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1 Acknowledgments

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2 Executive summary

The Cambodian mango industry has expanded rapidly with an estimated 65,250 ha of trees planted (MAFF 2016) in the last 10 years. Over 40% of Cambodia's mango production occurs in Kompong Speu province, which is approximately 100kms southwest from Phnom Penh, strategically located along Road No 4 which links the capital to Cambodia's major sea port of Sihanoukville.

Mango is Cambodia's major horticultural export product, with an estimated 139,000 tonnes of mainly green mangoes exported to Thailand, Vietnam and China in 2015/16 (*Vinning, 2016*). This "grey" export trade is difficult to measure as there are no official statistics gathered on cross border trade of mangoes. Returns to growers are poor, with average prices returned to Cambodian farmers by traders of between 1,500 and 2,000 riel/kg (around AUD $0.50). The major variety grown is Keo Romeat, which is highly sought after for not only the green mango market, but also the fresh mango trade. Small quantities of Keo Romeat are currently air-freighted to Europe, and buyers from countries such as South Korean are currently investing, or planning to invest in both farms and processing facilities, including Vapour Heat Treatment (VHT) plants

In December 2016, the Royal Government of Cambodia (RGOC) signed an MOU with the government of South Korea to develop mango exports. Since then, private investment in the Cambodia mango industry by South Korean companies such as Hyundai have increased, re-enforcing the move towards export. Despite this confidence shown in the Keo Romeat variety and local farmers' ability to produce export quality fruit, significant challenges still need to be overcome before successful exports of Cambodian mangoes can be realised.

Some of these challenges include the absence of internationally recognised pest and disease lists, haphazard use of pesticides to control pests such as fruit fly, inconsistent fruit quality standards, poor crop nutrition and the absence of cool chain infrastructure and packaging facilities.

Capacity building of the team in assembling internationally recognised pest and disease lists has been a focus. The aim of this training was to enable participants to develop accurate pest lists based on verified specimens held in recognised national collections to meet obligations under the World Trade Organisation Sanitary and Phytosanitary (SPS) Agreement. The workshops concentrated on specimen preparation and curation, long term storage, record keeping in databases, and diagnosis including verification by specialists. Training in use of DNA techniques for identification of pests and diseases was also conducted in November 2016 and March 2017. During these workshops, the molecular results from laboratory experiments conducted in November 2016 were discussed, including the molecular identification of *Fusarium mangiferae*, the fungus that causes mango malformation disease, a new disease record for Cambodia. NT DPIR is assisting with writing a scientific manuscript to report this new finding once the Cambodians approve for this information to be disseminated to the international scientific world.

Training workshops on a range of other topics from pest and disease identification and management, crop growth measurements, crop nutrition, postharvest management and nursery management have equipped the team to conduct project research activities.

A feature of the Cambodian mango industry is the widespread practice of producing two crops per year, otherwise known as double cropping. To achieve this, chemicals are used to induce flower production in the off season. Investigations into the current mix of chemicals used for flower induction for Keo Romeat in Cambodia will hopefully lead to safer alternative options being taken up by industry. The negative impacts on tree health with over use of paclobutrazol, and the reliance on thiourea (a suspected carcinogen) to induce flowering and its potential impact on human health has been the two main interest areas for the project team. Successful research conducted in the Northern Territory during the 2016/17 season using alternative treatments to thiourea such as mono-potassium phosphate and potassium nitrate has provided a good platform for a new approach in Cambodia.

Survival of Keo Romeat mangoes under long term storage conditions experienced during shipments by sea freight of up to 21 days is key to achieving successful exports. Control of postharvest fungal rots such as stem end rot is mandatory for successful out-turns of fruit. This project conducted the first series of export simulation trials on Keo Romeat in collaboration with a Korean partner.

The export simulation trials provided excellent experience in planning and managing commercial scale trials for the project team. The first trial was conducted in March 2017 using a dry season crop with fruit sourced from a large grower at Kompong Speu in cooperation with a Korean exporter. Over 300 mangoes were stored at 12^0C for a total of 21 days at the Cambodian Agricultural Research and Development Institute (CARDI) Postharvest Laboratory. Fruit was removed at 7, 14 and 21 days and assessed for skin and flesh colour, firmness, dry matter, skin defect and disease. An additional treatment of a postharvest fungicide Scholar ® (fluordoxinyl) vs no fungicide was also used.

A 2nd trial was conducted in November 2017 on fruit from a wet season Keo Romeat crop at Kompong Speu. Three trials were designed to test different postharvest treatments. These included fungicide, hot water plus fungicide, hot water alone, and a sanitised water treatment was also included. A smaller investigation was also carried out looking at Scholar ® dipping time of 4, 6, 8 and 10 minutes. Scholar ® proved partially effective at controlling stem end rot, and was more effective when sanitised water was used. The Scholar ® dipping was most effective when applied as a hot water treatment at 52^0C. However, this treatment was not acceptable commercially as it caused skin scalding. Further work is needed on postharvest treatments to develop effective export protocols.

In Australia, the PhD study within this project has identified a complex network of genes involved in the regulation of flowering in mango using *Arabidopsis thaliana* specific flowering genes. As such, the development of the specific Flowering Locus T (FT) gene using molecular probes to track induction was not feasible. Methodologies have been revised within the research program to evaluate the genes associated with the regulation of flowering in mango, how they are co-expressed and the network of positive and negative regulators of the flowering pathway from vegetative to flowering phase. The research is in the final year and techniques will be applied to phase II project to investigate similar flowering genes in Cambodian mango varieties.

In the Northern Territory, investigations into the effects of ethephon, potassium nitrate and thiourea on leaf flushing and flowering in Kensington Pride mangoes were carried out. The thiourea treatments were effective in increasing flowering in a season where high temperatures reduced normal flowering. Ethephon proved effective in removing unwanted

flush growth and maximising synchronised bud growth. Ethephon trials were also conducted in NSW

Fruitspotting bug research in NSW involved development of biocontrol release strategies. The NSW Entomology Team also provided support to this component looking at the ecology and initial evaluation of various biological control agents, including the egg parasitoids *Anastatus* sp. and *Gryon* sp. Pheromone traps for both species and trap crops were also tested as part of this component.

HORT/2012/003 has established a sound basis for development of a vibrant mango export industry. A follow up ACIAR project, which includes a Philippines component, will commence in mid-2018 and be led by the Northern Territory DPIR. The objectives of this project include improved nutrition of mangoes with an emphasis on the role of calcium, crop area mapping to determine more accurate statistics on tree numbers and production, introduction of DNA analysis for improved pest and disease management, and the development of an export development strategy for Keo Romeat mangoes, incorporating use of Good Agricultural Practices (GAP).

3 Background

The Cambodian mango industry has expanded rapidly since 2005. The most recent statistics from the Ministry of Agriculture, Forestry and Fisheries (MAFF) stated that the current mango area was 65,251 hectares in 2014/15 compared to 24,000 hectares in 2010. There are two major production regions, one in the south focused on the province of Kampong Speu, Kampot and Kampong Cham with approximately 50,000 hectares and the second region to the north-western provinces around Siem Reap and Battambang with approximately 7,600 hectares. Most of the trees were planted in Kampong Speu province with the total area of 39,500 hectares (60.5% of the total mango area) and production around 790,000 tons (Hean, 2015).

The “grey” export trade of predominantly green Keo Romeat mangoes is difficult to measure, as there are no official statistics on the quantities moving across the border. Based on border crossing observations, the ACIAR project HORT/2014/020 estimated that Cambodia exported around 135,000 tonnes of mangoes to Vietnam and Thailand in 2015/16. Prices to growers are reasonable at between 1,500 and 2,000 riels (or around AUD $0.50) per kilogram. However, growers are subject to fluctuating prices, and have no control of the fruit once it leaves the farm and is in the hands of the exporters and traders.

The Royal Government of Cambodia (RGOC) intent is to expand the domestic and export opportunities for mangoes. To meet this goal requires improvements to be made across the whole production/market continuum to enable Cambodia to compete at an international level. One of the key drivers for this is to meet phytosanitary requirements under the ASEAN agreements on export fruit crops by 2015. Critical to realising success is the need to build fruit science capacity in research, development and extension of both government and non-government agencies. This ACIAR project has been the major initiative in R&D to help address some of these challenges over the last four years.

Broadening the scope of the agricultural development agenda from annual cropping (rice and vegetables) into a perennial tree fruit crop research necessitates a significant investment in acquiring capacity by the different – research development and extension (RD&E) institutions to enable this. Appropriate investment in the best technology in the early stages of development of a perennial tree crop such as mango yields dividends in later years of fruit production.

Two scoping activities preceded development of this project. The first was a preliminary assessment to gain a better understanding of the constraints faced by the Cambodian mango industry, and identify areas of improvement that could be addressed through a joint project.

A more detailed nine month scoping study (HORT/2012/018) was conducted between May 2012 to January 2013 across all five production regions, to identify specific issues along the mango production and supply chain for future research and to provide a benchmark to evaluate progress from any future research. A survey of 100 farmers from the 5 key mango production provinces confirmed many of the issues identified in early studies, but provided additional detail.

The shortage of trained and experienced fruit crop researchers and advisers in Cambodia has been a constraint to the development of the local mango industry. For the RGOC to

meet its target of fresh mango exports, significant improvement is needed in all facets of production and supply chain management to enable the Cambodian mango industry to compete successfully with other mango trading nations.

Critical to the success of these aspirations is building the fruit science capacity in research, development and extension of both government and non-government agencies. Similarly, the Royal University of Agriculture and regionally-based universities need to be engaged to include fruit research into their science curriculums. The detailed survey conducted by Cambodian collaborators from CARDI, GDA and RUA as part of the SRA HORT/2012/018 served as an excellent introduction for the team to understand how a production systems approach to research compliments the discipline research method. All participants were challenged in understanding how stages in the plant's phenology are critical in determining when and what intervention has the greatest impact. This will continue to be the major strategy in the development of the capacity in Cambodia.

The shortage of high quality inputs required for quality mango production including pesticides, fertilisers, packaging materials is a well-recognised constraint. Pesticides in particular are variable in quality, and growers are often advised by re-sellers to tank mix pesticides with several other products to ensure the target pest in killed. During project surveys, the team observed up to seven insecticides and fungicides being mixed into the same spray tank. Not surprisingly these treatments were often ineffective, most likely due to incompatibility of the products when tank mixed, or more serious, levels of resistance development as a result of these practices in target pest populations.

The absence of modern plant and soil testing services in Cambodia has made it difficult to choose suitable sites for mango developments and develop appropriate nutrition programs. Ancillary services essential for establishment of GAP such as pesticide residue testing laboratories don't exist in Cambodia and fruit samples, for example need to be sent to Bangkok or Singapore for testing. Pesticide residue tests conducted for this project for example were submitted to SGS in Phnom Penh, but the fruit had to be shipped to the SGS Bangkok laboratory for testing. These were the first fruit samples SGS had processed in Cambodia. While there is a government testing laboratory which can test for fruit residues, it did not have the standards for the main pesticides used in fruit production and of interest to the project. For soil and leaf testing, there are no standards for leaf nutrient levels for varieties such as Keo Romeat.

The Australian mango production period commences in late August in Darwin and ends around late February with fruit from northern NSW or Gin Gin in WA. This spread in harvest window reflects the influence of variety and geographic distribution. Within one production region it is generally Kensington Pride that is harvested first, followed by R2E2, B74 (Calypso^{R}) and Honey Gold. Within each individual region or property, the harvest window may only extend for five to seven weeks depending on the climatic conditions in that season. Biennial bearing in mature mangoes has also a serious impact on the industry's capability to consistently (year to year) supply product into developed and volatile markets.

As property sizes increase and volumes of fruit within a region also increase the capacity to process and move significant volumes of fruit, whilst maintaining tight control on fruit quality, becomes very difficult and taxing on management and logistics. This is particularly critical as these larger orchards move to export markets. If there were techniques to advance or delay

fruit maturity on a property, to spread the harvest period, this would improve harvest, packaging efficiencies and profitability.

The ACIAR component of the program in Australia involved a blend of strategic (e.g. identification and refinement of methodologies to utilise the highly conserved gene, Flowering Locus T (FT gene)), adaptive (identifying current growth regulators that inhibit or stimulate the activity of the FT gene) and development (pulling all components to commercial practice). The outcomes from this work on the FT gene will have commercial benefits for Cambodia varieties, especially Keo Chen which does not respond to current manipulation strategies.

Fruitspotting bug (*A. lutescens and A. nitida*) remains one of the major pest problems of tropical and sub-tropical fruit crops. A Horticulture Australia Ltd funded multi-industry project MT10049 *A multi target approach to fruitspotting bug management* (2011- 2016) focussed on developing an integrated system of management for controlling this pest. In this project, one of the strategies is to identify and test a bio-control agent for FSB. Testing of the bio-control agents on farm in NSW and Queensland and finding ways to integrate them with current farm management practices was required.

The ACIAR component of this project component looked at the on farm adaption of the potential biocontrol for FSB, *Anastatus spp. Anastatus* is a recognised egg parasitoid of both species of FSB. This component also involved development of biocontrol release strategies. The NSW Entomology Team also provided support to this component looking at the ecology and initial evaluation of various other biological control agents, including the egg parasitoid *Gryon* sp. Pheromone traps for both species and trap crops were also tested as part of this component.

4 Objectives

The specific project objectives for Cambodia:

1. Develop and evaluate crop management strategies for productive, profitable and sustainable production of high quality mangoes.
2. Develop and evaluate sustainable practices for the integrated management of mango pests and diseases to maximise product shelf life and meet international phytosanitary requirements.
3. Identify and prioritise the key supply chain constraints, including postharvest losses, packaging, storage and transport to deliver mangoes into selected markets.
4. Design and implement a pathway to adoption of improved management options for the Cambodian mango industry encompassing both on farm production and the supply chain.
5. To build the capacity of the Cambodian research, development and extension system to deliver targeted and practical outputs to agribusinesses and farmers.

The specific project objectives for Australia:

1. Understand the function of the FT gene during the expression of flowering and incorporate these techniques into normal crop management for the manipulation of fruit maturity.
2. Achieve successful on farm adaptation of biocontrol agents as part of an improved system for integrated pest management for fruitspotting bug in macadamia, avocado and other sub-tropical tree crops.

5 Methodology

The nine month SRA HORT/2012/018 which preceded this project identified several key production constraints to mango production in Cambodia. These constraints included

- Heavy reliance on a single variety, Keo Romeat
- Poor tree nursery management practices
- Haphazard orchard design and layouts
- Variable soil types and poor understanding of crop nutrition
- Variable success with flower induction chemicals in double cropping Keo Romeat
- Poor skills in pest and disease identification and management
- Absence of harvest and fruit quality standards
- Poor understanding of existing domestic markets, and potential export markets
- Limited fruit research a capacity in either government or private sector

This information provided a sound basis for developing a research and development program for HORT/2012/003. Apart from the extensive grower survey of 100 farmers in 5 provinces during the SRA, government and NGOs working in the horticultural sector were consulted.

The specific activities addressed under the stated project objectives are detailed in *Section 6 Achievements against activities and outputs/milestones*.

1. *Develop and evaluate crop management strategies for productive, profitable and sustainable production of high quality mangoes.*

1.1 Nursery best practice: Production of consistently healthy and uniform seedlings of grafted mango stock is essential to building a strong tree crop industry such as mangoes. Project team visits to a range of mango nurseries, from on-farm nurseries to small commercial roadside nurseries revealed a similar range of issues. Poor choice of nursery site and layouts, poor quality potting media and minimal screening of seed suitability for planting were the most common issues observed. None of the nurseries practiced grafting, and although variety the Keo Romeat which makes up over 90% of the trees grown is polyembryonic, there appeared to be some variability in plant types.

In response to interest from some of the nursery managers regarding performance of various potting media used in the industry, the Plant Breeding Team at CARDI established a small potting media trial to investigate impact of some of the commonly used media on seedling growth rate and health. The summary trial report can be found in *Section 7 Key results and discussion.*

A workshop on nursery best practice was also conducted with the project team. The workshop involved an audit of the CARDI nursery and grafting demonstration which was carried out by Chan Savourn from GDA. The team were interested in trialling various potting mixes, and as a result a small investigation was carried out at the CARDI nursery comparing various media combinations as described above. A mango nursery best practice guide was also developed. A draft of the guide can be found in the *Appendices*.

1.2 Mango crop nutrition: The SRA Survey in 2012 and subsequent discussions with farm managers demonstrated a broad range of fertiliser practices used across the industry. A

common theme was to none of the farmers survey used leaf or soil tests to develop their nutrition programs and most were guided by market sellers or on the larger farms, Vietnamese or Thai agronomists who advised on managing the double cropping system in Keo Romeat, which is heavily dependent on the fertiliser program and timing to be successful. A typical fertiliser program consisted of the following:

First fertilizer application is normally applied in May/June, soon after the dry season crop is harvested and the 2nd fertilizer application is in September-October for the wet season crop which is harvested in December/January. Fertiliser is applied as either a dry mix of chemical fertiliser (NPK 0-22-200 1kg/tree) and animal manure (0.5kg/tree), spread by hand. In addition up to 1 kg per tree of potassium nitrate (KNO^3) is also applied as part of the flower induction program along with the chemicals paclabutrazol and thiourea. (See more detailed results from fertiliser experiments and explanation of flower induction in *Section 7 Key Results and Discussion*.)

1.2 Develop and evaluate sustainable practices for the integrated management of mango pests and diseases.

1.2.1 Cambodian Mango Pest List: During the course of the project a specimen based pest list (insects and diseases) has been developed for Cambodian mangoes to be used for international trade negotiations under the World Trade Organization's (WTO) Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS agreement). This list, and the insect and disease collections that underpin it, is maintained by GDA who are the National Plant Protection Organisation (NPPO).

Australian Plant Protection Team members, Dr Brian Thistleton and Andrew Daly initially held discussions with GDA to determine the current status of the mango pests list and existing gaps. Prior to commencement of this project, Cambodia had participated in an ASEAN – Australia Development Cooperation Program (AADCP) Strengthening ASEAN Plant Health Capacity Project which focused on developing pest list for mangoes across all ASEAN countries. One of the participants in this project was also a member of the current ACIAR project and had already made a start on assembling insects and pathogens for a pest list.

Two pest list training workshops were conducted. The first in November 2013 had been aimed at training the project collaborators (CARDI, RUA and GDA including provincial staff) in designing surveys and collecting pest and disease specimens. The second in April 2015, which consisted of a three day field trip and two days in the laboratory, continued on this theme but concentrated on specimen preparation and curation, long term storage, record keeping in databases, and diagnosis including verification by specialists. Dr Gary Kong (CRC National Plant Biosecurity), who is leader of the ACIAR project, "Enabling Improved Plant Biosecurity Practices in Cambodia, Lao PDR and Thailand" (HORT/2010/069), also participated with the pest list training.

There were also two molecular workshops conducted within the project.A three day DNA workshop was conducted in April 2016 at CARDI. The workshop was attended by eight participants from CARDI, Royal University of Agriculture (RUA) and the General Directorate of Agriculture (GDA). The program included lectures as well as practical sessions in the laboratories on deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) associated with various plant pathogens and insects, molecular tools and bioinformatics. With the assistance

of the Australian team members, participants collected mango leafhoppers, gall midges, mango fruit borer, mealy bugs and suspect diseased samples for their practical component. The previous project trips to Cambodia had identified two species of mango leafhoppers at CARDI, *Idioscopus clypealis* and *I. nitidulus*. During the practical sessions, participants extracted DNA from the insect specimens and leaf material. All DNA extracts underwent polymerase chain reaction (PCR) using a range of gene primers (i.e. DNA barcode for insects and fungal pathogens). Both species of mango leafhoppers were collected and participants were tasked to extract both species for identification using the DNA barcode. The final day was focused on preparing PCR products for sequencing in Australia and subsequent bioinformatics. Participants were introduced to the Geneious software and NCBI BlastN search engines for molecular identification. Once familiar on sequencing analyses, participants were provided with unknown sequences of both insects and mango plant pathogens and were tasked to use their newly acquired bioinformatics skills to identify the unknown specimens based upon highest sequence similarities using the two platforms. Almost half of the unknown sequences were identified during the workshop, with follow answers provided upon Dr Tran-Nguyen's arrival back to Australia. The second molecular workshop was conducted in March 2017 with 11 Cambodian delegates (7 RUA, 2 CARDI and 2 GDA). During this workshop, the molecular results from laboratory experiments conducted in November 2016 were discussed. The DNA barcoding of immature and adult specimens of cerambycids was discussed. Diagnostics was not clear cut and it was evident that this area needed further investigation for future research.

Figure 1: Drs Lucy Tran Nguyen and Brian Thistleton supervise project team members during a DNA Workshop practical

New specimens were photographed to aid in diagnostics and numerous specimens were brought back to Australia for identification and/or verification. Insects were brought back to Australia under the Department of Agriculture and Water Resources import conditions; in this case all specimens were preserved in 75% (or higher) ethanol. GDA also requested

that a series of dried herbarium specimens of plant pathogens be brought back to Australia for morphological diagnostics. For these to be safely brought into the country the DAWR import conditions specify that there must be an import permit, the specimens must be in triple sealed taped up plastic bags, a list on departmental letterhead must be presented at the airport, and that the specimens be irradiated before being released from quarantine. Updated lists of species were provided during the project and pinned specimens of fruit flies, identified by Professor Richard Drew and Dr Mark Schutze, and slide mounted specimens of scale insects and mealybugs, identified by Dr Michael Gorton, were returned to the GDA collection.

