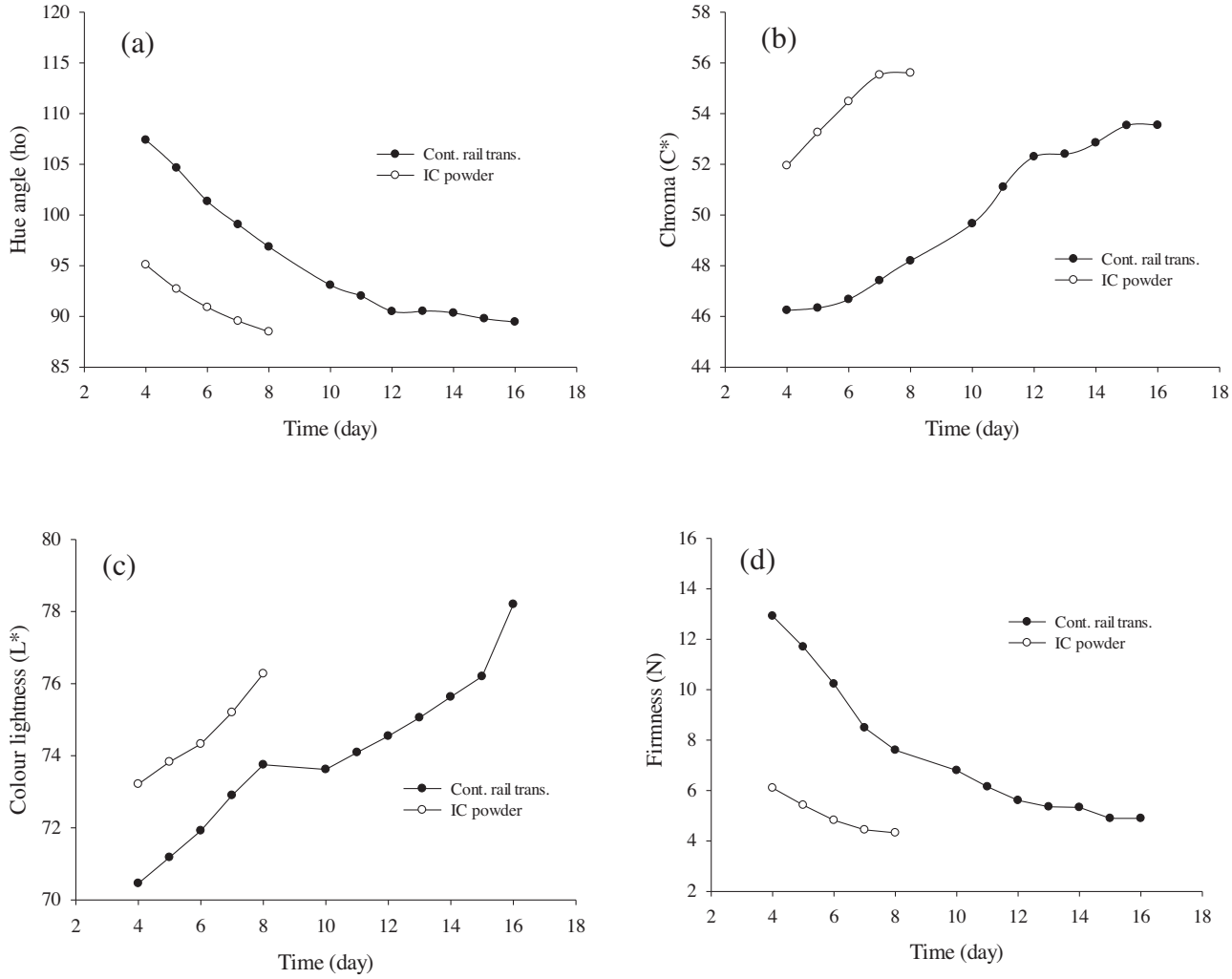


**Fig. 7.** Hue angle ($h°$) (a), Chroma ($C^*$) (b), Lightness ($L^*$) (c) and firmness (N) (d) of Calypso mango fruit treated with the IC powder (IC powder) and the untreated control (Cont. rail trans.) in in-transit ripening (18 °C). Quality assessments of initial green mango fruit (10 fruit) of the same batch for the in-transit ripening fruit in Katherine were Hue angle of 115.3–115.8°, Chroma of 45.9–46.3, Lightness of 63.6–63.9 and Firmness of 34.1–34.4 N. The 'Time (day)' in the *X* axis was the day after the fruit arrived at destination (Adelaide, Australia). Data were expressed with standard deviations.