At the end of the project an informal workshop was held at the GDA collection to show it to the CARDI and RUA team members, who had also participated in pest list training and surveys. This collection is now quite extensive, but will continue to be added to a new species are detected.

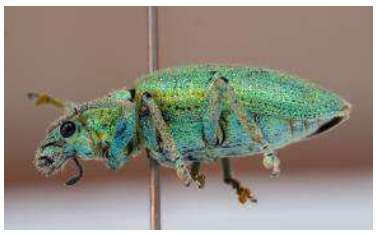


Figure 2: Mango pests in the GDA collection

1.2.2 Photography Workshops: Each partner organization was allocated a Nikon SLR camera at the commencement of the project. The primary purpose of equipping each team with a quality camera was to enable photographic records of pest and diseases to be taken as part of the pest surveys. Dr Brian Thistleton conducted two photography workshops with the team, with subjects covered including lighting, specimen preparation and cataloguing images. Apart from pest and diseases, photography of variety and fruit maturity images were also collated as part of the training. The images will be used in pest and disease guide and fruit maturity posters.

1.2.3 Mango fruit borer pheromones: The mango fruit borer, *Citripestis eutraphera*, was identified for the first time in Cambodia during the course of the pest list surveys. Research is underway by NT DPIR and The New Zealand Institute for Plant & Food Research Limited to identify its pheromones and to develop synthetic copies. One trial was carried out in Cambodia to test the pheromone there and to train staff in how to conduct these trials.

Figure 3: Citripestis eutraphera pheromone trials.

1.2.4 Other insect traps: During the course of the pest list surveys traps were used in an attempt to collect particular species of interest. One of these was a large longhorn beetle which bores into the trunks of mangoes. DNA analysis has been conducted on the larvae of this beetle but it is not present in the main DNA databases (BOLD and GenBank). The strategy has therefore been to collect adults which could be identified morphologically and then associated with the larvae through DNA. Water and pheromone traps were demonstrated and deployed to try to catch adults of this beetles and talks were given on the diagnostic features. It is believed that the species involved is *Batocera rufomaculata* (Coleoptera: Cerambycidae) but since this species has not previously been recorded in Cambodia (it is Myanmar, Vietnam and Thailand) it is necessary for the species to be identified and verified by specialists.

Figure 4: Adult longhorn beetle and pheromone trap

Similarly there is a species of mango gall midge in Cambodia which is new to science. In order for this species to be described it is necessary to obtain adults of this species. This can be done by rearing adults from the immature stages found inside the galls or by trapping the adults using small light traps (designed for sampling blood sucking midges) and linking the adults to the correct gall by associating the larval and adult DNA.

1.2.5 New fruit fly trap testing: The DPIR entomology section is currently participating in the testing of new fruit fly traps with Professor Richard Drew (International Centre for the Management of Pest fruit Flies, Griffith University) and AgNova. These are based on the female flies' attraction to various colours when they are seeking fruit for oviposition. The traps consist of disks of various colours with a lure and are currently being tested in NT to determine which colour attracts *Bactrocera jarvisi.* (It is already known that blue is the colour for *B. tryoni*). The aim in Cambodia is to determine which colour attracts *B. correcta* and studies on this with replicated trials will be conducted in the follow-up project (HORT/2016/190). On this trip four traps (red, yellow, blue and white) were demonstrated. A number of flies were collected and brought back to Australia for further study.

Figure5:Fruition® traps and caught flies

1.2.6 Pesticide residue testing: The pesticide residue testing carried out in the 2015 dry season crop provided an important baseline on specific chemicals which may be an issue for future market access, and raise awareness of any potential health issues. Ten farms in Kompong Speu, Kompong Cham and Siem Reap were tested as part of the survey. The chemicals screened for included the insecticides dimethoate, lamda-cyhalothrin, chlorpyrifos and cypermethrin, imidichloprid and fenthion, and the fungicide carbendazim.

2. *Identify and prioritise the key supply chain constraints, including postharvest losses, packaging, storage and transport to deliver mangoes into selected markets*

2.1 **Background**: Mangoes are an important fruit crop for Cambodia. This is evidenced by: (1) an increasing area under mango production; (2) capacity to sell Cambodia's most common mango variety, Keo Romeat, when green or ripe; and (3) increasing demand for the Keo Romeat in the export market, particularly to Vietnam, China and Thailand for consumption or on-sale to other markets such as the European Union. At the time of the Census of Agriculture in Cambodia 2013 the area planted to mango orchards was reported to be 42,000 hectares (MoP and MAFF, 2015). Recent news reports also detail growing and new export markets for fresh mangoes. For example, in 2015 the Cambodian government signed a memorandum of understanding with South Korea to formalize the direct export of fresh mangoes (Kang, 2015) and in 2016 fresh mangoes were shipped direct to the European Union for the first time (Cheng, 2016). With this growth come opportunities to contribute to regional development. To leverage on economic and development opportunities associated with mango production in Cambodia and to build competitiveness and resilience in the industry we require an understanding of on-farm production practices and the key stakeholders in and spatial flows of the supply chain.

The emerging Cambodian mango industry faces many challenges including dependency on a single variety (Keo Romeat), lack of export protocols, competition with regional neighbours (Thailand and Vietnam), variable quality and declines in productivity. Despite this demand from regional neighbours, particularly in the off-season remains strong. As part of an Australian Centre for International Agricultural Research (ACIAR) project, this study seeks to identify and prioritise the key supply chain constraints, including postharvest losses, packaging, storage and transport to deliver mangoes into selected markets. To that end, 3 surveys have been undertaken to better understand Cambodian mango supply chains and identify opportunities for intervention:

- In 2014, a semi-structured survey of supply chain actors was undertaken in Battambang province to gain insight into mango flows, supply chain constraints and opportunities for intervention;
- In 2015, a trader survey was undertaken in the Neak Meas and Deum Kor wholesale markets in Phnom Penh to understand product flows for both domestic and imported fruit, quality requirements and collect some preliminary price data;
- In 2016, a semi-structured survey of supply chain actors was undertaken in Kampong Speu province.

As part of an earlier ACIAR SRA in 2012, one hundred mango farmers across 5 provinces (Kandal, Kompong Speu, Kompong Cham, Battambang and Siem Reap), stratified by farm size, were surveyed to understand current production practices and constraints. This provided us with a good understanding of at the farm end of the chain and so this was not included in this study.

Approach and Methodology

a. Pilot study (March 2014)

Survey checklists from AGB/2012/006 *Eastern Indonesia Agribusiness Development Opportunities – Mango Value Chains* (developed by Tiago Wandschneider) were obtained, discussed and refined to tailor them for use in Cambodia. These checklists were also translated ready for pre-testing in Battambang and Siem Reap. Much discussion centred on the selection of collectors and other supply chain partners, with the core difficulty being able to locate collectors and aggregators. This is particularly problematic for mango supply chains where limited provincial resources have been invested in fruit production.

Pre-testing of the check-lists was undertaken in both Battambang (4 interviews) and Siem Reap (3 interviews) – interview transcript reports were prepared by Lim Sophornthida and used to review and refine the check lists. Meetings with Provincial Departments of Agriculture (PDA) were also held in both provinces to get a broad overview of the mango industry and to facilitate contacting contractors, collectors and wholesalers.

Following the pre-testing significant re-working of the check lists was needed to make them more suited to Cambodian conditions. To determine an effective sampling strategy we needed to: 1) identify major production districts in Battambang and sample contractors within this; 2) identify collectors/aggregators as these are the key people to understanding product flows and 3) visit key border posts to see first hand

the cross-border trade and identify traders. The pilot study enabled us to gain sufficient insight into mango supply chains to determine the approach needed for the design of our Battambang study, we then refined this approach further for the Kampong Speu and Phnom Penh wholesale market studies.

b. Battambang supply chain study (2014):

Our overall objective was to gain insight into mango flows, supply chain constraints and opportunities for intervention. To that end we used the following approach:

- Direct observation – market/field/export gate visits during the main production season – enabling us to follow the mango chain from farm to market
- Visits and interviews of exporters/importers at 3 border gates - Prum (Pailin province), Liem, and Phnom Deiy (Battambang province).
- Conducted a semi-structured survey in Battambang province of wholesalers, collectors and contractors using refined checklists from *AGB/2012/006 Eastern Indonesia Agribusiness Development Opportunities – Mango Value Chains.*

Full interview transcripts were then prepared (available upon request) and this was used to analyse the supply chains. The approach used is qualitative in nature and primarily designed to gain insight into the supply chains understudy. Further quantitative work would be recommended to gain an understanding about marketing margins etc. – however our attempts to include this in the semi-structured surveys overcomplicated the surveys resulting in data that was difficult to use in an effective way.

The contractor/farmer interviews were conducted in three main parts. Firstly, information was collected on contractors'/farmers' experience, sources of information for management practices, and relationships with the tree owners. Secondly, information was collected on crop management including type and timing of fertiliser and chemical application and the associated input costs. Thirdly, they were asked about mango yield and price information and information regarding mango buyers. The collector interviews were separated into two main parts: (1) detailing the collectors' relationship with contractors/farmers and wholesalers (in the domestic or export market) and (2) establishing the collectors' scale of operations and margins.

c. Phnom Penh Wholesale market survey (2015):

In Phnom Penh, there are two major wholesale markets where mango trade is conducted – they are Neak Meas and Deum Kor markets. To enable us to obtain representative data we first needed to estimate the population of wholesalers (large, medium, small) in these two major wholesale markets. This then enabled us to determine the number of interviews that would provide us with a representative sample. These population estimates were undertaken during the height of the mango season (April 2015) with the actual survey then undertaken post-mango season as we felt that wholesalers would be better placed to answer questions at this time. Also undertaken at the height of the mango season was an observational study at the markets to enable us to document postharvest handling practices, estimate traded

volumes and obtain wholesaler contact details. Following the mango season we then undertook semi-structured interviews with 15 wholesalers in Neak Meas and 10 wholesalers in Deum Kor markets.

d. Kampong Speu supply chain study: A similar approach was used to that detailed in (4) above.

For more detail on the supply chain studies see Section 7 Key Results and Discussion and a full report in Appendix 3.

2.2 Export simulation trials: Two export simulation trials were conducted at the CARDI postharvest laboratory. The first was during the on-season in April 2017, and the second was during the off-season in November 2017. The Korean company Foodya has been cooperating with the project during 2017, and is planning to be the first company to export mangoes by sea freight to Korea from Cambodia.

The first export simulation trial in April 2017 was from a Cambodian owned farm in Kompong Speu. Over 400 mangoes were harvested into padded cartons and transported by 4 wheel drive vehicle to CARDI, where a postharvest fungicide dip (Scholar @ 260mls/100litres for 1 minute) was applied. Fresh fruit weights, dry matter % and scores for external defects were measured before placing the fruit in trays into the CARDI coolroom at 12^0C. Fruit was removed at 7, 14 and 21 days and assessed for neck rot, body rots or other skin defects. A sample of fruit was also assessed immediately after harvest. All samples were ripened at room temperature for 7 days prior to assessment.

In November 2017, Mr Jay Ahn, the Foodya representative engaged a collaborator farmer at Kompong Speu, and 300 mangoes were harvested by the team. Fruit bags to protect the crop from fruit fly had been used on the fruit, and the early fruit numbered. Supplementary bagged fruit was also harvested, as fruit for several postharvest trials were required. The fruit was transported in padded cartons to CARDI and postharvest treatments using Scholar® (fluoxydil) fungicide were applied. Several rates and dipping time were used in the trial along with a water only dip treatment prior to the fruit being placed in the coolroom at 12^0C. Fruit was assessed after storage at 0, 7, 14 and 21 days plus 7 days ambient storage to allow fruit to ripen. Following team discussion, a further 150 mangoes were harvested for inclusion in a hot water dipping trial, as a comparative treatment to fungicide. Various dipping times of the fungicide (2, 4, 6, 8 and 10 minutes) were also included. The Felix F750 quality meter was used for testing dry matter content of Keo Romeat. This instrument uses near infra-red (NIR) technology developed at Central Queensland University to non-destructively measure dry matter content of fruit, and provided an opportunity to develop new calibration models for varieties other than Kensington Pride. For *more detailed results in Section 7 Key results and discussion*)

3. Design and implement a pathway to adoption of improved management options for the Cambodian mango industry encompassing both on farm production and the supply chain.

3.1 Determine knowledge gaps in mango production and supply chain management. The SRA survey of 100 farmers revealed major gaps in knowledge across all production regions. Most of the information was either derived from sellers in the market, or for the larger farmers, consultants from Thailand or Vietnam. Very little information was derived

from government researchers. Virtually no locally generated information on locally grown varieties was available, as there was no research being conducted which was publically accessible. A baseline survey of provincial (PDAFF) staff and farmers on identification of major pests and diseases was carried out in the first year of the project. Farmer's ability to recognise the key pests and diseases was reasonably good, with 70% giving the correct identity of samples given to them. This information provided a good basis for workshop planning in Years 3 and 4 of the project. Other areas of need for information for farmers identified by the project team included pruning, in particular management of young trees, fertiliser selection and rates, management of pest and diseases and appropriate choice of pesticide/fungicides.

3.2 Identify potential collaborator farmers willing to test new ideas and technology in up to 3 provinces. Collaborator farmers were identified in Battambang, Kompong Cham and Kompong Speu during the pest list surveys in the first 18 months. Several of these farms were used for farmer workshops and pruning / spraying demonstrations. However, logistics of regular site visits to Kompong Cham and Battambang were difficult due the long travel times to field sites, so the majority of the field trials were located in Kompong Speu. Fertiliser and crop regulation trials were carried out on a farm in Kompong Speu, and fruit for the export simulation trials were sourced from farmers in the same vicinity.

3.3 Conduct provincial workshops on topics nominated by farmers in target districts. Workshops on a range of topics were conducted in all five provinces. Pest and disease identification and management workshops were carried out in 2015/16, involving Australian and Cambodian team members. Additional workshops in Kompong Cham and Kandal were carried out by the Cambodian team. A pruning workshop was conducted in Battambang 2016, and a farm walk to inspect fertiliser trials and a spraying operation were carried out in Kompong Speu in 2016/17. Several farmers were involved in collection of pest/disease specimens, and provided data for the project team for crop regulation and nutrition experiments. *See Section 7 Key results and discussion for further details.*

4. To build the capacity of the Cambodian research, development and extension system to deliver targeted and practical outputs to agribusiness and farmers.

This ambitious objective encompassed all aspects of mango production, from nursery practices and hygiene, pruning of small and large trees for better productivity and pest and disease control, understanding of pest and diseases and understanding of these pests, disease life cycles and pest and disease lists and postharvest management. Also included is the market chain and an understanding of fruit quality to enable better prices for the grower.

4.1& 4.2 Provide scientists with a training program to build capacity in mango RD&E: Initial workshops went about building capacity of the numerous project researchers who had vast experience in carrying out research in annual crops such as rice and vegetables but were unfamiliar with fruit tree crops. The priority was to give these staff a knowledge of mango crop cycles and an understanding of year round crop production and including the double cropping practices commonly used in Keo Romeat production. This activity assisted all parties to provide a baseline for an industry that had no documented baseline to work from. In understanding the phenological baseline the researchers could then proceed in matching different pests and diseases to stages of growth for the plants life cycle. This involved physically going out to the orchards and catching and identifying pests and diseases.

As part of the plant disease diagnostics and pest identification training program, equipment was also supplied to the project. Two PCR units were donated by the NTDPIR to help with pest and disease diagnostics as part of the DNA Workshops, Dyna lights and specimen collection equipment along with other entomological supplies were also provided. A motorised sprayer was also supplied for use in insecticide trials and farmer demonstrations on efficient spray application methods.

4.3 Link up and use existing extension services to enhance skills development and information flow to PDAFFS, NGO and agribusiness in mango productivity and quality: Each of the collaborating organisations, CARDI, GDA and RUA were allocated one or two of the five target provinces of Kompong Speu, Kandal, Battambang, Kompong Cham and Siem Reap to be responsible for. This would allow for a reference guide for current and future researchers, and contribute to the pest and diseases list being assembled for market access purposes.

Local agribusiness such as United Cambodia Agri (UCA) were involved in several field visits with the team along with field demonstrations. The project team also contributed to a pruning video produced by UCA. The Korean company Foodya also worked closely with the team on the export simulation trials and this collaboration is ongoing.

Later, extension material was planned where gaps in knowledge were considered to be obvious. Gaps in knowledge involved both industry and all of the collaborators. Input of all material and workshops was a direct result of the learnings over time that started to fill the gaps in knowledge. Once the baseline was determined we could then set protocols. Examples of development of protocols and outputs which are still in progress include:

- Quality assurance posters, product description, grading standards for marketing fruit
- Mango variety description sheet
- Pruning videos and fact sheets small and large trees
- Regional pest and disease workshops
- Basic growing calendar
- Nursery best practice guide
- PBworks, giving a database of all the resources gathered through the project.

Example pages from the Mango Nursery Manual and the Mango Pest and Diseases Guide can be found in Appendices 5 & 6.

4.4 A series of guest lectures delivered to the Royal University of Agriculture by the Australian team. A total of seven lectures were delivered to RUA students throughout the project. Generally one Australian team member was delegated to deliver a lecture on each team visit. The Cambodian team also delivered occasional lectures on mango related subjects to 3rd and 4th Year students. A total of 10 RUA students also assisted with pest and disease surveys and the export simulation trials and various stages during the project.

In Australia

5. *Understand the function of the FT gene during the expression of flowering and incorporate these techniques into normal crop management for the manipulation of fruit maturity.*

There were three components to the Northern Territory based research conducted in the project. This work was complemented by concurrent research with the Hort Innovation

funded mango project, *MG12012 Manipulating mango flowering to extend the harvest window*. The three components were as follows;

5.1 *Investigate and characterise the FLOWERING LOCUS T (FT) gene and its homologues in a greater diversity of mango cultivars:* A PhD project was created to conduct this work. Stacey Cook was based at the University of Queensland, with Dr Lucy Tran Nguyen acting as one of her supervisors throughout the project. While the molecular work was carried out at UQ, field experiments were conducted in the Northern Territory. As part of the field work, a glasshouse pot trial was conducted to evaluate the effect of temperature, potassium nitrate and thiourea on the expression of flowering related genes in mango. Two Australian mango varieties (Kensington Pride and NMBP1243) using mature scions grafted onto a common rootstock was subjected to six treatments. These were cold temperature (<20C), 4% KNO_3, 1% thiourea, cold plus KNO_3 and cold plus 1% thiourea.

5.2 *Develop in vitro systems to label and track the FT gene using fluorescent molecular probes during floral induction in mango:* An aeroponic culture of mangoes was established at Berrima Research Farm under glasshouse conditions to conduct nitrogen studies. In additional work related to the PhD study, *Arabidopsis thaliana* was used to identify a complex network of genes involved in regulation of mango flowering. Development of a specific FT gene using molecular probes to track induction was deemed not feasible. Methodologies had to be revised to within the research program to evaluate the genes associated with the regulation of flowering in mango, how they are co-expressed and the network of positive and negative regulators of the flowering pathway from vegetative to flowering phase.

5.3 Investigate the role of chemical treatments, inductive temperatures and rootstock on the expression of the FT gene and flowering in mango:
Investigations were carried out into the effects of ethephon, potassium nitrate and thiourea on leaf flushing and flowering in Kensington Pride mangoes. The thiourea treatments were effective in increasing flowering in a season where high temperatures reduced normal flowering. Ethephon proved effective in removing unwanted flush growth and maximising synchronised bud growth. Ethephon trials were also conducted in NSW. The tip pruning trial was repeated using Honey Gold and B74 in Katherine in 2016-17. Trees were pruned at monthly intervals from April till September and monitored at weekly intervals. This covered the period prior to and after inductive temperatures occurred.

A detailed presentation of the results from these investigations can be found in *Section 7 Key results and discussions.*

6. Achieve the successful on farm adaptation of biocontrol agents as part of an improved system for integrated pest management for fruitspotting bug (FSB) in macadamia, avocado and other sub- tropical tree crops.

6.1 *Optimising the monitoring system and assessment of biological control options for fruitspotting bug (FSB):* ACIAR funds were utilised to help fund additional studies into adaptation of biological controls for FSB, use of trap crops and effectiveness of pheromone lures for monitoring of FSB.

As part of this study we looked at 3 different native egg parasitoid species; *Anastatus* sp. (Hymenoptera: Eupelmidae), *Gryon* sp. (Hymenoptera: *Scelionidae*) and *Centrodora darwini* (Girault) (Hympenoptera: Aphelinidae). *Anastatus* sp. was selected for commercialisation and was successfully reared on eggs of *Antheraea pernyi*, Chinese oak silkmoth.

Pheromones for both *Amblypelta* spp. were investigated in previous studies under various ACIAR projects. For *A.l. lutescens* (found only in Queensland and the Northern Territory) an aggregation pheromone lure which lasts six weeks in the field, was fully optimized. A prototype sticky panel trap has also been developed. An optimum trap density has been established at 10 traps/ha. A monitoring protocol has been developed and treatment threshold has been determined at 0.5 bugs/trap/fortnight. The crops in which the thresholds were specifically proven to work were avocados and custard apples. The launch of a commercially available *A.l. lutescens* pheromone trap took place in March 2017.

A number of trap hedges at the Centre for Tropical Horticulture (CTH) at Alstonville and 4 trap hedges on and adjacent to commercial farms (macadamia, avocado and custard apple) located in different regions, including Alstonville, Nambour and Bundaberg. We developed a monitoring protocol and treatment threshold of ≥ 30% of 5th instar nymphs in the FSB population recorded at any particular date, which can be applied to the main crops (macadamia, avocado and custard apple).

End of Project Review: In November 2016, ACIAR commissioned an End of Project Review of HORT/2012/003. The review made a range of recommendations, including funding of a project extension from April 2017 to December 2017, and funding of a phase 2 of the ACIAR Cambodia Mango Project. Other key recommendations included the following:

- It is recommended that, where possible, the key relevant personnel from the mango project in all agencies continue to be involved in the follow-on SRA and ACIAR Cambodian/ Philippines mango project.
- Repeat key research where essential, for example determining the mango harvesting maturity indices, and refining crop control strategies for key varieties in major regions of production in Cambodia.
- Provide key soils data (physical and chemical) including depth and drainage to help address the high calcic, high soil pH levels > pH 8-8.5plus problems.
- Continue to take over more of the Technology Transfer (information, training, extension, workshops, demonstrations etc.,) from the Australian Team, which Cambodians have already commenced in a very positive way.
- Strongly support much needed realistic determination of Gross Margins for farmers/contractors and clearly define value-added margins for farmers/contractors, collectors, traders, exporters, retailers etc.
- The project team should articulate the adoption pathways for affordable, low cost technologies for mango production that can be successfully introduced to farmers. In the next phase of the project, the future research, development, extension and training activities should strongly encourage strong involvement of larger producers, private traders or firms in the supply chain (e.g. chemical suppliers, exporters, etc.).

Other ACIAR Project Collaborations: The HORT/2012/003 team enjoyed good collaboration with other ACIAR projects, including the Gary Kong's ACIAR project *"Enabling*

Improved Plant Biosecurity Practices in Cambodia, Lao PDR and Thailand" (HORT/2010/069), which includes Cambodia partners CARDI, GDA and RUA and Grant Vinning's project *AGB/2014/020 Value chain analysis of the Cambodian mango industry* which studied the cross border trade into Vietnam and Thailand. Joint workshops were conducted with Gary Kong on development of pest lists, and included training of the team in the use of the database *PestPoint,* and Grant Vinning utilized project team members on data gathering field trips to border crossings. Grant also presented updates at the 2015 and 2016 project planning workshops.

6 Achievements against activities and outputs/milestones

Objective 1: To develop and evaluate integrated crop management strategies for productive and profitable high yielding orchards that produce quality fruit that meet international export standards.

no.	Activity	outputs/ milestones	completion date	comments
1.1	Improved mango nursery practices	a) Establish model nurseries b) Develop best practice nursery manual	Dec 2017	a) A draft of the mango nursery best practice guide was completed by December 2017 and presented to the project team for input. *An updated version of the manual can be found in the Appendix 5. Also see potting mix trial report in Appendix 3.*
1.2	Germplasm collection & evaluation	a) Establish two a collection of scion and rootstock trials in regional provinces and CARDI. b) Evaluate performance of grafted v's non grafted plants c) Collect performance data on Cambodian varieties and collate into extension material	Dec 2017	a) Propagation of introduced rootstocks and various combinations of the scions including Kensington Pride and R2E2 were established during 2016 and planted out in the CARDI orchard. Approximately 50% of trees failed to establish and replacement trees are now being propagated. Comparative planting not yet commenced. c) A draft of a varietal description manual including the major mango varieties grown in Cambodia is in development.

1.3	Regional experimental trials	a) Establish regional orchards. b). Develop crop phenology models for each region. c). Implement best management practices for agronomy and plant protection practices. d). Conduct training programs for researchers, extension officers agribusiness and grower.		a) Regional orchards are yet to be established b) Comparisons between Keo Reach and Keo Tep demonstrated that Keo Reach had four flushes per year, with a significant late flush, while Keo Tep only had two flushes beginning in the rainy season in 2015/16. Observations of flushing/flowering times were also collected from the Kompong Speu collaborator site. Weather data from CARDI will be matched to the growth data. c) Pruning trials and demonstrations have been carried out in Battambang. A workshop and field demonstrations were completed as part of the November 2016 project review.
1.4	Crop manipulation	a). Document current practices growers use to manipulate flowering. b). Evaluate alternative practices to move the harvest window forward and/or backwards to maximise profit and acceptable to the consumer		a) Double cropping practices are now well understood. A combination of paclobutrazol, thiourea and potassium nitrate are used in varied concentrations in different regions to induce out of season flowering. Impacts on tree health are significant in some locations, particularly where high rates of paclobutrozol are used. b) An ethephon trial at Kompong Speu was conducted and appears to check flush growth in early wet season. Alternatives to using thiourea also to be examined in the 2016/17 crop.
1.5	Economic analysis	Determine the profitability of mango production in Cambodia		Economic analysis of important components such as the flower induction system commenced. Costings for specific inputs gathered in May 2016 visit. Gross margin analysis also being compiled.

1.6	Survey of major pests and beneficials in mango crops to assess impact on yield and fruit quality in the on season and off season Specimen collection, taxonomic ID, photos, life cycle specimens	Publication describing lifecycles, pests and beneficials Survey report including pests list and natural enemies Initial survey report		a. Training in methods (2 workshops which included regional staff) in collections and taxonomy. b. Surveys conducted and insects and pathogens collected from all five provinces covered by the projects. c. Official collection held at GDA. d. Specimens brought to Australia for specialist identification and then returned to the GDA collection. e. Plant clinics – 4 workshops completed in provinces. f. Molecular training in pest and disease diagnostics. g. Donated 2 PCR units to CARDI j. Purchased Dyna-lights and collecting equipment for CARDI, GDA and RUA.
1.7	Conduct survey of diseases in mango crops in on season and off season. Conduct laboratory isolations to confirm record of locations	Publication describing diseases list based on survey		See 1.6 above. Pest and disease list nearing completion. Cross checking with regional pest and diseases lists will be required, along with previous records. In view of the international market access implications involved with pest lists (which includes invertebrate pests and diseases), GDA have decided that data on new pest species be kept confidential until the identifications have been verified by a specialist. While they are still keen for projects such as this one to assist with verification of identifications they have introduced a new protocol for obtaining permission to take specimens out of the country. (See also 1.8)

1.8	Develop an IPM/IDM program based on major pests and diseases from survey - test softer chemicals to conserve beneficials - use of biological controls & encourage beneficials - test pheromones (insects) and trichoderma (diseases)	Development of draft IPM guidelines	Dec 2017	It is planned to have a draft pest and disease field guide prepared and translated into Khmer for review at the start of the following project (HORT/2016/190). One of the first activities of that project will be to field test this with grower groups before publication. GDA also asked us to use the information to develop posters in Khmer Testing of Agnova Fruition ® fruit fly traps was commenced in November 2017. Specimen flies were brought back to Australia for identification. Pheromone trial conducted for *Citripestis eutraphera*. Development of a synthetic pheromone still underway by NTDPIR and NZIPFR.
1.9	Conduct residue testing program for pesticides to identify problem chemicals	Residue testing completed and confidential report available to project team.	June 2016	Completed

PC = partner country, A = Australia

Objective 2: To Identify and prioritise the key supply chain constraints, including postharvest losses, packaging, storage and transport to deliver mangoes into selected markets.

no.	**Activity**	**outputs/ milestones**	**completion date**	**comments**
2.1	Map social, economic and bio-physical components of mango supply chains	c) Report of survey of key supply chain actors (collectors, wholesalers, retailers and exporters). d) Identify where losses are occurring along the supply chain and determine appropriate interventions together with supply chain actors e) Market Information report detailing price and quality data	May 2017	Survey Highlights: To capture information on current production practices and the flow of goods and services in the supply chain we interviewed a sample mango growers and collectors in Kampong Speu Province, Cambodia. The objective of the interviews was to understand the status quo in mango value chains in Cambodia. Also to identify opportunities to improve future management and marketing decisions for growers and consequently improve farm-level productivity and profits. Given this objective, the interviews were limited to the production and consolidation activities in the supply chain and not extended to retailers or consumers. Information gained from interviews with mango contractors and collectors in Kampong Speu Province, Cambodia indicates that the mango industry is growing and has further growth potential if

		received for mango in selected markets (provincial, urban and export (Ho Chi Minh) markets.		productivity an on-farm and post-farm management practices can be improved. Thailand and Vietnam are important and growing markets for fresh mangoes grown in Kampong Speu Province. To capture the opportunities associated with these markets there is a need to improve on-farm management and information flows in the Cambodian mango industry. At present the information flow between contractors and collectors is restricted to price and timing of harvest for the current season. Frequent discussions about expected yields, quality, seasonal conditions and market demands between collectors and contractors could aid both to modify their operations to take advantage of market opportunities.
2.2	To determine and deploy appropriate postharvest technology for reducing losses and improving quality out-turn	b) Review ASEAN and other fruit standards and develop appropriate fruit standards for Cambodia c) Train Cambodia researchers in postharvest evaluation of mango d) Develop and trial appropriate low-cost technology that can be adopted by farmers and other supply chain participants e) Recommend appropriate interventions to suite of supply chains under study (refer 4.3)	June 2017	c & d) The export simulation trial provided excellent experience in planning and managing commercial scale trials for the project team. Fruit was sourced from a large grower at Kompong Speu with cooperation from the Korean exporter. Over 300 mangoes were stored at 12^0C for a total of 21 days at the CARDI Postharvest Lab. Fruit was removed at 7, 14 and 21 days and assessed for skin and flesh colour, firmness, dry matter, skin defect and disease. An additional treatment of a postharvest fungicide (Scholar ®) vs no fungicide was also used. See the report in *Section 7 Key results and discussion.*

2.3	To initiate, develop and monitor 3 demonstration supply chains – two domestic and one export chain	a) Determine 3 supply chains to implement b) Link with commercial partners to implement identified interventions c) Report on 'road testing' of supply chain interventions	May 2017	The Kompong Speu surveys identified potential supply chain collaborators to work with in the follow up project, commencing early 2018. There was very limited funding to implement this study in the current project.

PC = partner country, A = Australia

Objective 3: To design and implement a pathway to adoption of improved management options for the Cambodian mango industry encompassing both on farm production and supply chain.

no.	Activity	outputs/ milestones	completion date	comments
3.1	Determine knowledge gaps in mango production and supply chain management with PDAFF extension workers and farmers	Complete knowledge audit of PDAFF staff and farmers on province by province basis	July 2016	Baseline knowledge surveys carried out during pest and disease workshops in four provinces. Identification of the major pests and diseases was reasonably good with farmers able to correctly identify 70 to 80% of samples presented to them.
3.2	Identify potential collaborator farmers willing to test new ideas and technology in up to 3 provinces (ie Kandal, Kompong Speu and Kompong Cham)	At least 2 Collaborator farmers identified per province. Demonstration orchards will be established at these sites	Dec 2017	Nitrogen fertilizer trials and investigations into use of ethephon to control flush and flower timing were continued with the farmer collaborator in Kompong Speu. Timing and rates of paclobutrazole were also investigated on this farm and found to be very high dosages, up to 10 times the normal rates applied. Control trees on the block served to provide a useful comparison for tree health. These control trees were also used for follow up N rate trials in the 2017 wet season crop.
3.3	Establish grower interest groups in collaboration with PDAFF staff	Groups established and operating		See 3.4 below

3.4	Conduct provincial workshops on topics nominated by farmers in target districts	1st workshop held early 2014 in key provinces	October 2016	CARDI, GDA and RUA staff collaborated with Provincial Department of Agriculture Forestry and Fisheries staffs carry on the famer workshops on pest and disease control were delivered in Kompong Speu, Kandal, Battambang, Kampong Cham and Siem Reap Province. A total of 158 farmers participated in the workshops which were held in September/October 2016.

PC = partner country, A = Australia

Objective 4: To build the capacity of the Cambodian research, development and extension system to deliver targeted and practical outputs to agribusiness and farmers.

no.	Activity	outputs/ milestones	completion date	comments
4.1	Provide scientists with needed fruit RD&E training through graduate degree programs, mentors, or alternative options, to build their capacity to undertake RD&E . Annually review the program	Series of team workshops to build capacity in tree crop agronomy, pest and disease	April 2017	Training workshops specifically designed to build R&D capacity of the team continued during 2016/17. Two DNA training workshops were conducted in December 2016 and March 2017. Two pest list training workshops conducted: November 2013 trained project collaborators (CARDI, RUA and GDA including provincial staff) in designing surveys and collecting pest and disease specimens: April 2015, three day field trip and two days in the laboratory, specimen preparation and curation, long term storage, record keeping in databases, and diagnosis including verification by specialists. Two molecular workshops were conducted with 11 Cambodian delegates (7 RUA, 2 CARDI and 2 GDA) in April 2016 and March 2017. During this second workshop, the molecular results from laboratory experiments conducted in November 2016 were discussed. This included the molecular identification of plant pathogens. The DNA barcoding of immature and adult specimens of cerambycids was discussed. Diagnostics was not clear cut and it is now important for the Cambodian research team to continue to survey and collect these longicorns that cause damage to their mango trees. Two photography workshops were conducted with subjects covered including lighting, specimen preparation and cataloguing images. Apart from pest and

				diseases, photography of variety and fruit maturity images were also collated as part of the training. Pest and disease lists progressing well. Collections near completion. Building on training delivered during 2015. Specimens brought to Australia for specialist identification. Provided diagnostic equipment. Impact of P&D in yield and quality not commenced. Plant clinics – 4 workshops completed. (2016). Total of 139 growers attended. Molecular training in pest and disease diagnostics. Donated 2 PCR units to CARDI Purchased Dyna-lights and collecting equipment to CARDI, DGH and RUA. Good progress in team and provincial staff capacity building in this component. Training ongoing. A training workshop was also provided on designing and conducting a pest management trial. A trial was designed to test insecticides for control of mango leafhopper, and a property identified for this to be carried out. Unfortunately the pest, which had been in large numbers the previous year, was uncommon when the trial was planned and the trial did not go ahead.
4.2	Develop modules and conduct training courses and workshops for researchers and extension agents in general orchard management practices and in disease and pest management strategies.	PDAFF involved in 2nd Phase workshops and commence training farmers	Dec 2016	The project team in collaboration with PDAFF coordinators conducted a field day as part of the Project Review in November 2016 at Dr Vuthy's farm in Kompong Speu and a pruning workshop at Battambang. In Kompong Speu the team presented the results to date on the nitrogen fertilizer trial, and the ethephon treatments which were applied on the same block of trees. Farmers were also addressed on spray application principles. In Battambang, 35 farmers attended a workshop on pruning mango trees, which was followed by a farm walk and practical demonstration. The farmers also inspected a block of mangoes that had been "staghorned" 12 months previously on the Beung Rieng Research Station

4.3	Link up and use existing extension services, to enhance skills development and improve information flow to NGOs, agribusiness services growers, contractors and field workers in mango productivity and quality.	a) Nursery production guide, growing manuals and postharvest posters etc are published and available for use in training workshops b) Training of private sector commences	Dec 2017	A draft of the nursery manual was completed by December 2017. The pest and disease guide and the quality guide drafts should also be completed. Meetings were also held with major local growers who were keen to get involved with the export development component of the project going forward. Meetings were also held with Korean investors who already owned farms in Cambodian including Hyundai Agro who were importing suitable orchard machinery were and were interested in collaborating with the team to develop management and export strategies for Cambodian mangoes. Developed of Cambodia Mango Standard (ASEAN) GDA also worked closely with private sectors especially extension the MoU of export Cambodia Mango to Korea.
4.4	Guest lectures are delivered to the Royal University of Agriculture and Regional Universities by local and Australian team members	Lecture program developed in 2013. Delivery of lectures continues throughout the project		A total of seven lectures were delivered to RUA students throughout the project. Generally one Australian team member was delegated to deliver a lecture on each team visit. The Cambodian team also delivered occasional lectures on mango related subjects to 3rd and 4th Year students. No ACIAR Mango Team lectures were delivered at RUA during 2016/17 due the time constraints.

PC = partner country, A = Australia

Objective 5: To develop management options to manipulate the mango harvest window.

no.	Activity	outputs/ milestones	completion date	comments
5.1	Investigate and characterise the FLOWERING LOCUS T (FT) gene and its homologues in a greater diversity of mango cultivars	a). Identify FT and homologous genes from selected mango cultivars b). Commencement of PhD student (alternative funding source)	April 2017	Data mining from mango next generation sequencing data (obtained from the small trees project) is ongoing and primers targeting flowering genes from the experimental host, *Arabidopsis thaliana*, have been designed and will be used to identify similar sequence reads in the mango genome. This work will allow the expression levels of mango flowering genes to be monitored during trials. Glasshouse trials based upon temperature and chemical treatments have been conducted at Berrimah Research Farm on potted mango trees (KP and NMBP1243), a large number of samples have been collected at different times and tested using real time PCR technology allowing the expression of each gene to be monitored. This trial is currently being repeated. See more detail in *Section 7*

				Key results and discussion
5.2	Develop *in vitro* systems to label and track the FT gene using fluorescent molecular probes during floral induction in mango.	a). Establish *in vitro* systems using experimental host (tobacco and/or *Arabidopsis thaliana*) and mango-derived FT gene probes. b). Replicate (a) in selected mango cultivars		Aeroponic culture of mangoes commenced under glasshouse conditions for nitrogen studies. The PhD study within this project has identified a complex network of genes involved in the regulation of flowering in mango using *Arabidopsis thaliana* specific flowering genes. As such, the development of specific FT gene using molecular probes to track induction was not feasible. Methodologies have been revised within the research program to evaluate the genes associated with the regulation of flowering in mango, how they are co-expressed and the network of positive and negative regulators of the flowering pathway from vegetable to flowering phase. The research is in the final year and techniques will be applied to phase II project to investigate similar flowering genes in Cambodian mango varieties.

5.3	Investigate the role of chemical treatments, inductive temperatures and rootstock on the expression of the FT gene and flowering in mango	Report on trial detailing treatments (chemical, temperature and rootstock) on FT expression and flowering		Continued investigations into the effects of ethephon, potassium nitrate and thiourea on leaf flushing and flowering in Kensington Pride mangoes. The thiourea treatments were effective in increasing flowering in a season where high temperatures reduced normal flowering. Ethephon proved effective in removing unwanted flush growth and maximising synchronised bud growth. Ethephon trials were also conducted in NSW The tip pruning trial was repeated using Honey Gold and B74 in Katherine in 2016-17. Trees were pruned at monthly intervals from April till September and monitored at weekly intervals. This covered the period prior to and after inductive temperatures occurred. These results are currently being analysed and are consistent with responses in Darwin. Previously we had not focussed on the period after cessation of inductive temperatures. However, it is evident that any cool experienced by the tree prior to tip pruning does not contribute to the floral induction process. This is consistent with induction occurring when there is active bud growth In the NT, a glasshouse pot trial was conducted to evaluate the effect of temperature, potassium nitrate and thourea on the expression of flowering related genes in mango. Two Australian mango varieties (Kensington Pride and NMBP1243) using mature scions grafted onto a common rootstock was subjected to six treatments. These were cold temperature (<20C), 4% KNO_3, 1% thiourea, cold plus KNO_3 and cold plus 1% thiourea. Samples were taken at time of chemical treatment (0h) then 1, 3, 6, 18 and 36 hours post treatment and stored at -80C. RNA extractions were conducted at the University of Queensland, and reverse transcriptase PCR (RT-PCR) was conducted using a range of flowering genes primer sets including positive and negative regulator genes. The results from this trial showed that the treatments had an impact on the expression of the chosen flowering genes. Differences in expression between the two cultivars was also observed. In particular, thiourea treatment increased the expression of the positive flowering regulator FLD, thus supporting observations that thiourea is a floral inducer. Overall, the trial supported the hypothesis that the expression of flowering regulation genes is influenced by chilling and application of KNO3 and Thiourea. NB- once the potted plants were removed from the glasshouse following the trial, flowering was observed in some of the plants. This trial was repeated and processing the data is underway and incomplete at the time this report was compiled. This research could be applied to evaluate the effectiveness of these treatments on Cambodian mango varieties and is proposed for the next project. It will need to be modified, in particular, the temperature as <20C would not available in Cambodia environment. As such, a minimum temperature for Cambodia mango varieties still needs to be determined. In 2017, investigation to identify when flowering occurs in mango in a NT commercial orchard and correlate the gene expression levels of candidate mango genes responsible for floral induction was conducted. Year 1 of this trial indicated no clear pattern of target flowering regulators and will be repeated in 2018.

PC = partner country, A = Australia

Objective 6: In Australia, to achieve the successful on farm adaptation of biocontrol agents as part of an improved system for integrated pest management for fruitspotting bug in macadamia, avocado and other sub tropical tree crops.

no.	Activity	outputs/ milestones	completion date	comments
6.1	Appoint a full time technical officer to support the adaption of biological controls in the fruitspotting bug project.	Technical officer appointed		The HIA fruitspotting bug project concluded in September 2017. ACIAR funds were used to employ a NSW DPI technical officer to draft the Pest and diseases manual in late 2016.
6.2	Optimise a monitoring system for FSB	Evaluation of pheromone/trap systems for *A. nitida* and trap crop evaluation complete		The *A. lutescens* pheromone trap was released for commercial sale by Organic Crop Protectants (OCP) in March 2017. Work on development of a similar trap for *A nitida* continued with further evaluations at CTH Alstonville.
6.3	Evaluate the effectiveness of *Anastatus* and other egg parasitoids of FSB			*Anastatus* is used on a limited scale commercially. The wasp continues to be evaluated on commercial orchards under the new HIA funded macadamia IPM Program along with other biological options including *Oencertis spp.* and *Gryon spp*.