After 72 h, all ethylene treated fruit had significantly lower firmness of 15.7–16.4 N compared to untreated fruit (23.0 N) ($P < 0.01$). After 10 days, ethylene treated fruit softened to 4.3–4.5 N, while the control fruit softened to 7.0 N ($P < 0.001$). It took 15–17 days for the untreated control mangoes to reach 4.3 N firmness, which is a similar firmness (3.9–4.5 N) to Calypso mangoes displayed on a major retail chain display shelf and supplied by the same grower and commercially ripened and distributed fruit (Woolworths, Brisbane, Australia).

Exogenous ethylene consistently promotes climacteric fruit softening during ripening (Lèlievre et al., 1997). During mango fruit ripening, starch hydrolysis, and pectin and cellulose degradation in the cell wall are major changes associated with fruit softening (Prasanna et al., 2007; Tucker, 1993; Voragen et al., 2006). Enzymes involved in these textural changes include glycanases, glycosidases, endomannanase and α-mannosidase (Yashoda et al., 2007).

### 3.2.4. Flesh attributes

DTFC was significantly less at 10.0–10.2 days after harvest for ethylene-treated mango fruit than for non-treated fruit (15.3 days; $P < 0.01$). This difference is similar to the response of Kensington Pride mango that ethylene treated fruit had 5 days shorter in ripening time after harvest (7.9 days after harvest) than the untreated control mango (13.6 days after harvest) (Hofman et al., 2001). DTFC of all treated Calypso mango fruit was between 9 and 11 days after harvest, meanwhile it was 12–17 days for the untreated control. Moreover, ANOVA of the standard deviation (SD) of DTFC showed that untreated mango fruit ripened has significantly higher SD (1.3) than all ethylene treated fruit (0.60–0.75). This suggests that ethylene treated fruit had their ripening more uniform and quicker than untreated control mango.

### 3.3. In-transit ripening of Calypso mango fruit

In the 2011 season, mango fruit in the control and in the ethylene treated containers maintained pulp temperatures of 17.4–19.1 °C during a transport (Fig. 6). The $h°$ of ethylene-treated mango fruit decreased from 95.1 to 88.5° by 10–13 days after harvest from Katherine (Northern Territory, Australia) (Fig. 7a). For the untreated control fruit, it took up to 15–19 days for fruits to change to 90 $h°$. Fruit treated with the IC powder in-transit had higher $C^*$ and $L^*$ values than did the untreated control (Figs. 7b and c).

The IC-treated fruit in both the laboratory and in-transit trials exhibited higher $h°$ value than the untreated control. In a study on

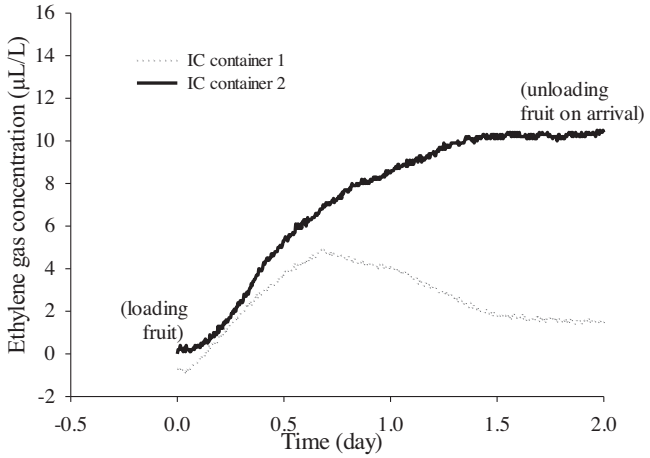


**Fig. 8.** Ethylene gas concentration ($\mu L\,L^{-1}$) in the truck containers (1 and 2) using complex powder during the in-transit ripening.

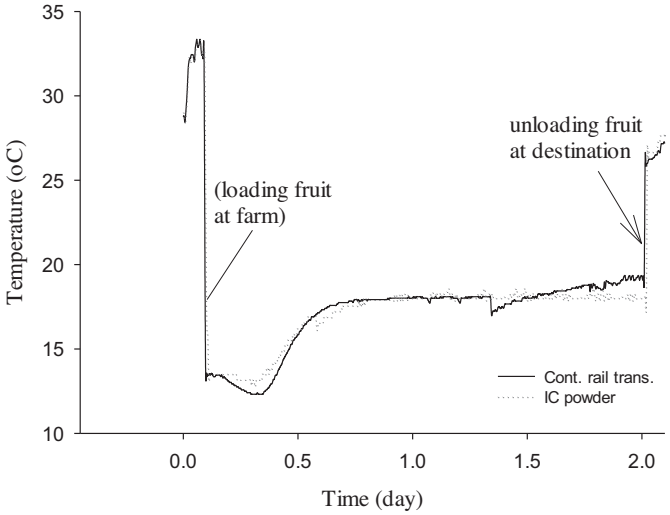


**Fig. 9.** Recording of pulp temperature (°C) of mango fruit in the truck containers of IC complex powder treatment (IC powder) and untreated control during the in-transit ripening (Cont. rail trans.).

in-transit ripening of Bartlett pear, those pear treated with 100–150 $\mu L\,L^{-1}$ ethylene over 60 h at 10 °C showed similar changes in skin colour as did ethylene-treated fruit exposed in a comparative laboratory experiment to 100 $\mu L\,L^{-1}$ ethylene for 48 h at 10 °C (Mitcham et al., 2000).

Fruit treated with ethylene decreased in firmness from 34.4 N to <6 N in 11 days of ripening after harvest (Fig. 7d). In contrast, it took 16 days for untreated control fruit to soften to 6 N. DTFC of ethylene treated mango fruit were 11.1 days compared with 17.3 days for untreated fruit. These results are similar to the laboratory experiment, indicating that the ethylene release system in the