PC = partner country, A = Australia

7 Key results and discussion

Throughout the course of the project there were numerous outputs from the project that generated a high level of interest from farmers, and provide a good platform for follow up research and commercial implementation. Some of the more significant of these include the following, and are discussed in more detail below:

- Initial flush and flower growth measurements of Cambodian mango variaties that can form the basis for future development of standardised crop models specific to local varieties.
- Development of variety seasonality tables for Cambodian mango varieties.
- Development of comprehensive pest and disease lists and improved understanding of the major pests and diseases in Cambodian mangoes through accurate identification improved understanding of the damage caused and control methods.
- Improved skills in extraction of DNA from insect and leaf material for use in molecular laboratory based techniques for identification of pests and diseases.
- Practical techniques in pruning and shaping of young trees to develop sound tree architecture, and re-working of large older trees to re-invigorate under-producing orchards.
- As a result of several trials in Cambodia and Australia, progress towards viable alternatives to use of thiourea for flower induction and leaf flush management , including use of ethephon and potassium nitrate.
- Initial nitrogen rate trials have provided as basis for future mango nutrition guidelines. Leaf nutrient analysis is also being carried out to establish some baseline values for the key major and minor nutrients.
- Recommendations on suitable potting media for production of fast growing nursery seedlings.

Under this key results section, some of the highlights from the project are presented below.

7.1 Physiology Studies: Growth measurements in Keo Reach and Keo Teap

Basic physiology studies by Srey Nech from the CARDI Plant Breeding Team were conducted in the CARDI varietal collection at CARDI in 2014. The studies were carried out on Keo Tep, which is a selection of the variety Keo Chen, the 2nd most popular variety in Cambodia behind Keo Romeat. The comparisons between Keo Reach and Keo Tep demonstrated that Keo Reach had four flushes per year, with a significant late flush, while Keo Tep only had two flushes beginning in the rainy season. Weather data from CARDI was matched to the growth data.

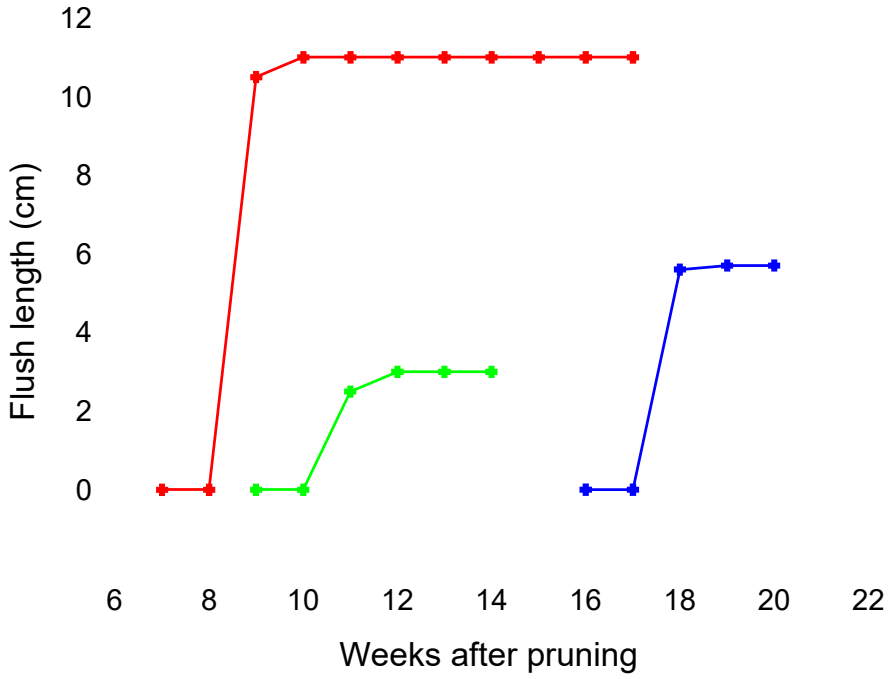


Figure 6: Flush length measurements on Keo Reach at CARDI

From the shoot meausrements it is possible to develop a simple growth model across the season for Keo Reach grown at CARDI. See Figure 7 below for a typical flushing pattern for the variety Kensington Pride.

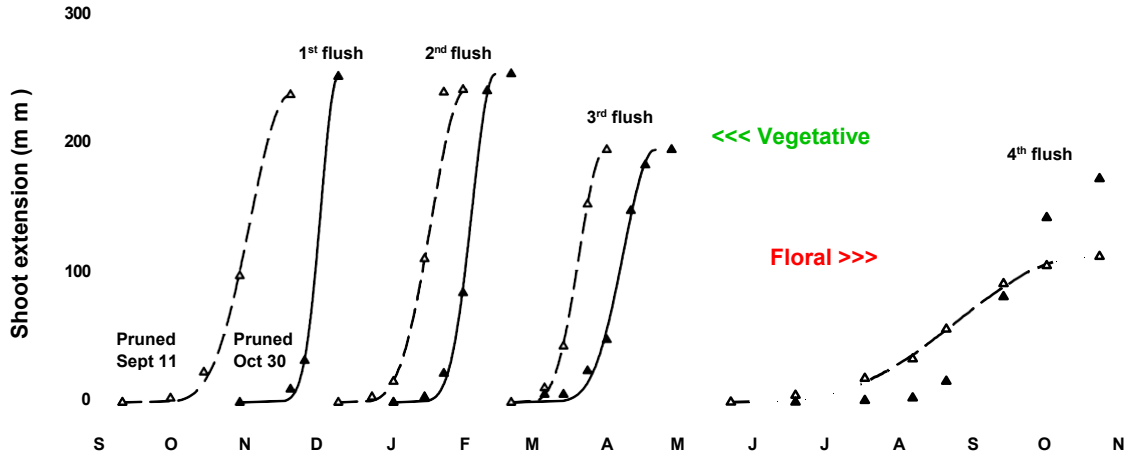


Figure 7 : Indicative flushing pattern for the variety Kensington Pride

7.2 Seasonality

In Cambodia, mangoes are available from January to May but the peak harvest is from March to May. The fruit is often eaten ripe and the popular varieties among growers and consumers including Keo Romeat, Keo Chen, Prum Sen, etc. Keo Romeat accounts for more than 80% of production in the on-season (March to May), and almost 100% during the off-season harvest (November to December). The off-season harvest during the September

and November period as mango is manipulated to flower after the dry season crop is harvested. Keo Romeat is the only variety so far produced in the off-season.

Table 1: Harvesting cycle of different mango varieties

Variety	**Peak harvesting months**											
	J	F	M	A	M	J	J	A	S	O	N	D
1. Keo Romeat			1	1	1							
									2	2	2	
	3	3										3
2. Keo Chen	1	1	1	1	1	1						
3. Thai mango					1	1	1	1	1			
4. Keo	1	1	1	1								
5. Prum Sen	1	1	1									
6. Kh'tis		1	1									
7. Kh'tis Durian					1							

Source: Cambodian Marketing of Mangoes: An Estimate of Volume (Bo, Theang, Kay, Pann, & Newman, 2015)

*** Remarks for Keo Romeat variety:

1. Mango harvested On Season
2. Mango harvested Off Season 1st
3. Mango harvested Off Season 2nd

7.3 Cambodian Mango Supply Chains

Key results

a. Battambang supply chains

In Battambang, mango occupies 2224 ha, comprising 889,600 mango trees (400 mango trees/ha). Mangoes are produced in 6 districts including Rattanak Mondol, Banan, Samlot, Bavil, Kam Reang and Phnom Prek districts. The districts of Kam Reang and Phnom Prek, are recent additions with production in this region only starting a few years ago. There are 5 main retail markets including Toul Ta Ek, Leur, Thmey, Nat and Chrey market.

Table 2: shows the number of semi-structured interviews undertaken with different actors in the chain.

Details	Battambang province					Pailin province		Siem Reap province		Total
	Sangkei and Banan districts	Boeung Chouk market	Theiy market	Phnom Deiy market	Liem market	Pailin Town	Prum market	Banteays rey district	Samaky market	
collector	5						2			7
collector/ grower/ producer off season	2					1				3
collector & wholesaler				1	2	1				4
Contract farming	9							1		10
Wholesaler		6	2		1		1		2	12
Total	16	6	2	1	3	2	3	1	2	36

Table 2: Semi-structured interview summary by function and location

Fig 8 shows pictorially our current understanding of the mango supply chain in Battambang. The supply chain on the right represents the export supply chain with limited supply going on to the domestic market. The chain on the left represents the domestic mango supply chain in Battambang with product largely supplied from outside the region. Additionally some of this product is also sold to Thai retailers.

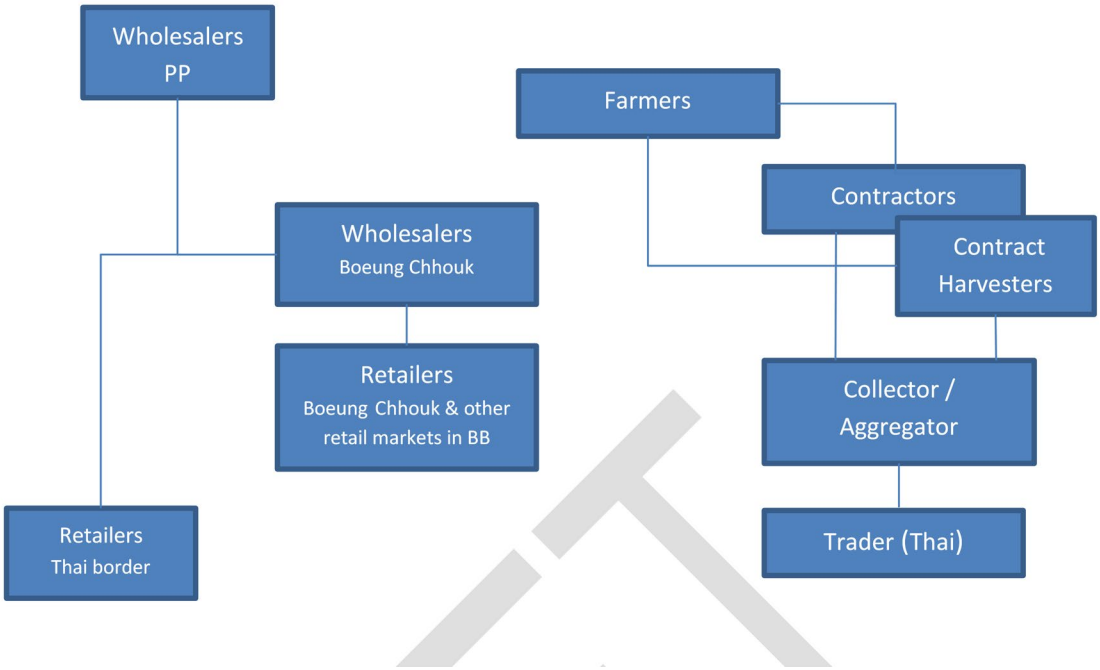


Figure 8: Battambang mango supply chain

i. Variety availability

Keo Romeat is the main variety exported to Thailand and Vietnam. In the off-season (December) collectors typically sell the fruit for R3000/kg, whereas on-season (April) prices are typically R1000/kg. Fig. 9 shows the availability of the two key varieties – Keo Romeat and Keo Chen.

Variety	J	F	M	A	M	J	J	A	S	O	N	D
Keo Romeat												
Keo Chin												

Fig. 9 Availability of key mango varieties (note: other varieties are sold in small quantities on the local market including: Khiev Savoeu, T Chau Anant, and Keo Morokot.)

ii. Contractors

In Battambang, contractors play an increasingly important role, with more farmers relinquishing their trees to contractor management. Contractors typically undertake production, flower manipulation and harvesting with each contractor managing 2-5 farms (415 -1450 trees). Depending on tree age, contractors typically pay $15 per tree/year for a 7-9 year old tree. Contracts may be entered into on an annual or triannual basis. Typically mangoes are produced in 2 main seasons: 1) on-season – April/May and 2) off-season – October-January.

Flower manipulation for off-season production is critical to ensure a profitable business, however from our discussions with contractors there appears to be a wide variety of techniques employed. Given the strong

returns for off-season production, contractors tend to try and produce fruit predominantly in the off-season. For example they may look to produce 100 tonnes during the on-season and 130 tonnes in the off-season. Contractors appear to have learnt techniques from Thai or Vietnamese traders and largely do not understand the phenological cycle of mango and how they might get the best off-season performance. Indications are that they are also stressing the tree resulting through over-production and limited nutrient replenishment. Part of this is likely to be due to unsuccessful attempts to induce flowering.

Contractors are likely to be a strong intervention point – given their diverse role and relationships with other key players in the chain.

iii. Traders/collectors/exporters

Battambang wholesalers in Boeung Chhouk wholesale market (and the smaller Thmei wholesale market) are small scale (500 kg/day) and therefore need to source mangoes from PP wholesalers (Neak Meas) rather than from Battambang where mangoes are exported directly to Thailand.

Mangoes are typically sorted into 2 grades. Grade 1 mangoes are large (3-4 fruit/kg), well coloured and no pest or disease damage. Grade 2 fruit are typically smaller (5-6 fruit/kg) and small defections are permitted. Mangoes for export are packed in 25 kg plastic crates. Whereas product for the local market tends to be packed into plastic bags. Typically Khmer traders purchase the product following a visit to the farm. They are often responsible for harvesting and packing. Transport to the Thai border by ute (3-4 T) is USD100/trip, whilst transport by truck (8T) costs around USD200/trip. At Leim gate/border - 3 large collectors/traders selling directly to Thai traders - 100-200 t/day during on-season, 20-40 t/day during off-season; many small collectors/traders. A similar situation exists at other border gates. Exported produce is sold into both the fresh and processing sector. On-season production is mainly used by the Thai processing sector 650 riel/kg (US16c/kg), whereas off-season production is mainly sold into the fresh sector 1200-1300 riel/kg (US30c/kg) Product is sourced from throughout Cambodia, traders/collectors at border supplied by network of collectors from other provinces. Most medium- high quality Battambang produced mangoes are being directly exported (limited locally produced fruit in Battambang markets).

Full interview transcripts prepared by the CARDI Socio-economic team have been provided in earlier reports.

b. Kampong Speu supply chain

The following descriptions of mango production, inputs, and prices and geographical spread of buyers are based on interviews with 10 contractors and 15 mango collectors in Kampong Speu Province, Cambodia.

i. Contractors

The majority of the contractors interviewed were relatively new to the industry with a maximum of 10 years of experience as a contractor. However, half of the contractors interviewed (five) also owned trees that they manage alongside the trees they contract. The average number of trees managed by each contractor was 6,000 (range: 800 – 23,300). The contracted trees ranged from three to 15 years old. The older trees were commonly more expensive to rent however, tree rents were also dependent on the relationship between the farm owner and the contractor. One contractor was able to rent 10 year old trees for only $2.58[1] per tree because he worked for the farm (and tree) owner for a long time. Other contractors in the sample paid an average of $11.15 for a tree. The range of prices paid across trees that were 3 years old up to 15 years old was $2.58 to $20 per tree. Higher prices are paid for older trees (10-15 years old) and lower prices were paid for younger trees, e.g. $3 per tree for a 3 year old trees.

All contractors interviewed were growing the Keo Romeat variety to harvest and sell in the on-season, typically in April, and off-season which typically runs from October to March. No contractors were growing other mango varieties or targeting other harvest/sale times.

Important inputs to mango production include fertiliser, chemicals to manipulate flowering, fungicides, pesticides, fuel and labour. Inputs such as fertiliser and chemicals were sourced locally or from as far away as Phnom Penh. Fuel was commonly sourced from within the Kampong Speu province. The contractor typically supplements their own labour with local sources of labour for tree management activities that are time critical. Harvest labour was commonly described as the responsibility of either the contractor or the buyer, depending on the buyer and mango price. Information inputs especially related to chemical use decisions tend to be sourced from the chemical sellers or the contractors' previous experiences.

Without specifying the split between on- and off- season production, the annual production of each interviewed contractor for 2015 is listed in Table 1, with quantities sold separated by quality grades. Fruit are classified as Grade 1 if they are large (3 fruit per kilogram) and have no skin blemishes and classified as Grade 2 if they are smaller (5-6 fruit per kilogram) with few skin blemishes. The largest producer was able to sell 610 tonnes of mangoes in 2015 however, the large majority of these were sold as Grade 2 (Table 1). Alternatively some of the smaller producers were able to sell 100% of their mangoes as Grade 1 (Table 1).

Table 3. Tonnes of mangos sold in 2015 as separated by quality grades for each contractor interviewed.

Contractor	Grade 1	Grade 2	Ungraded	Total
1	60	20		80
2	150	460		610

[1] *All dollar figures are in 2016 US Dollars.*

3		400		100	500
4		30			30
5				45	45
6				40	40
7		40			40
8		260	60		320
9		96		144	240
	10	200		200	400

Maximum, minimum and average farm-gate prices for 2015 as reported by the interviewed contractors are provided in Table 2. Grade 1 mangoes attract higher prices than Grade 2, small and ungraded fruit whereas ungraded mangoes attract higher prices than Grade 2 and small fruit (Table 2). This indicates that unless the fruit is Grade 1 it may not be worth grading before sale. Packaged mangoes attract the highest price at the farm-gate as this reduces packaging costs further down the supply chain. All interviewed contractors reported that their mangoes sold on farm to a collector or large export traders. For the 2014 and 2015 season there was a 50:50 split in mangoes sold at the farm-gate to Cambodian collectors and Vietnamese traders.

Table 4. Maximum, minimum and average mango prices (USD/kilogram) at the farm-gate in the on-season and off-season for 2015 as separated by quality grades.

	On-season			Off-season		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Grade 1	0.43	0.33	0.38	0.55	0.38	0.46
Grade 2	0.20	0.20	0.20	0.33	0.20	0.25
Small	0.15	0.08	0.11	0.15	0.08	0.11
Ungraded	0.50	0.18	0.30	0.58	0.35	0.44
Packaged	0.95	0.50	0.66	-	-	-

ii. Collectors

Of the 15 mango collectors interviewed four were classified as small, five as medium and six as large. These classifications were based on the scale of mango collecting operations and the capital owned and operated by the collector. For example, a small collector typically collects 600 tonnes per year and sells on to a larger collector or trader whereas a large collector typically collects more than 7000 tonnes per year and will collect from smaller collectors and farms. Larger collectors also tend to own a truck to transport mangoes to the Cambodian border to access export markets.

The collectors interviewed had a range of relationships and experiences with traders and wholesalers. All collectors on-sold mangoes to a larger collector or trader, i.e. no collectors sell directly to consumers. Based on on-sale information from collectors, Thailand is the key market for mangoes grown in Kampong Speu Province (Table 3). The Thai market is followed by Vietnam, local sales to larger collectors and China (Table 3) however there is anecdotal evidence for much of the product entering the Vietnamese market continuing on to China. The different markets also tend to purchase different products. The Vietnamese tend to select good quality fruit (e.g. grade 1) and pay different prices for different quality grades. Whereas much of the fruit that collectors send to Thailand is ungraded with one price.

Table 5. Average percentage of mangoes, acquired by interviewed collectors, that is on-sold to different buyers for 2013-2015.

Buyers	2013		2014		2015	
	On-season	Off-season	On-season	Off-season	On-season	Off-season
Collector at Thailand border	63%	63%	66%	66%	48%	43%
Vietnamese trader	29%	29%	34%	34%	32%	36%
Chinese trader	-	-	-	-	9%	9%

Cambodian collector	8%	8%	-	-	11%	12%

iii. Chain structure and product flows

Mango producers in Kampong Speu Province typically have two options to sell their fresh fruit. The first option could be to sell direct to a large-scale international trader (Figure 2). This arrangement is most common with Vietnamese traders. The arrangement is established at the beginning of the season with decisions on provision of inputs such as paper bags to cover fruit to protect against fruit fly and the provision of labour for bagging and harvest activities. Traders working directly with producers tend to seek the best quality fruit and thus are engaged throughout the season to ensure they maximise the access they have to high quality fruit.

The alternative option is to sell to a collector (Fig. 4). The collector will approach the grower toward the end of the season when the mangoes need to be harvested. There is rarely a pre-arranged contract to buy from a grower at the beginning of the season. The collectors vary in their scale of operations. Once a collector aggregates the products from multiple farms they have two options, one is to sell to a larger collector (this is common if the collector is small scale and collects relatively small quantities e.g. 600 tonne per year) the alternative is to sell direct to a trader. The collectors do not sell to retailers or consumers. If the trader is based at the border, it is the responsibility of the collector to transport the fruit to the border. For this reason it is often the large collectors that sell fruit to traders based at the border. Much of the fruit collected by the interview sample was sold on to Thailand or Vietnam. Though, anecdotal evidence was provided to indicate that fruit sent to Vietnam may eventually be on-sold to retailers and consumers in China.

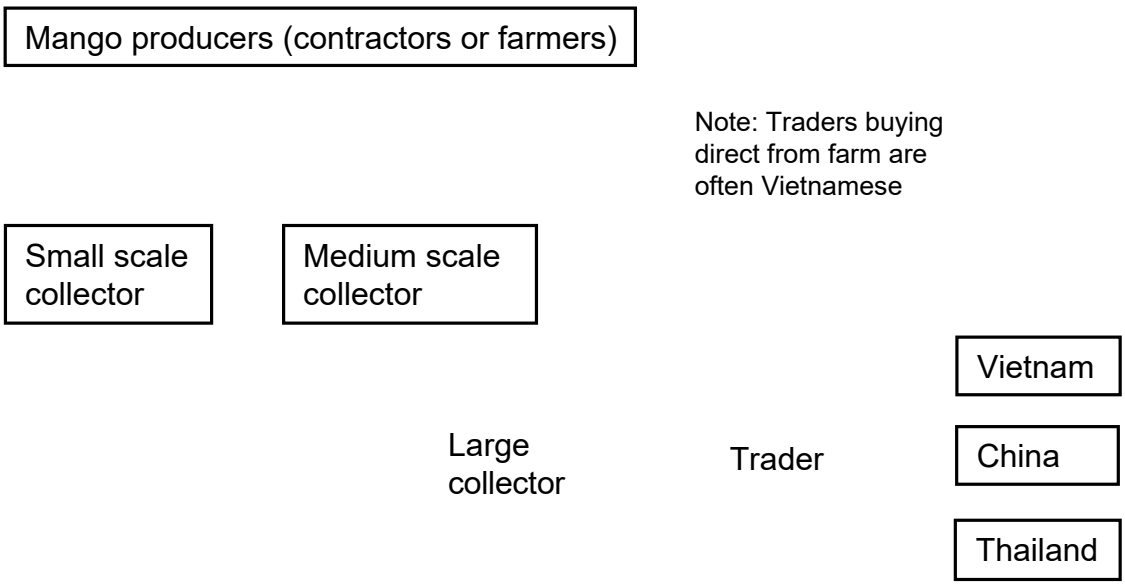


Fig. 10 Simplified supply chain for fresh mangoes produced in Kampong Speu Province, Cambodia.

iv. Supply chain issues

Key issues that arose during interviews with contractors and collectors include: mango price variability, relationships between supply chain actors and information flows. When respondents were given the opportunity to express concerns or future opportunities for the industry price was mentioned most often. For the three-year recall period, 2013 to 2015, many contractors described the mango price as a concern as it had been very variable. On the contrary, a small sample of the collectors described the mango price as increasing with increasing opportunities to enter the export market. This may indicate that any increase in price is not being passed on to the grower.

Contractors are motivated to manage leased mango orchards in a sustainable manner. They are aware that their opportunity to renew leases depends on the owners perceptions of their management. With one to three year leases being typical it is important for the contractor to renew their lease in order to learn about their environment, get returns on investments and build relationships upstream and downstream in the supply chain. The tree rent agreed is variable across farms with tree age the main driver of rent prices. Rents were lower for the contractors that knew the owners and were trusted by the owners. It was difficult to ascertain any other situations in which the relationship between owner and contractor was important. For example, none of the interviewed contractors were offered extended lease times or management advice or assistance from owners if they knew each other well. Contractors sell to collectors that offer the highest price. As a consequence it is rare to sell to the same collector for multiple consecutive harvests and thus difficult to establish trust, expectations and certainty in the market.

Information exchanges between contractors and collectors tend to be limited to price and harvest date; the collector sets the price and date and the contractor responds with whether they can deliver to those conditions. There appears to be little forward contracting or negotiation of price and timing of sale between the contractors and collectors. There is also little communication between the land-owner and the contractor outside of the negotiation of the lease. The contractor appears to get most information about farm management practices from their own experiences or input suppliers.

c. Phnom Penh wholesale markets

There are 2 main wholesale markets in Phnom Penh for mangoes – Deum Kor and Neak Meas. Deum Kor sources mangoes from Kandal (Kean Svay), Kampong Speu (Kirirom), Takeo, Vietnam and Thailand. Mangoes are then sold to other Phnom Penh markets and provincial markets such as Kampot, Kampong Chhnang, Kampong Cham, Sihanouk Ville, Prey Veng, Takeo, Kampong Speu and Kandal, The market has 15 mango wholesalers in and around the market and 50 mango retailers. The main selling times – early morning (3am) and late evening (9-10pm).

Neak Meas sources mangoes from provinces throughout Cambodia and is considered the primary wholesale market for mangoes. Within the market there are exporters marketing to Thailand, Vietnam and in-direct to China. Within the market there are
10-15 large wholesalers selling 30-100 tonnes/day, 20-30 small wholesalers selling <10 tonnes/day,

i. A Neak Meas wholesaler – one of the large wholesalers

For off-season trade (September – December) their focus is Thailand (50 tonnes/day), Vietnam (20 tonnes/day) and China (18 tonnes/day). They classify fruit into 2 grades: Grade 1 – 2-3 fruit/kg and Grade 2 – 4-5 fruit/kg. For Grade 1 fruit in 2015 they typically receive 1500-2000 riel/kg (Thailand), 2000 riel/kg (Vietnam) and 2800-3000 riel/kg (China). Each market has different packaging with Grade 1 fruit destined for Thailand packed in 25 kg plastic crate, whilst a 70 kg box is used for Vietnam and a 40 kg plastic crate for China.
For on-season trade (March-May) they typically export 100-180 tonnes/day to Thailand, 20 tonnes/day to Vietnam and 16 tonnes/day in-direct to China.

Note: Interview transcripts have been previously supplied in earlier reports.

6. Conclusion

The Cambodian mango industry is expanding rapidly and this is primarily been driven by regional export trade of Keo Romeat. Despite its popularity in regional markets there is limited domestic trade due to Khmer consumers strong preference for Keo Chen and other varieties. For future growth to be sustainable there is a need for a greater diversity of varieties. The strong regional trade networks into Thailand, Vietnam and China provide significant opportunities for the industry to capitalise on these. However with ASEAN trade agreements (AEC) this is likely to have implications for product standards, food safety standards and also result in greater official trade. Ultimately this will drive improvements across the sector but for Cambodia to remain competitive these changes need to be implemented now.

Information gained from interviews with mango contractors and collectors in Battambang and Kampong Speu Province, indicates that the mango industry is growing and has further growth potential if productivity an on-farm and post-farm management practices can be improved. Thailand and Vietnam are important and growing markets for fresh mangoes grown in both provinces. To capture the opportunities associated with these markets there is a need to improve on-farm management and information flows in the Cambodian mango industry.

There is much variability in the on-farm management practices and use of inputs across mango contractors. Information from the contractors interviewed also indicated that inputs are sourced from a number of markets and suppliers. Much information regarding the use of chemicals and fertilisers was said to come from the seller or previous experience. This raises a number of concerns. Overuse or inappropriate use of such inputs could represent a waste

of resources and drive up the cost of production for growers. Further, chemicals are essential to achieve commercial yields of mangoes in provinces however overuse or ignorance of maximum residue limits and withholding periods will increasingly threaten export opportunities as international consumers demand safe products. It will be important for extension agents or local government groups to provide information to contractors and farmers on the appropriate use of chemicals and the market access issues that are associated with produce that is contaminated with chemicals.

The relationships between key supply chain actors are short-term with many one-off interactions. For example, collectors often buy mangoes from different orchards each year. Without continued relationships with the same producers it is difficult to build rapport and trust. On-going relationships could improve the flow of information between buyers and sellers of mangoes and allow forward thinking or planning to meet changing market requirements. At present the information flow between contractors and collectors is restricted to price and timing of harvest for the current season. Frequent discussions about expected yields, quality, seasonal conditions and market demands between collectors and contractors could aid both to modify their operations to take advantage of market opportunities.

7.4 Development of pest lists

At the request of the National Plant Protection Organisation (GDA) in Cambodia full lists of pests will not be presented in this report. However the key results from the pest list studies were:

- Many new pests (insects and diseases) were added to Cambodia official list. These include known pests of mangoes, often present in neighbouring countries but which had not yet been recorded in Cambodia.
- Specimens of these are now held in the official national Cambodia collection at GDA.
- Many specimens were brought to Australia for verification by specialists including scale insects, mealybugs, fruit flies and fungi, and many of these have been returned to the official Cambodian collection (this work is still ongoing).
- In several cases, where it was not possible to breed the insects to adult for morphological identification, DNA analysis on the immature stages was used to identify the species.
- A species of mango gall midge (Diptera: Ceccidomyidae) which occurs in Cambodia and in neighbouring countries is new to science. Attempts were made to collect adults of this species so that it could be officially described and named (this work is ongoing).
- The results of the pest list surveys were used to:
    - For trade - based on SPS agreement, need to provide training partners with complete list of insects and pathogens on the crop, official lists should be maintained by National Plant Protection Organizations (NPPOs) and lists should be specimen based – voucher specimens should maintained in an official national collection.
    - For production – from the complete lists it was possible to determine the most damaging insects and diseases for which control measures are necessary. These will form part of the field guide under development to train farmers to recognise these pests and trials in the phase 2 project will determine ways to control them compatible with IPDM principles.

7.5 Regional Experiments: Field experiment on response of Keo Romeat mango to different rate of NPK fertiliser application

In order to develop more accurate fertiliser recommendations, experiments were carried out at Kompong Speu by the CARDI Soil and Water Group.

1.1. Objective

The experiment was conducted to assess effectiveness of current fertiliser practices on mango crops in Kompong Speu Province, Cambodia. It was conducted in 2015-2016 at Dr. Vuthy's farm, located at Teuk Thla village, Beoung Roung commune, Phnom Srouch district, Kampong Speu province (picture 1).

Figure 11: Experimental field and its location on the farm.

1.2. Treatment and experimental layout

Based on baseline survey information and a group discussion with Australia team, the fertilizer trial was conducted at the above location with 5 fertiliser rates as following:

-T1: Farmer practice 1kg/tree of 15:15:15 NPK (control)

-T 2: Farmer practice 1 kg/tree of 15:15:15 NPK + Boron + Zinc + foliar

-T3: 2 kg/tree of 15:15:15 NPK

-T4: 2 kg/tree of 15:15:15 NPK + Boron + Zinc + foliar

-T5: 1.5 kg/tree of 15:15:15 NPK+ Boron + Zinc + foliar

All fertilizer treatments 15:15:15 NPK was applied on 09 October 2015, excluding Boron, Zinc and foliar application (figure 1).

Table 6: Experimental plot layout

Rep1	T0	T0	T0	T0	T0
	T3	T4	T2	T1	T5
	T0	T0	T0	T0	T0
Rep2	T2	T3	T5	T4	T1
	T0	T0	T0	T0	T0
Rep3	T5	T1	T2	T3	T4
	T0	T0	T0	T0	T0
Rep4	T1	T5	T4	T2	T3
	T0	T0	T0	T0	T0

T0:	Untreat tree buffer
T1:	Farmer practice 1 kg of 15:15:15 NPK (Control)
T2	Farmer practice 1kg of 15:15:15 NPK+ Boron+ Zinc+ foliar
T3	2kg/tree of NPK 15:15:15
T4	2kg/tree of NPK 15:15:15+ Boron+ Zinc+ foliar
T5	1.5kg/tree NPK + 0.5 /tree + Boron+ Zinc+ foliar

2.3. Data collection

- Soil sample were collected before fertiliser application and at harvesting day.

- Flower assessment was performed by counting flower branches per m^2 and 3 samples in each tree (see in table 1).
- Tree canopy volume was measured by measuring thee dimension of plant canopy including height, width and length. Canopy volume was calculated by using the following equation:
  $V = (\pi^2 * x * y * z)/6$ where
  x is canopy width perpendicular to the row,
  y is canopy length perpendicular to the row and
  z is tree height (Charles-Edwards et al., 1986)

  **Charles-Edwards, D.A.Doley, D. and Rimmington G.M 1986. 'Modeling plant growth and development.' (Academic Press: Sydney).*
- The trial was harvested on 29th April 2015. Data to be collected on harvesting day were total fruit weight per tree and number of fruit per tree.
- Leave samples were also collected for nutrient analysis after harvesting to analyse for nutrient concentration in the mango lease.

Figure 12:. Harvesting mango crop on fertilizer experiment in 2016.

1.4. Result

Soil property before fertiliser application

Before fertiliser application, soil pH was on both layers was moderately alkaline (8.1-8.2). Organic carbon was low for layers, 0.35 % for topsoil (0-30 cm depth) and 0.28% for subsoil (30-50 cm depth). Total N and Olsen P of both layers were very low (Table 1).

Table 7: Soil properties before fertiliser application

No	Soil Depth	pH (1:5 H_2O)	EC	Organic C	Total N	Olsen P
	(cm)		(mS/cm)	(%)	(%)	(mg/kg)
1	0-30	8.2	0.05	0.35	0.04	0.84
2	30-50	8.1	0.03	0.28	0.03	0.76

Due to unavailability of micronutrients (Boron and Zin), data for treatment 2 and 4 were omitted from experiment results.

Without fertiliser application, number of flower braches was only 5 branches/m^2. Applying 1.5 or 2 kg of 15:15:15 NPK/tree significantly increased number of flower braches to 7 branches/ m^2. Fruit weight per m^3 canopy was only 0.41 kg/m^3 for untreated mango tree. It was increased to 8.6 or 9.0 kg/m3 when 15:15:15 NPK fertiliser was applied 1 or 2 kg/tree, respectively. There was no significant effect of 15:15:15 NPK fertiliser rate on fruit size. The fruit weight per fruit was ranging between 0.27-0.31 kg/fruit (table 2).

Table 8. Mango flowers and fruit response to fertilizer application. Data are a mean of four replication.

Treatment code	Fertilizer rate (15:15:15 NPK) (kg/tree)	Flower branches per m^2 canopy (No/m^2)	Tree canopy volume (m^3/tree)	Total fruit weight (kg/tree)	Number of fruit (No/tree)	Fruit weight per m^3 tree canopy (kg/m^3)	Weight per fruit (kg/fruit)
T0:	Untreated tree	5	26.58	10.25	39.00	0.41	0.27
T1:	Farmer practice 1kg/tree of 15:15:15 NPK	6	21.29	18.39	64.50	0.86	0.28
T3:	2kg/tree of 15:15:15 NPK	7	22.90	20.01	71.25	0.90	0.27
T5:	1.5kg/tree of 15:15:15 NPK	7	26.88	14.32	44.75	0.60	0.31
LSD (P ≤ 0.05)		**1.4***	**ns**	**ns**	**ns**	**0.32***	**ns**

Note: Soil and leaf sample analysis after harvesting has not completed yet.

7.6 Field trial on the effects of ethephon on Keo Romeat in Kompong Speu

The trial was conducted at the same farm at Teuk Thla village, Beoung Roung commune, Phnom Srouch district, Kampong Speu province from 28 May to 27 June 2016. Aim of this trial is to determine the effects different concentrations of ethephon has on immature flush of Keo Romeat mango.

5 treatments were identified depending on different concentration levels of ethephon as given in below:

- T1: Control 0ml + wetter
- T2: Low 0.5ml/L + wetter
- T3: Medium 1ml/L + wetter
- T4: High 1.5ml/L + wetter
- T5: Very High 2ml/L + wetter

60 flushing branch apices at stage 2-3 of Keo Romeat mango were selected for the trial and divided into 5 groups of 12 each representing a treatment. The 60 flushing branch apices were sprayed, tag and taken images.

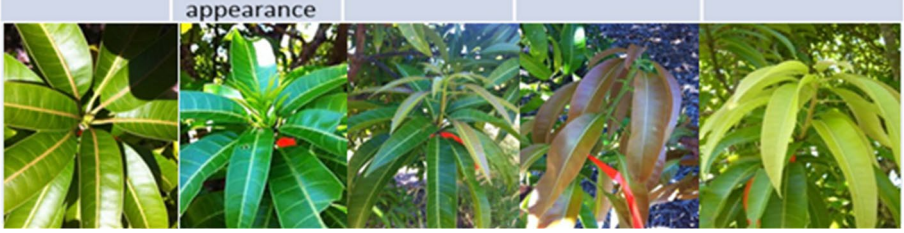
Development Stage	1	2.	3.	4.	5.
Description	Swollen vegetative bud, no stalk visible	Elongate of shoot commenced , young eaves, visible, spiky appearance	Lamina on leaves expanded	Leaf fully expanded but red in colour. leaves floppy	Fully expanded light green leaves
Appearance					

Figure 13: Stages of mango flush development

Result

The results showed that except control (T1: no ethephon), all immature flushes were abscissed even though spraying with low concentration of ethephon (T2). However, praying with high (1.5ml/L) and very high (2.0ml/L) concentration ethephon also caused some mature leaves to drop down (picture 4-8)

Figure 14: Mango flushes on application day (left) and four weeks (right) after applying with 0 concentration of Ethephon (T1: control).

Figure15: Mango flushes on application day (left) and four weeks (right) after applying with low concentration of ethephon (T2: 0.5 ml of 72% ethephon/L water).

Figure 16. Mango flushes on application day (left) and four weeks (right) after applying with medium concentration of ethephon (T3: 1.0 ml of 72% ethephon/L water).

Figure17: Mango flushes on application day (left) and four weeks (right) after applying with very high concentration of ethephon (T4: 1.5 ml of 72% ethephon/L water).

Figure 18: Mango flushes on application day (left) and four weeks (right) after applying with very high concentration of ethephon (T5: 2.0 ml of 72% ethephon/L water).

7.7 Export simulation trial of Keo Romeat mangoes

Objective 2.2 To determine and deploy appropriate postharvest technology for reducing losses and improving quality out-turn

A key objective of the Cambodian Government is to develop export markets for mangoes. As part of achieving this objective, in December 2015, the Government signed an MOU with the Government of South Korean to develop mango exports. Korean companies such as Hyundai Corporation have also rapidly increased their activities in agriculture since 2015, and in 2016 announced plans to build the first Vapour Heat Treatment plant in Cambodia. Development of export protocols is an essential component of building an export market, and information relating to the suitability of Keo Romeat for fresh export to markets in the region is required.

The export simulation trials at CARDI were undertaken in partnership with the Korean Food Processing Company, Foodya, who are developing protocols for mango exports. Two experiments were carried out in April 2017 (on season) and November 2017 (off season) to

measure the performance of Keo Romeat mangoes under medium term cool storage conditions.

In the 1st trial, a sample of 340 mangoes from a collaborator farm at Kompong Speu was harvested following dry matter testing, washed in Mangowash ® and then 50% of the fruit was treated with a postharvest fungicide Scholar® (fluordoxinyl) at 260ml/100L dip for 2 minutes. The fruit was then packed into cartons supplied by Foodya and placed into the CARDI coolroom, which was set at 12^0C. Initially the fruit was placed on the coolroom floor but after consultation with the Australian team it was decided to stack the cartons on a pallet to optimise air circulation. Trial protocols were developed by the team.

Fruit was removed at 7, 14 and 21 days and kept at room temperature for another 7 days prior to assessment. Fruit from the 7 day storage can be seen in the photo below, right. The results from the trial were assessed, and a report provided to the Korean company.

Figure 19: Export shipment trial fruit is sorted and packed into sleeves prior to storage in the CARDI coolroom. Right: Fruit removed after 7 days cool storage and 7 days ripening at room temperature is assessed. Note the level of breakdown on some of the fruit.

Dry matter testing was conducted on fruit from the trial block at harvest time. Dry matters ranged from 20 to 24%, which is high compared to Australian mangoes. Most of the fruit had started to colour at harvest, although it was firm, and there were no visible signs of disease on the fruit. Figure below shows the range of dry matter % across the various treatments.

Figure 20: Dry matter of Keo Romeat mangoes from samples of each storage treatment time of 7, 14 and 21 days.

Each fruit was individually scored for stem end rot and body rots. Although rots were not apparent on most of the fruit on removal from cold storage, rots developed during the 7 day storage period at room temperature. Rots were particularly severe after the 7 day storage followed by 14 days ripening, with only seven fruit remaining at assessment stage.

The fungicide treatment was ineffective at controlling both stem and body rots. Although the rate of fungicide fluordoxinyl at 260mls/100 is the standard rate used successfully in Australia, the dipping time of 2 minutes is inadequate as most of the literature suggests a dipping time 5 minutes is required for control of fungi such as anthracnose. Fungicide resistance is also a possibility, but has not been demonstrated with fluordoxinyl in this part of the world.

The 2nd set of postharvest storage trials was conducted in November 2017 on fruit from a wet season Keo Romeat crop at Kompong Speu. A 3rd set of trials will be conducted in March 2018, and will include Vapour Heat Treatment in a commercial plant in Vietnam. An outline of the November 2017 trials described below.

Objective: Trial various post-harvest protocol to identify which effectively prevent fruit rots and therefore enable long term fruit storage.

Trial 1 – The effect of postharvest fungicides on Keo Romeat mangoes for control of stem end rot (*Colletoctricum sp.)*

Background: Stem end rot is a major constraint for successful exports of mangoes, particularly where use of field applications of fungicides is limited. During an earlier experiment in April 2017 looking at cool storage of Keo Romeat, stem end rot and body rots (usually caused by *Colletotricum sp*. or Anthracnose) were a major cause of losses in fruit from a long term storage trial at CARDI. Use of pre-harvest fungicides is rare in Cambodia, and none had been used in the crop used for this trial.