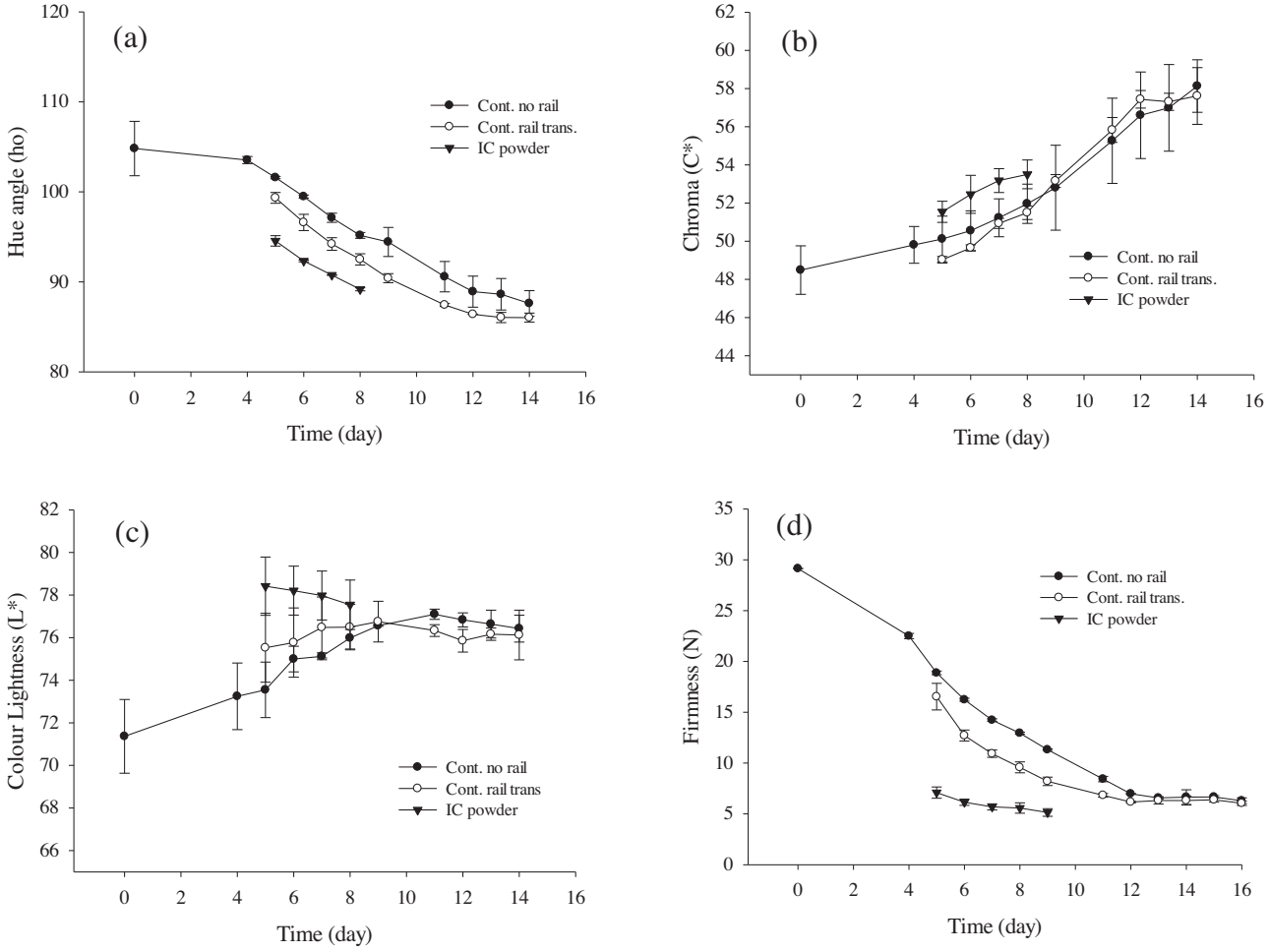


**Fig. 10.** Hue angle ($h^{\circ}$) (a), Chroma ($C^*$) (b), Lightness ($L^*$) (c) and firmness (N) (d) of Calypso mango fruit treated with the IC powder (IC powder) and the untreated control (Cont. rail trans.) and untreated control without rail transport (Cont. no rail) in in-transit ripening at a set temperature of 18 °C. Data were expressed with standard deviations.

truck container was effective despite the absence of reliable ethylene concentration data.

For the 2012 season, ethylene was detected at near the middle of the load in both road/rail containers after 2 h. The levels then increased after 16 h to 4.9 $\mu L L^{-1}$ in container 1 and 10.5 $\mu L L^{-1}$ after 24 h in container 2 (Fig. 8). The ethylene concentration in container 2 maintained concentrations at about 10 $\mu L L^{-1}$ until removal (51 h). This ethylene concentration data demonstrated the capacity of the IC powder to release sufficient ethylene to ripen mango fruit during transport. Ethylene concentrations in container 1 decreased to 1.5 $\mu L L^{-1}$ at unloading. As truck container 1 and 2 contained similar ethylene release systems, it is possible container 1 had higher leakage rates. During the transport of mango fruit, both ethylene treated and untreated control truck containers were set for 18 °C and the fruit pulp temperatures were recorded within 14–19 °C (Fig. 9).

Fruit quality parameters as assessed showed similar treatment effects in the 2012 as per the 2011 trial. Thus, ethylene treatment was associated with more rapid loss of green colour, higher $C^*$ and a more rapid loss in firmness as compared with no ethylene treatment control fruit (Fig. 10a, b and d). The treated fruit reached full colour within 8 days after harvest compared to within 11 days for non-treated in-transit fruit and 13 days for non-treated fruit that were not rail transported.

The in-transit studies in 2011 and 2012 were conducted to validate the use of the IC powder for in-transit ripening, and the results across the two consecutive mango seasons suggest that the concept is indeed commercially feasible. They suggest effective ethylene release and maintenance during transit at concentrations that significantly enhance ripening. The lower ethylene concentrations in the truck container as compared to calculated (30 μL/L) could be due to gas leaking during transport and/ or the calculations based on inaccuracy of assumption. Nonetheless, mango ripening can be triggered by ethylene gas at 5 μL/L within 24 h (Bhandari and Ho, 2014).

## 4. Conclusion

These results confirm that the ethylene-α-CD IC powder is an effective ethylene release system and can produce similar mango ripening effects like those associated with ethylene from conventional sources such as commercially available pressurised cylinders. The IC powder was also an effective release system in in-transit applications, wherein the novel formulation resulted in quicker ripening, including attractive skin colour. Differing ethylene concentrations in two containers suggest that container leakage rate could be an issue. Overall, the IC powder shows clear promise for in-transit fruit ripening during transport.

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