Method: In trial 1, fruit was harvested from a commercial farm at Kompong Speu, 100kms west of Phnom Penh, packed transported and treated on the same day. 240 fruit was harvested by hand, packed into padded boxes and transported back to CARDI. Fruit stems were removed from half the fruit at harvest, with 10cms of stem retained on the remaining fruit. Just prior to postharvest treatments, stems were carefully removed from this fruit. A

postharvest fungicide fluordoxinyl Scholar® treatment. A split plot design trial design was used to accommodate the stem retention treatments.

- Main plot
  - 2 Treatments of Scholar (Nil and 260ml/100l for 2 min)
  - 4 Removal times from storage at 12 ˚C (0, 7, 14, 21 days)
- Sub plot
  - 2 methods of removing stem [5 fruit for manually removal at harvest (a) and 5 fruit for removal with cutter at packing house (b)]
- 3 replicates (5 fruit)
- 240 fruit= 2 scholars x 4 times x 2 sub plots x 3 rep x5 fruit

Trial 2: Effect of hot water treatment on shelf life of Keo Romeat mangoes.

Background: In the variety Kensington Pride, use of hot water treatments of postharvest fungicides has improved efficacy in controlling postharvest rots including stem end rot and body rots. Under certain conditions, hot water (at 52^0C) for 5 minutes on its own has also significantly reduced the incidence of postharvest losses.

Method: A Randomised Complete Block Design (RCBD) trial including 5 treatments was established. The five treatments were as follows:

1. Control (no treatment, storage at 25 ˚C)
2. Room Temperature Water dip
3. Room Temperature Scholar dip
4. Hot Temperature (52 ˚C) for 5 minutes Water dip
5. Hot Temperature (52 ˚C) for 5 minutes plus Scholar dip

Three replicates of 10 fruit making a total sample of 150 fruit were used. This fruit was stored at 12 ˚C for 21 days then ripened at 25 ˚C for 7 days before being assessed for quality.

Results and Discussion: Postharvest fungicides:

Stem and body rots were severe in the untreated fruit at the ripening stage, suggesting that high levels of fungal spores were present on the fruit at harvest, and infections developed in storage. While visible rots were minimal on removal of the fruit from the coolroom, they developed during the 7 days ripening period at room temperature. No preventative fungicides were applied to the crop prior to harvest, which could explain the prevalence of both stem and body rots on the fruit. Postharvest fungal infections are also more likely to develop in the wet season, though there wasn’t an unusually high level of fungal infection observed in the crop prior to harvest.

The hot water treatments when combined with Scholar fungicide improved control of stem end rot and body rots in Keo Romeat when compared to cold water Scholar or just hot water on its own. However, hot water at 52^0C for 5 minutes caused scalding on the skin of all fruit in those treatments. The scalding was so severe, the fruit would not be marketable in a commercial situation. (See comparative images of treatments overpage).

Figure 21: Effects of hot water (52^{0}C) treatment for 5minutes on Keo Romeat mangoes (left) compared to Scholar ® fungicide cold treatments (right).

Two additional pilot trials were also carried out and are described below.

Pilot trial 1: Effects of using sanitised water

- All fruit treated with Scholar® at 260ml/100l for 2 min
- 2 treatments of water (Tap water and tap water + 100 ppm of chlorine)
- All fruit stored at 12˚C for 21 days and ripen 25˚C for 7 days
- 3 replicates [5 fruit of (a) and 5 fruit of (b)]

Pilot trial 2: Effects of double fungicide concentration and longer duration dipping time.

- 2 concentrations of Scholar® (260ml/100l and 520ml/100l)
- 4 Treatment times (2, 4, 6 and 10 mins)
- Storage at 25˚C for ripen

The fully analysed results from these trials will be combined with the third set of trials which will be conducted in March 2018, and reported on early in the new project.

7.8 Pesticide residue testing

Of the samples tested only 10% of samples had residues present, and these were well within acceptable limits. One fungicide in particular was detected in several samples, and it is suspected that this fungicide is applied close to harvest to manage diseases such as fruit anthracnose which can develop postharvest. This information has drawn attention to use patterns for certain chemicals that will require follow up to assist farmers to better manage such chemicals to prevent residues occurring in future.

Australian Component

7.9. Investigate the role of chemical treatments, temperature and rootstock on expression of the FT gene in mango flowering

In the NT, a glasshouse pot trial was conducted to evaluate the effect of temperature, potassium nitrate and thiourea on the expression of flowering related genes in mango. Two Australian mango varieties (Kensington Pride and NMBP1243) using mature scions grafted onto a common rootstock was subjected to six treatments. These were cold temperature (<20C), 4% KNO_3, 1% thiourea, cold plus KNO_3 and cold plus 1% thiourea. Samples were taken at time of chemical treatment (0h) then 1, 3, 6, 18 and 36 hours post treatment and stored at -80C. RNA extractions were conducted at the University of Queensland, and reverse transcriptase PCR (RT-PCR) was conducted using a range of flowering genes primer sets including positive and negative regulator genes.

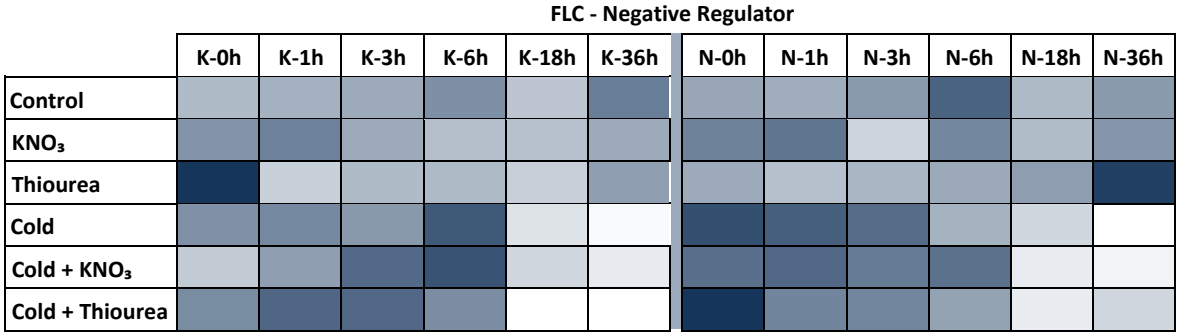


Figure 22: Heat map showing expression of the FLC gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest.

The results from this trial showed that the treatments had an impact on the expression of the chosen flowering genes. Differences in expression between the two cultivars was also observed. In particular, thiourea treatment increased the expression of the positive flowering regulator FLD, thus supporting observations that thiourea is a floral inducer. Overall, the trial supported the hypothesis that the expression of flowering regulation genes is influenced by chilling and application of KNO3 and Thiourea. NB- once the potted plants were removed from the glasshouse following the trial, flowering was observed in some of the plants. This trial was repeated and processing the data is underway and incomplete at the time this report was compiled.

See full update on the PhD Project in Appendix 9

7.10 Improved use of weather data to manage flowering and cropping in NT Mangoes

The Maddern Julian Oscillation is an atmospheric disturbance that travels eastward around the tropics and is associated with anomalous rainfall events. In the Territory major use for the MJO appears to be anticipate rainfall events in October –March and has little or no influence on the occurrence of low evening temperatures. Since there is no relationship between the

MJO and cool evening temperatures in the Darwin region or Katherine it was not possible to prepare an article for Mango matters.

The cool evening temperatures that occur in Darwin and Katherine are associated with the anti-clockwise rotation of winds arising from high pressure systems in the Great Australian Bight and over Victoria (Fig 1.) These winds bring the cool temperatures from central Australia to the northern areas of the Territory.

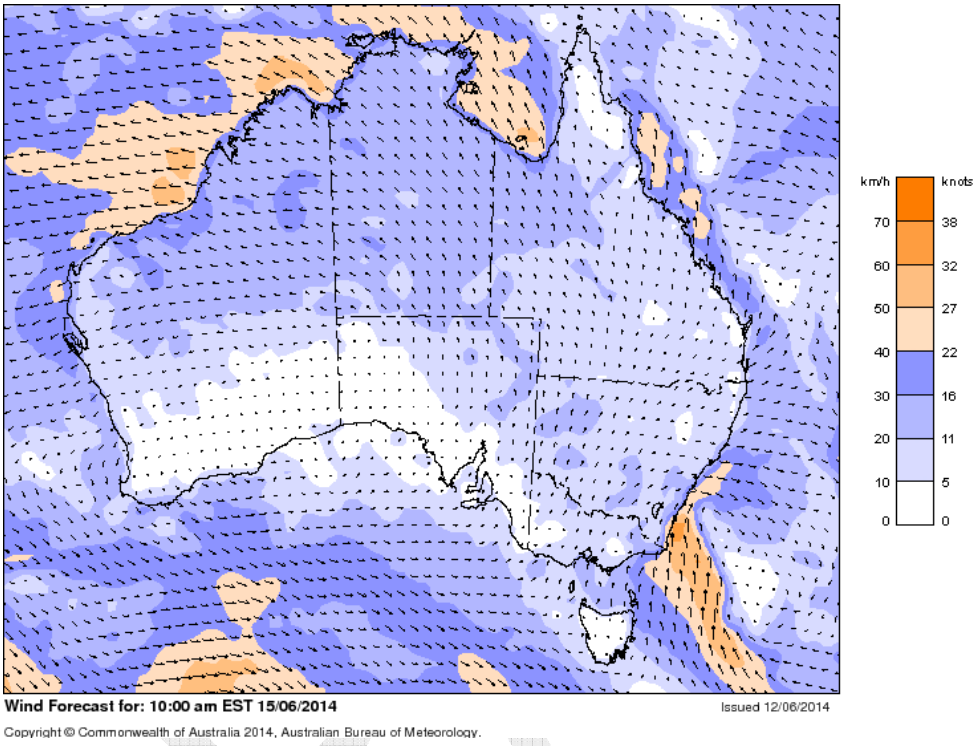


Figure 23. Wind map of Australia for 15th.May 2014 showing the south easterly winds. Wind strength is proportional to the size of the arrow and indicates the direction of air flow. The high pressure system is in white and with low wind speeds. Wind spirals outward counter clockwise.(http://www.bom.gov.au/marine/wind.shtml)

The arrival of high pressure systems can be anticipated by viewing the Bureau of Meteorology website (http://www.bom.gov.au/australia/charts/indian_ocean.shtml)

(Fig.2). Further consolation is required to predict the amount of cooling and duration of chilling (hours below 20^{o}C) in Darwin that a high pressure system developing over the Indian Ocean will bring. In the interim the appearance of a high pressure system off Perth results in chilling in Darwin (>20oC) 5-7 days later.

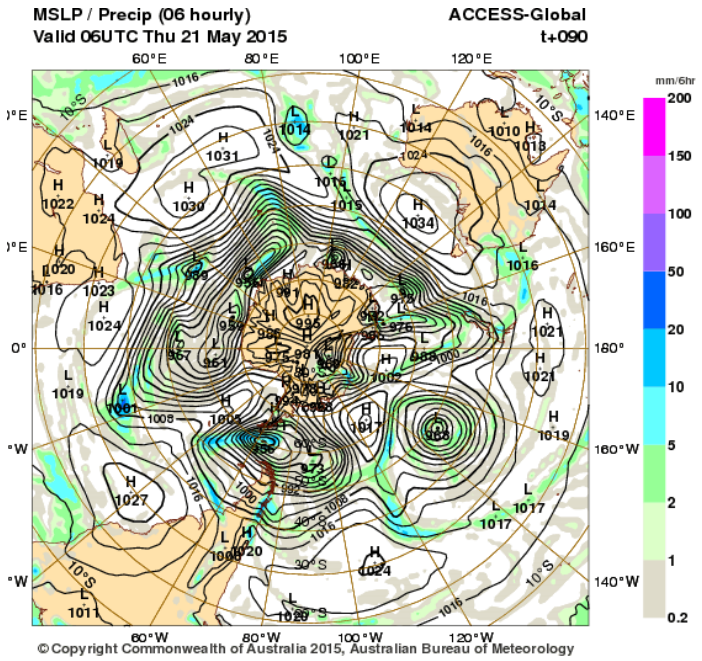


Figure 24: Map of barometric pressure showing areas of high pressure developing in the Indian Ocean and travelling eastwards. The high pressure system shown over the Australian Bight at this time (21st May 2015) produced south easterly wind in Darwin and night temperatures that went below 20°C in regional production areas such as Noonamah and Barry Springs.

7.11 Develop models to describe relationship between temperatures and plant growth

Mangoes grow by repeated periods of shoot extension referred to as flushes. These can be either vegetative or floral, though floral flushes can be composed of mixtures of leaves and flowers. If growth occurs during suitable inductive cool temperatures or subject specific chemical treatments then a flush can be induced to be floral. The aim of this work was determine if it was possible to predict the period and frequency of flushing by monitoring flush growth and ambient temperatures. Using this information it was intended to target specific stages of growth based on these models Analysis of the results for 2013-2014 indicate that it is not possible over a year even in an irrigated orchard with or without paclobutrazol to predict the flushing cycles as waterlogging and irrigation deficits have much greater impacts than temperature.

This work was performed by monitoring tagged branches in orchards of B74 (Acacia Hills) and Kensington pride (KP) (Lambells Lagoon). In the original proposal of doing multiple KP was not possible because trees of an appropriate size/age could not be located. Temperature loggers were established at both sites. At fortnightly intervals three trees had all branches tip pruned and 20 branches tagged on each tree. This treatment was repeated to separate groups of three trees on six occasions. This meant that flush regrowth was staggered over a three month period and a total of 18 trees and 360 branches were monitored. At fortnightly intervals the length of regrowth was measured, and notes made if the regrowth was vegetative or floral.

B74

During the study period B74 trees went through up to 5 flushing cycles if they did not flower immediately after pruning. The time taken to start a flush was shortest after pruning but the duration of the cycle extended to several weeks over the wet. Since there is two weeks between the pruning treatments it was expected that the number of flushes would have been ranked in descending frequency as the year progressed (Fig 3). There was no unifying heat sum model that described the flushing cycle and it would appear environmental conditions towards the end of the dry and waterlogging during the wet, slowed the flushing.

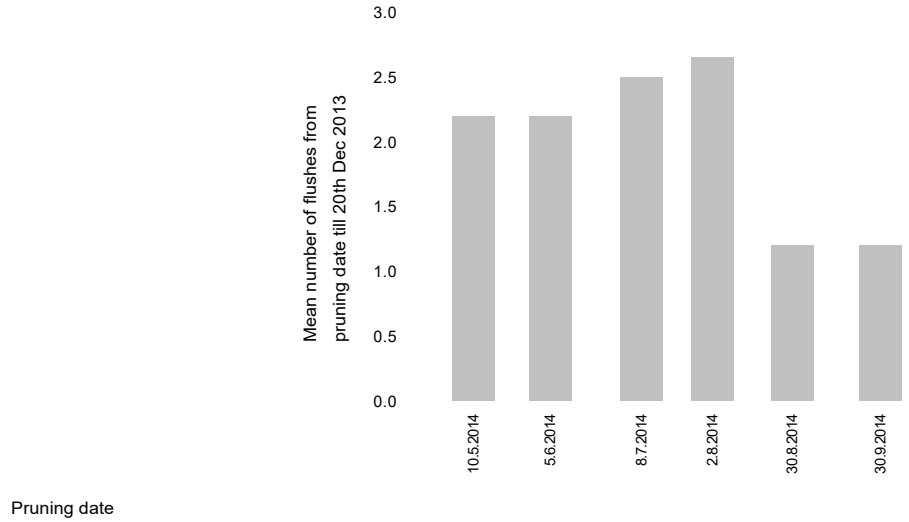


Figure 25: Mean number of flushes in B74 initiated during the study period after pruning on the date indicated. These should have been ranked in descending magnitude if cumulative heat sum was the major driver of flushing duration and frequency.

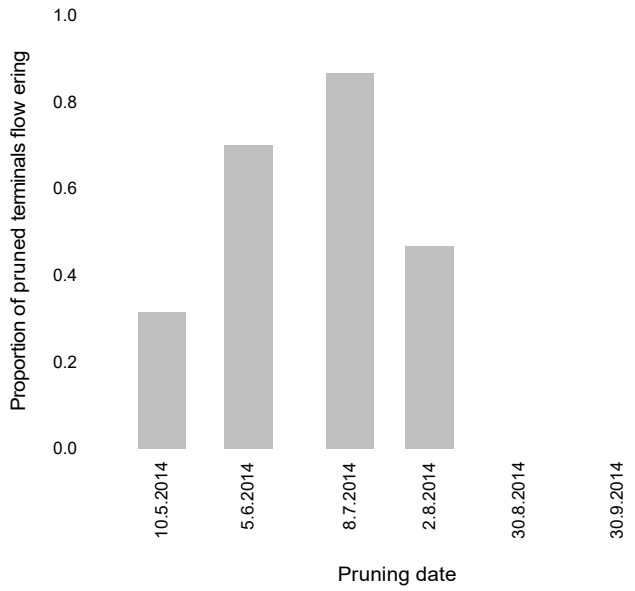


Figure 26: The proportion of branches that developed inflorescence after pruning differed greatly during the study period. This was related to the temperatures during flush emergence

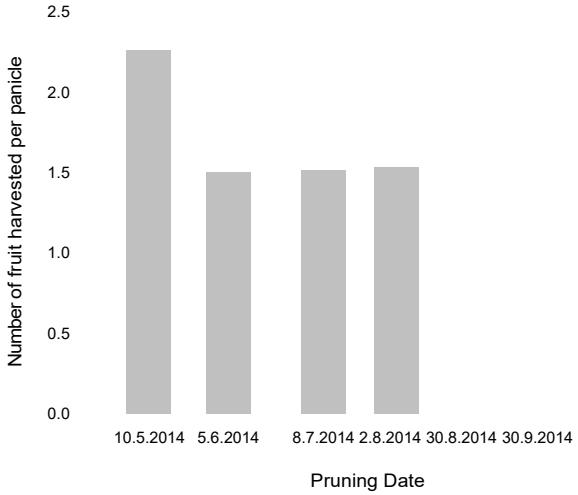


Figure 27: The fecundity of the inflorescences as measured by the number of mature fruit harvested was greatest early in the year but was comparable after this time. However many more fruit were harvested from early season flowering as a greater proportion of the branches flowered.

This trial provided insights into the number of hours below 20°C during flush development that resulted floral initiation (Fig. 5). There are some outlying points that occurred on the west side of the tree. It would appear that around 100 h below 20°C were required for B74 flowering. This relationship was not improved by including the number of degree hours below 20°C.

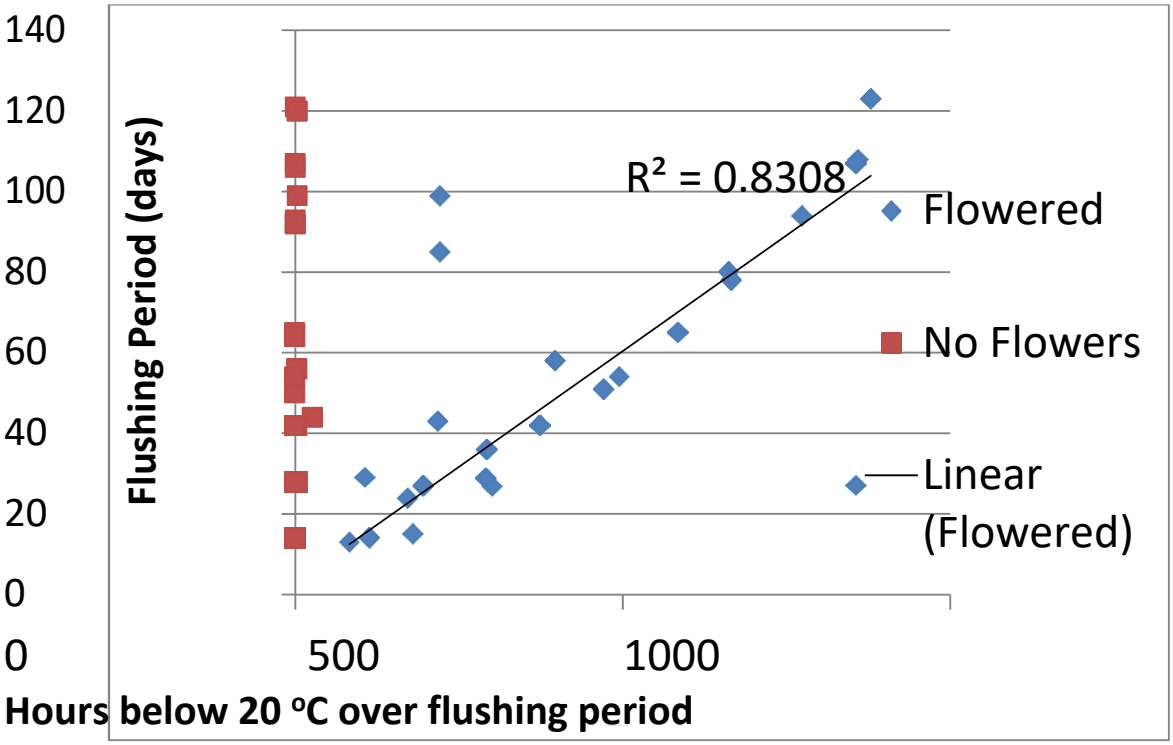


Figure 28: Relationship between the duration of the flushing period and the number of hours that were below 20°C during this period. Note the flushes that were exposed to greater than ~100h flowered.

Kensington Pride

More cycles were observed in Kensington Pride (Fig 7) over a shorter monitoring duration compared to the B74 but there was no heat-sum model that described the pattern of growth. The trees were routinely sprayed with a Pryrethrum based insecticides to control leaf eating pests but flushing was frequently reset due to defoliation. During the Wet of 2013-2014 the site was flooded for 3 months and no growth occurred for 14 weeks. This was instructive in the capacity of the high water table to stop growth and synchronise flushing once the soil dried but emphasised that prevailing weather conditions either excessive wet or dry had greater influence on flush growth than heat-sum on flush emergence and duration of flush cycle.

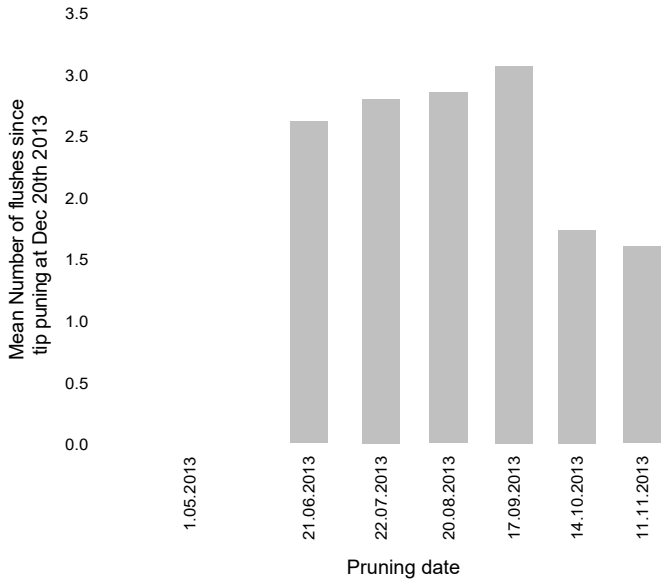


Figure 29: The mean number flushes after being tip pruned on the date indicated. The number of flushes in Kensington Pride was greater than B74 despite commencing treatment a month later. It would have been expected that the number of flushes initiated would have decreased as pruning progressed as cumulative heat-sums diminished. This was not observed.

7.12 Quantify paclobutrazol uptake in Kensington Pride using different rootstocks aeroponic system

Initial trials to grow mango seedlings in 2013-2014 appeared successful in shade house conditions (Fig.7). The clonal plants of the same selection all During the Dry in 2014 it became evident that the trees grown in the aeroponic system were developing foliar symptoms of nutrients imbalances (Fig.8). The nutrient solutions were modified but this did not correct the problem. It was concluded that the temperatures in the shade house and nutrient solution were extreme and possibly contributing to this problem.

Figure 30: Mango aeroponic system established within a shade house at Berrimah farm Darwin. The temperatures with the shade-house regularly exceeded 40°C while in the bin temperaturesexceeded 45°C.

Figure 31: Three different mango rootstock varieties grown through 2-3 flush cycles with in the aeroponic system while established in the shade house. While growth of the same cultivar was comparable all varieties developed foliar symptoms of nutrient disorders.

A successful submission was made to the NTDPI&F and a glasshouse was equipped with two 10kW air conditioners that are capable of holding the glasshouse daytime temperatures at around 32°C (Fig 9.). This is comparable to ambient orchard conditions in rural Darwin. Plants were established in aeroponic to determine whether the nutrient imbalance problems have been solved and plants appear normal after two flush cycles (Fig. 10).

Figure 32: The upgraded glasshouse with two 10 kW air conditioners and a ceiling air vent to keep the day temperatures below 35°C. A similar system is currently being installed at the other end of the building to enable the night temperatures to achieve inductive temperatures (>20°C).

Figure 33: Mango seedlings of a single Kensington Pride selection growing in the newly renovated controlled temperature shade-house in Darwin. Note the under-bench outlet for the air conditioner (right) directed at the nutrient solution bins (foreground) ensuring the nutrient solutions remain cool. Using this system the air temperature does not exceed 32°C during the day. Plants have grown through two vegetative flushes and not developed any foliar nutrient deficiencies.

7.13 Uptake and growth response of mango on paclobutrazol under aeroponics conditions

Objectives

The objectives of this study were to elucidate the relationship between varying dose of paclobutrazol and uptake by mango, determine the concentration of paclobutrazol that affects growth of mango, and verify whether paclobutrazol can modify the ability of mango to absorb nutrients.

This study aimed to answer the following questions:

1. What is the effect of paclobutrazol on root and shoot growth of mango?
2. What are the uptake rate of paclobutrazol by mango and the concentration of paclobutrazol that affect mango growth?

Methodology

Preparation and culture medium of mango plants

Fruits of mango cultivar Kensington Pride will be collected Jabiru Tropical orchards. Seeds were extracted and grown in potting mix. Two-month-old plants were transferred to aeroponics tubes with perlite and growth medium. Plants will be grown under aeroponics condition by misting the roots for two seconds every 8 minutes interval during the day and 40 minutes during the night.

The component of aeroponics solution is shown in Table 1. During each preparation, the pH of the solution is adjusted to 6.5 and electrical conductivity to 2.0 – 2.1 mS/cm. Plants were allowed to grow for three months before treatment application.

Table 9: The nutrient composition of aeroponics culture solution

Element	Total Concentration (mg/L in solution)
Nitrogen	225
Phosphorous	51.5
Potassium	280.08
Calcium	190
Magnesium	28.4
Sulphur	46.2
Iron	2.05
Manganese	0.38

Boron	0.27
Zinc	0.12
Copper	0.05
Molybdenum	0.01

Treatment

The concentration of paclobutrazol taken by the plant will was varied by allowing the plants to absorb paclobutrazol at different times (0, 12, 24, 48, 72, 96 hours after exposure) in aeroponics culture solution with 1 mg/L active ingredient of paclobutrazol. Each treatment was replicated three times. The experiment was laid out in complete randomised design. After exposure, plants were transferred into a new bins and arranged according to the layout. The bins will be supplied with untreated (no paclobutrazol) aeroponics culture solution. To obtain plants with homogeneous characteristics, root length, plant height will be measured. Root and shoot will be uniformly cut based on the lowest root length of the experimental plants.

Data gathered

The following growth parameters were measured at fortnightly interval:

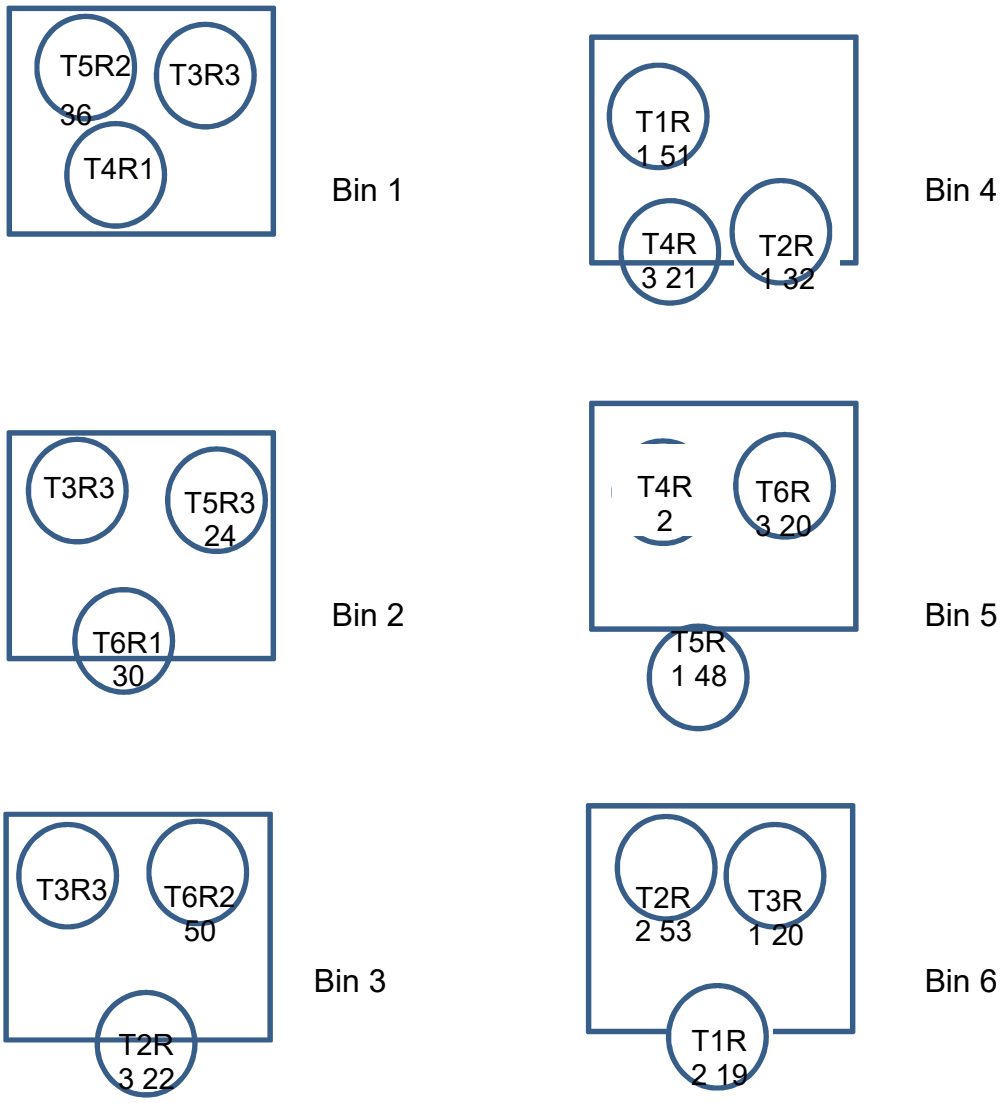
1. Shoot length was measured from the base of the plant to the tip of the bud.
2. Plant diameter was be measured 1cm from the bottom of the plant
3. Number of leaves were counted and flush status recorded
4. Root length was be taken from the bottom part of the vase to the longest root tip.
5. Stem diameters were measured at the base and tip of new stem (growth unit).

Layout

Treatment

1. Control
2. 12 hrs exposure
3. 24 hrs exposure
4. 48 hrs exposure
5. 72 hrs exposure
6. 96 hrs exposure

Figure 34:: The aeroponics trial layout with duration of treatments



Statistical analyses

Data was analysed using Statistica version 13.1. Treatment were determined by analysis of variance (ANOVA) and treatment means will be compared using the least significant difference (HSD) test.

Results

Plant height

Our results show that overall growth of test plants were influenced by PBZ exposure and absorption time. Plant growth decreased under Paclobutrazol treatment. An exposure time of 12hrs or more was seen to have an effect $P< 0.01$ on inhibiting shoot growth/plant height. Post-hoc Tukey's HSD tests showed that exposure times greater than 24hrs had less growth than the control and 12 hr treatment groups at the 0.05 level of significance. Exposure to 1mg/active ingredient of PBZ for greater than 24hrs did not exhibit any further significant reduction in plant height.

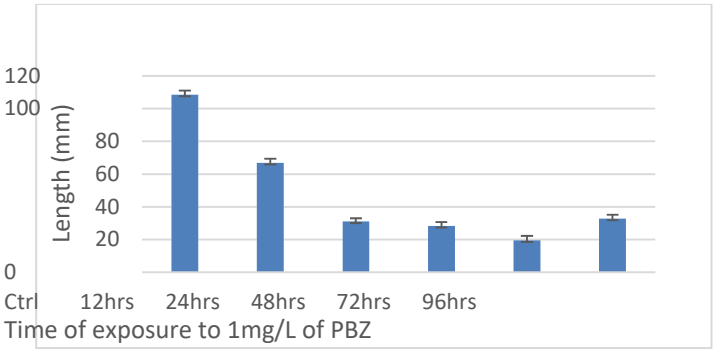


Figure 35: Comparison of growth in height of Kensington pride Mangoes exposed to a 1mg/L Paclobutrazol aeroponic culture solution. Height of Kensington Pride Mangoes were monitored for 2 months after an exposure time of either 12, 24, 48, 72 or 96 hours. Table reflects the mean of observations.

Root length

In our experiment, root growth seemed to be inhibited by Paclobutrazol which was contrary to what was expected. Post-hoc Tukey's HSD tests showed that the control group had greater growth at the 0.05 level of significance than the various exposure rate treatments.

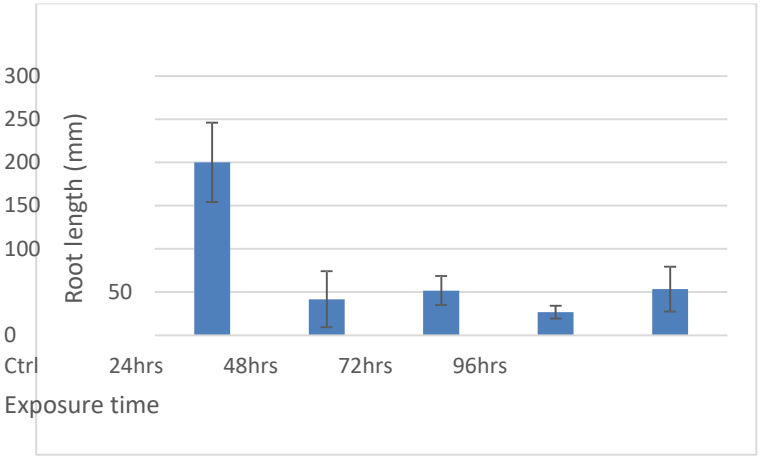


Figure 36: Mean Root growth of Kensington Pride mangoes two months after being exposed to a 1mg/L aeroponic solution for various lengths of time.

Leaf growth.

After 2 months it was observed that Paclobutrazol exposed trees developed fewer leaves than control group however this was not statistically significant.

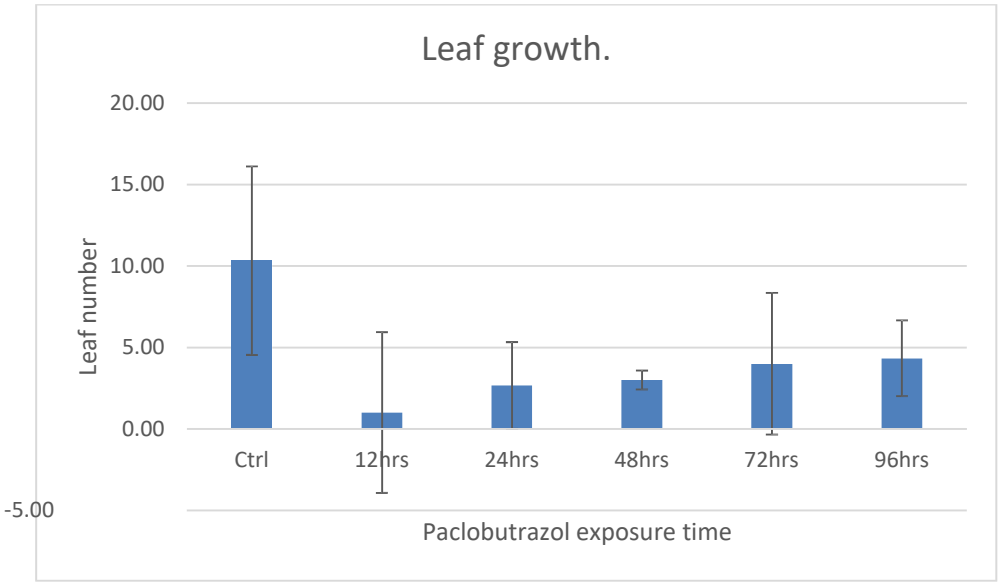


Figure 37: Average number of new leaves developed by Kensington Pride trees based on various rates of exposure to an aeroponic solution containing 1mg/L of Paclobutrazol

7.14 Improve the use of ethephon to manage mango flushing cycle

In April 2014 after the granting of a permit to investigate the use of ethephon on mangoes by the APVMA a field trial was established at Lambells Lagoon on 20 year old Kensington pride trees. The trees were planted at a spacing of 8 X 10 meters and were approximately 6-7 m tall. A Greentech 6000L mist sprayer was used that was calibrated to apply 4 L per tree. Initial spray formulations were difficult because previous published trials had used back pack sprayers and only noted the concentration of ethephon and indicated that trees were sprayed to run-off. In the trials that were conducted to register ethephon trees 485 g ai/ha was applied, using 9L per 100 m^2 of canopy. This amount of spray was almost double the volume

used by the collaborating grower and it was considered using this quality of spray would have resulted in a considerable amount running-off. In addition applying this volume of spray would have double the labour cost for the grower as he applied half the volume of spray normally.

While the commercial formulations of ethephon do contain a wetter, previous investigations have shown the inclusion of an additional adjuvant improves the efficacy of ethephon. Ground water used for spraying in the Northern Territory is generally alkaline which may have led to hydrolysis of the ethephon releasing the ethylene prior to application. LI700 was used as it acidifies the spray while increasing uptake by it corrosive action on the leaf cuticle. The ethephon spray alone was pH 3.5 while with the LI700 was pH 4.0.

The following 5 treatments were developed to investigate 2 concentrations of ethephon, with and without LI700.

1. Water only
2. 145g ai/ha ethephon
3. 145g ai/ha ethephon + 0.25 ml/L LI700
4. 290g ai/ha ethephon
5. 290g ai/ha ethephon +0.25 ml/L LI700

All 5 treatments were applied to separate sets of 25 trees within a single row. Each treatment was separated by 2 buffer trees. This process was replicated in four rows with the treated rows separated by two untreated buffer rows. The order of treatments within rows was randomised to account for special trends within the block. Two trees within each set of 25 trees had 10 branches tagged at different stages of flush development to monitor responses to the spray treatments. All leaves were raked out from under trees so that the timing and amount of leaf drop could be measured. Due to the high day temperatures in Darwin, and the label directions for other crops specifying ethephon should only be applied at temperatures between 18 ^{o}C and 32^{o}C, sprays were applied between 7 and 10 pm. The response to the spray treatments were monitored at weekly intervals for 3 weeks after spraying by observing the effects on the tagged flushes, and collecting and drying at 60^{o}C and weighing the leaf drop for the two monitored trees per treatment (total 100 trees). The lay-out of the trial is shown in Figure 11.

Figure 38: Layout of the April 2014 trial at Lambells Lagoon investigating the effects of ethephon application levels and a commercial wetter (LI700) on leaf abscission and vegetative flush development. White= Water, Pink = 145 g ai/ha ethephon, Yellow = 145 g ai/ha ethephon +0.25ml/L LI700, Blue = 290 g ai/ha ethephon, Red = 290 g ai/ha + 0.25 ml/L LI 700.

The ethephon treatments had little or no effect on the development of flushes. Few if any immature leaves were abscissed by any of the treatments. However, the ethephon did appear to promote leaf abscission at 290 g ai/ha with the highest mean weight of leaf drop being recorded after 2 weeks from the spray with 0.25 ml/L LI700 added (Table 1). Note these results have not been corrected for tree size with more leaves being shed from larger trees.

Table 10: The effects of ethephon spray treatments on the weight of dried leaf collected from under trees 1 and 2 weeks after treatment. This was performed using Kensington Pride mangoes at Lambells Lagoon in April 2014. Note tree size has not been included in these results.

Treatment	Mean weight of leaf fall tree^{-1} (g) Week 1	Mean weight of leaf fall tree^{-1} (g) Week 2	Mean total leaf fall tree^{-1} (g) after 2 Weeks
Water	163	654	817
145g ai/ha ethephon	168	662	830
145 g ai/ha ethephon + 0.25 ml/L LI700	139	309	447
290 g ai/ha ethephon	231	1306	1536
290 g ai/ha ethephon + 0.25ml/l LI 700	256	1599	1855

These results were disappointing as the ethephon treatments were intended to reset the flushing processes by chemically removing existing new growth. Based on these results a new spray program was developed with increased concentrations of ethephon. To provide linkage between the experiments the highest ethephon spray formulation from the April trial was retained but the content of LI700 was increased from 0.25ml/L to 1 ml/L. A treatment including KNO_3 was added as cool inductive night temperatures were expected. Sprays were applied at 4 l $tree^{-1}$ .The lay out of the trial was similar to the earlier experiment but a new set of trees were selected As previously described the 5 spray treatments were applied to groups of 25 trees within a row. Treatments were separated by 2 tree buffers. These treatments were repeated in four rows that were separated by two untreated buffer rows. The allocation of treatments was randomised within a row (Figure 12).

The revised spray treatments were as follows

1. Water plus LI700 1ml/l
2. 290 g ai/ha ethephon plus 1ml/l LI700
3. 580 g ai/ha ethephon plus 1ml/l LI700
4. 870 g ai/ha ethephon plus 1ml/l LI700
5. 15 kg KNO_3 /ha 1ml/l LI700

Figure 39: Layout of the May 2014 trial at Lambells Lagoon investigating the effects of ethephon application levels and the response to KNO_3. Orange rectangles indicate trees treated with additional KNO_3 sprays.

Twenty branches at different stages of flush development were tagged per spray treatment using the stages of flush development are shown in Figure 13.

Development Stage	1	2.	3.	4.	5.
Description	Swollen vegetative bud, no stalk visible	Elongate of shoot commenced , young eaves, visible, spiky appearance	Lamina on leaves expanded	Leaf fully expanded but red in colour. leaves floppy	Fully expanded light green leaves
Appearance					

Figure 40: Stages of buds tagged to identify stages affected by different ethephon and KNO_3 spray formulations

All sprays were applied after 7 pm in the evening. The first spray treatments were applied on the evening of 8th May 2014, with a second spray on 21st May 2014 using the same spray formulations. The spray treatments reapplied that night.

On the 10th of June the trees were assess for the percentage of terminals flowering. That evening 12 trees at the northern end of every block of trees was sprayed with 15 kg KNO_3 /ha (3%) with 1ml/l LI700 to stipulate flowering. Two days later 12th June the same KNO_3 sprayed tree were sprayed again with 7.5 kg KNO_3/ha (1.5%) with 1 ml/l LI700. On the 10th August trees were assessed for percentage of terminals flowering.

Table 11: Mean percentage of terminals developing inflorescences on the east and west side of trees on 10th June 2014, following 2 repetitions of the spray treatments.

Treatment	East	West
Water	0.0	0.0
KNO_3 15kg/ha	4.8	5.4
Ethephon 290 g ai/ha ethephon	0.0	0.0
Ethephon 580 g ai/ha ethephon	0.0	0.0
Ethephon 870 g ai/ha ethephon	0.1	0.0

Only the KNO_3 treatments formed flowers (Table 2). The couple of trees that flowered in the 870 g treatment were close to the KNO_3 treated and could have be affected by drift. There was insufficient hours below 20°C prior to June 10th to have caused this flowering (Fig.14) even if the effect of KNO_3 was only cause bud break. This suggests that KNO_3 can induce flowering in Kensington Pride mangoes although at a lower level than reported internationally in other mango cultivar.

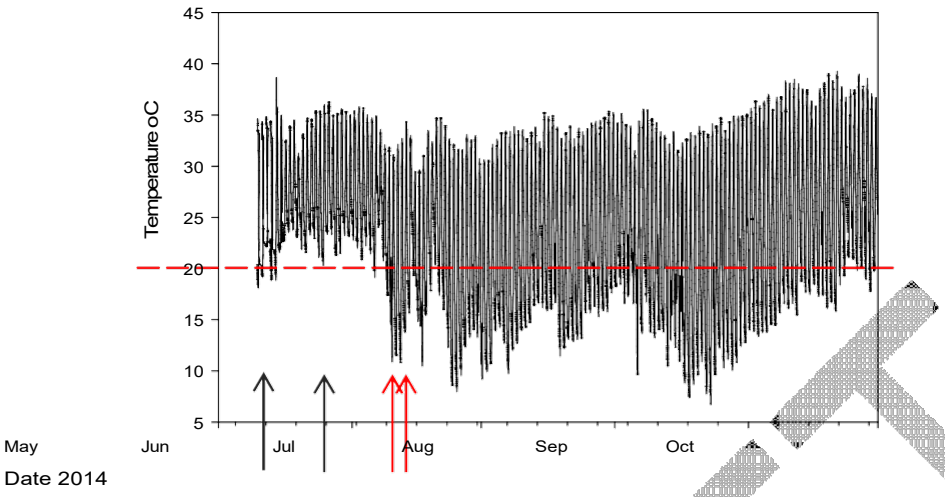


Figure 41: Maximum and minimum temperatures (°C) monitored at Lambells Lagoon during the period when ethephon and KNO_3 treatments were applied. The threshold temperature below which flower induction in believed to occur in mangoes indicated. The black arrows indicate the evenings that ethephon treatments were applied. The red arrows indicate the evening on which the KNO_3 were applied to the 12 trees at the northern end of the 25 trees within a treatment.

Later evaluation became more complex because inductive temperatures continued till September so that new flowers were potentially being constantly recruited over this period. When assessed on 10th August 2014 the KNO_3 treated trees consistently better than the non-KNO_3 treated trees (Table 3). Similarly the ethephon treated trees also performed well. More detailed statistical analyses have been done but are not presented here.

Table 12: Mean percentage of terminals that formed inflorescences assessed on 10th August 2014. Flowering scores are given for both the west or east sides of trees that were treated with different spray formulations of ethephon or potassium nitrate. Note the plus or minus KNO_3 treatment were applied on the 10th and 12th June 2014.

	Plus KNO_3		Minus KNO_3	
Side of tree	West	East	West	East
Water	44.4	56.4	26.9	51.0
KNO_3 15kg/ha	41.9	38.3	35.0	36.0
Ethephon 290 g ai/ha	54.7	61.1	42.9	57.2
Ethephon 580 g ai/ha	50.5	55.9	32.5	32.1
Ethephon 870 g ai/ha	45.0	53.8	41.0	48.1

The effects of different spray concentrations on flush are shown in Table 4. Note additional information on the effects of 1160 g ai/ha ethephon due to a double spraying of one treatment with 580 g ai/ha. Leaf flushes on the 290 g treatment were defoliated unlike in the April which appears to be due to the increase in content of LI700.

Table 13: Summary of the effects of different ethephon treatments on vegetative flush on Kensington Pride mangoes in 2014 at Lambells Lagoon. OK= no effect, X? = outer buds scales lost, X = shoot defoliated or most buds scales shed, XX= Leaves shed and shoot stalk killed.

	Flush stage				
Treatment	1	2	3	4	5
Water	OK	OK	OK	OK	OK
Ethephon 290 g ai/ha	X?	X	X	X	OK
Ethephon 580 g ai/ha	X?	X	X	X	OK
Ethephon 870 g ai/ha	X?	X	X	X	X
Ethephon 1160 g ai/ha	X	X X	X X	X X	X

In NSW, a trial using a commercial formulation of the plant hormone ethephon to delay flower development in Honeygold mangoes was established to examine the benefits of delaying flowering several weeks in Spring to a more reliable timeslot. The collaborator grower was also interested in looking a delayed harvest to take advantage of historically high prices alter in the season when other mango production regions had ceased harvest. Ethephon was applied every two weeks from mid-May through to the beginning of August at a concentration of 1000ppm delay flower panicle development on 5 year old mango trees. By mid-July 2016, the treatments appeared to be successful in delaying development of flower panicles. This trial complemented the trials conducted in Cambodia aimed at removing leaf flush in the lead up to wet season flower induction.

Also see draft paper on tip pruning in Appendix 5.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The development of pest lists (insects and pathogens) based on verified specimens held in an official Cambodian collection (at GDA) has increased the knowledge of the pests of mangoes on Cambodia. Many species were recorded in Cambodia for the first time and there is at least one species new to science which needs to be described.

With follow up training in April 2017, the use of DNA techniques for identification of pest and diseases training, and provision of 2 PCR machines to CARDI have improved the ability of the Cambodian team to conduct their own diagnostics. The participants from three organisations who attended the molecular training workshops included undergraduate, postgraduate and scientists. Compilation of the pest and disease identification guide has further improved the teams understanding of the key species impacting mangoes in Cambodia. Photography training conducted by Dr Brian Thistleton has enhanced the ability of the team to build their own image libraries for use in publications, and as records for pest lists.

The molecular skills acquired in this project will be applied and reinforced in the next project potentially to expand to identify and link genotype with phenotypic traits of mango varieties of interest. The PhD research conducted within this project is a first of its kind for mangoes and has provided a glimmer into the complex networks of the regulation of numerous flower genes. The results from the glasshouse pot trial to induce flowering and being able to track the up or down regulation of flowering genes is novel and correlates well with field trials conducted with thiourea.

The **export simulation** trial assessments conducted in April 2017 and November/December 2017 were largely carried out by the Cambodian team, which involved staff from GDA and RUA and the CARDI Postharvest team. Analysis of the results is underway and the exercise has been beneficial in terms of experienced gained in planning and implementation of postharvest experiments. Involvement of the Korean exporter and the grower in the trial was invaluable. The team is now also better connected to some of the larger commercial players helping to develop capacity in Cambodia for exports. The team will conduct a third export simulation trial in March 2018, on this occasion assessing fruit which has been exposed to vapour heat treatment at the VHT plant in Ho Chi Minh City Vietnam. This trial will replicate the actual conditions required for protocol markets such as Sth Korea.

The CARDI Soil and Water Group have also conducted some excellent work on the crop nutrition and use of growth regulators to control unwanted leaf flush in their on farm trials at Kompong Speu. Crop productivity measures from the NPK fertiliser nutrition trial at Kompong Speu demonstrated an increase in the fruit numbers on fertilised trees, but no increase in individual fruit weight. The use of ethephon to remove leaf flush in the lead up to flower induction was successful, and demonstrated the labour saving potential in removing unwanted flush. Outputs from these trials will assist with development of future alternative recommendations on double cropping.

Two presentations were delivered on outputs from the Australian component of the project at the 12 International Mango Symposium in Guangxi, China in July 2017. The papers were

entitled *"Effect of temperature, potassium nitrate and thiourea on expression of flowering related genes in Magnifera indica" by Stacey Cook, and "Chemical control of mango flower initiation in Darwin (Australia)" by Cameron McConchie, Amy Dobell and Robert Williams.* Another paper was delivered at the 11th Australian Mango Conference in Bowen in May entitled *"Managing mango flowering and implications for growers"* by Cameron McConchie. See abstracts from the 11th International Mango Symposium in Appendices 1 and 2.

8.2 Capacity impacts – now and in 5 years

Given that fruit crop research is relatively new to the project team, the emphasis during the early stages of the project was on capacity building in areas such as tree physiology, nutrition, pest and disease management and supply chain analysis. An emphasis on training activities during the first 2 years of the project transitioned to more practical applications of new skills and knowledge in the final 2 years of the project, with small scale research activities commencing, and steady progress and growth in confidence by the project team in identifying the issue, designing appropriate research trials to address those issues, measuring results and analysing outcomes. Training workshops on a range of topics from pest and disease management, pest and disease diagnostics (including molecular techniques), crop growth measurements, crop nutrition, postharvest management and nursery management have equipped the team to conduct these research activities.

Capacity building also involved the Cambodian researchers and extension staff delivering workshops (supported by Australian research and development staff) to each of the growing district. This gave the researchers confidence and a greater understanding of the pests and diseases. In fact the majority of work over the four years of the project involved developing the capacity of the research organisation, extension bodies and the Cambodian mango industry. The project has been successful in developing our Cambodian partners to have appropriate level; of skills in tree crop research. The farming community also gained further knowledge and the interaction at these workshops was a valuable indicator of their success.

Figure 42: Dr Minh Nguyen from the FAVRI, Hanoi delivers at the CARDI Postharvest Workshop

Figure 43: Drs Brian Thistleton and Ruth Huwer inspect GDA insect collections with the project team.

Challenges along the way.

Goals were appropriate if all went to plan however in many cases all did not go to plan. Gathering an understanding of the complex production system within Cambodia was difficult and took time. Pest trials were planned but not performed due mainly to the absence of the pest for the year of the trial. Other projects also being run by Cambodian counterparts interfered with the mango project.

Outcomes. Key results and how this work will benefit the Cambodian mango industry.

Building capacity of the team was a priority. The development of the mango pest and disease manual is clear evidence that capacity building is happening. The Cambodian partners are continually adding to the catalogue through collecting, identifying and auditing to the point of identifying through DNA processes, using skills acquired during the DNA Workshop Training program: Delivery of workshops on a diverse range of topics including pest identification and management , crop production and postharvest was also an effective way to build capacity in the team. Cambodian researchers teamed with Australian counterparts to deliver talks to growers, generally in Khmer without translation. With the baseline being developed the needs of industry has been identified. Linkages to pest and disease and canopy management have been realised and information delivered in country regarding this area. Spray application workshops which highlighted safe spray application practices were run. Better coverage and a more open tree will lead to better fruit quality and greater income to the grower

Figure 44: Farmer training in pest and disease identification and management at Kompong Speu conducted by Dr Khay Sathya, Dr Brian Thistleton and the Plant Protection Team April 2016.

Feedback from participants on workshop activities has been overwhelmingly positive, and there has been an appropriate balance of team training and delivery to farmers for this stage of the project. As the team gain in confidence with mango production and postharvest practices, more delivery of information to farmer groups will be built into the next phase of the project. The pest and disease workshops and the pruning workshop in Battambang in 2015 were well received by farmers and similar workshops were run in 2016/17. In terms of impact to farmers, a poll taken at the conclusion of the pruning workshops for instance revealed that 90% of the growers attending have the intention of changing their practice as a result of the workshops in Siem Reap and Battambang.

An important part of building future capacity in fruit crop research was the collaboration with the Royal University of Agriculture (RUA). A guest lecture series was built into the original project plan, and over the 4 years of the project, a total of seven lectures were delivered to RUA students throughout the project. The topics ranged from pests of mangoes, disease management, biological control case studies, mango crop nutrition, and mango production systems in Australia, aswell as pruning mangoes. Generally one Australian team member was delegated to deliver a lecture on each team visit. The Cambodian team also delivered occasional lectures on mango related subjects to 3rd and 4th Year students. No ACIAR Mango Team lectures were delivered at RUA during 2016/17 due the time constraints.

Figure 45: Bob Williams delivers a presentation on pruning to Year 2 students at RUA in May 2016.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

If the Royal Government of Cambodia's goal of developing a mango export market to sophisticated markets such as South Korea is achieved over the next 5 years, there will be significant benefits to the Cambodian economy. Apart from legitimate export revenues, potentially thousands of jobs could be created in the growing, packaging, transporting and exporting sectors.

Returns for green mangoes in the current "grey trade" to Thailand and Vietnam are low with prices fluctuating between 1,000 and 1,500 riel/kilo. Returns for mangoes going into the fresh export market is likely to be higher, and diversion of the current Keo Romeat crop into fresh exports is likely if Cambodia is successful in negotiating export trade on a regular basis. Increased demand for the product will hopefully result in price increases for the farmer.

With growers moving into supplying fruit for export, its likely to result in wider adoption of GAP and overall improvement in fruit quality reaching the domestic and export markets. This should lead to an overall increase in the value of the Cambodian mango crop. Demand for higher quality inputs such as pesticides, fertiliser and packaging will also have knock on effects for the agricultural supplies industry.

Although there are no direct impacts on policy from project activities, the signing of an MOU with Sth Korea in December 2016 on cooperation to work towards future mango trade between the two countries is significant. The Royal Government of Cambodia is committed to providing support to mango research to support this initiative which will benefit the project (and the economy) in the medium to long term.

8.3.2 Social impacts

Farmer workshops conducted in four provinces have successfully engaged local farmers with the project and the project team. Valuable feedback from farmers received during the workshops has assisted the team with planning and priority setting for future workshops.

Some of the topics highlighted for future training include advanced pruning techniques, spray application and timing for effective pest control, and crop nutrition.

An improved understanding of the value chain, particularly of the smaller provincial based market agents is being developed by the Socio economics group through their survey work. Trading volumes can range from less than 1 tonnes per day for some of the smaller wholesalers during the off season, to hundreds of tonnes per day for the larger traders during peak season. The surveys are helping to understand the various actors in the chain and inter-dependency of the farmers, collectors, traders and wholesale/retailers. Identification on new market opportunities will potentially benefit a large number of participants from the farm and along the supply chain

One of the largest impacts of opening up new markets for Cambodian mangoes will be the increased demand for labour and quality inputs. Construction of packing facilities, treatment plants, cool chain equipment and better quality transport will result in new jobs and improved employment opportunities for Cambodian living in regional areas.

Women are employed in various roles along the supply chain, from nursery management, harvesting, packaging and market sales. Creation of new businesses focussed on export crops should result in more jobs within the horticultural sector.

Safer use of pesticides is likely to have benefits for communities living in mango production regions. Adoption of GAP, a major component in the next project, should result in safer practices, not only in use of pesticides, but also operation of machinery and handling of fertiliser.

8.3.3 Environmental impacts

The Kompong Speu market surveys highlighted some concerns regarding quality of inputs, such as pesticides and fertilisers. Many farmers report the main source of information regarding the rates, timings and type of chemicals and fertilisers came from market sellers or from their own previous experience. This raises a number of concerns. Overuse or inappropriate use of such inputs could represent a waste of resources and drive up the cost of production for growers. Further, chemicals are essential to achieve commercial yields of mangoes in Kompong Speu however overuse or ignorance of maximum residue limits and withholding periods will increasingly threaten export opportunities as international consumers demand safe products. It will be important for extension agents or local government groups to provide information to contractors and farmers on the appropriate use of chemicals and the market access issues that are associated with produce that is contaminated with chemicals. As Cambodia moves towards export of fresh commodities such as mango, it's critical that the principles of Good Agricultural Practice (GAP) are implemented and practiced by those participating in the export market trade. Record keeping and documentation of on-farm practices will need to be mandatory, and it's likely that choice of chemicals and spray application methods, including worker safety will come under scrutiny. The next phase of the

ACIAR Mango Project has a significant component on GAP, and hopefully this will result in changed attitudes and practice over time.

The pesticide residue testing carried out in the 2015 dry season crop has provided an important baseline on specific chemicals which may be an issue for future market access, and raise awareness of any potential health issues. Of the samples tested only 10% of samples had residues present, and these were well within acceptable limits. One fungicide in particular was detected in several samples, and it is suspected that this fungicide is applied close to harvest to manage diseases such as fruit anthracnose which can develop postharvest. This information has drawn attention to use patterns for certain chemicals that will require follow up to assist farmers to better manage such chemicals to prevent residues occurring in future.

Figure 46: A typical spray operation on a Kompong Speu farm. Worker PPE is very basic.

Investigations into the current mix of chemicals used for flower induction for Keo Romeat in Cambodia will hopefully lead to safer alternative options being taken up by industry. The negative impacts on tree health with over use of paclobutrazol, and the reliance on thiourea to induce flowering and its potential impact on human health are the two main interest areas for the project team. Successful research conducted in the Northern Territory during the 2016/17 season using alternative treatments to thiourea such as mono-potassium phosphate and potassium nitrate has provided a good platform for a new approaches in Cambodia.

8.4 Communication and dissemination activities

As mentioned in the body of this report there was a number of formats for communication to the collaborators and also out to the mango industry of Cambodia. Part of the capacity building throughout this project was related to giving CARDI and RDA officers the ability to communicate relevant information to the growing regions regarding mango production. In

order to do this they needed to fully understand the subject matter they were to present. This was clearly achieved through the workshops mentioned. Impact from such events was measured through surveys. The farmer workshops were the best format for delivering information. Resources employed encompassed all modes of learning as it can never be assumed that growers learn in any one format. Video messages such as the “How to prune mango” series proved extremely popular for the group. Key to its success was to physically go out and prune trees after the video was run so that the information was fresh in the grower’s heads. Literature was also distributed to reinforce the key points of the video. There was also time for questions and answers which proved to be more important than first thought. Questions were not only related to mango pruning but other aspects of crop production including nutrition, pest management etc. these highlighted key gaps in knowledge for industry and were noted and would be followed up on for the next information delivery session.

Now that a baseline of knowledge has been established the next phase of the project will build on this in developing further publications, videos and eventually a grower manual. This would lead to better quality fruit achieving increased prices which could feed ino a benchmarking project.

Internal Project Communication: At the commencement of the project Dr Brian Thistleton created a shared drive using the file sharing website PBWorks. This site operates in similar fashion to Google Drive, with each team member being allocated a password to be able to access workshop presentations, meeting agendas, specimen photos and publications. The website was particularly useful for the team to post insect and disease specimens when seeking help from Dr Thistleton with identification.

9 Conclusions and recommendations

9.1 Conclusions

While the focus of the ACIAR Cambodia Mango Project on capacity building of Cambodian researchers in fruit tree crop research has established a sound basis for good quality, self-directed research there is still more work to be done. Newly acquired skills in problem identification, research design, implementation and communication/extension of the results has the Cambodian team well placed to conduct future research and development activities independently and support growth of the horticulture sector. The RGOC objective of establishing trade with South Korea and other countries in the region is a driver for future growth in mango R&D, and HORT/2012/003 has been a major contributor to achieving this goal.

The partnership with the Korean company Foodya and interest from other investors in Cambodian horticulture is an important aspect of the work, and this partnership will hopefully grow stronger in the next project. Private investment is critical to sustainable growth of the industry, and establishment of a fresh mango export trade will be a basis for this future growth. The mediocre returns and lack of control of the product by Cambodian farmers from the green mango trade with Thailand and Vietnam is not a sound basis for growth. Opportunities in processing and other forms of value adding mangoes also needs to be considered. In the future, with a centralised packaging shed for mango exports, there may be an opportunity to develop a farmer cooperative. This would give the growers, as a unified group more power to set prices rather than when represented as a single farmer the price taking mind set.

9.2 Recommendations

It is important that the fruit R&D commenced under HORT/2012/003 is continued. There is now a core group of trained researchers in the three collaborating Cambodian organisations CARDI, GDA and RUA and a high level of enthusiasm to continue the work. While good progress has been good in some areas such as development of pest and disease lists, understanding the markets and double cropping system, there is a need to put further effort into identifying suitable regions and soil types for future development, crop nutrition, alternative mango varieties and integrated pest and disease management IPDM. A better understanding of the economics of mango production for both the domestic and export markets is required. For instance, the choice to grow Keo Romeat for the fresh export market, with potentially higher returns than selling onto the green mango market, will require a higher level of management and increased financial investment to be successful. Development of a financial decision support tool might be a useful tool for industry.

The next phase of the ACIAR Cambodia Mango project will commence in mid-2018. The NT DPI&R, will lead the project and continue working with all three organisations CARDI, GDA, and RUA. The project will also have a Philippines component. In summary, the new project objectives will include the following;

- Improved nutrition of mangoes with an emphasis on the role of calcium. Ionomics, or the measurement of total elemental composition of the plant, will be used in this study
- Crop area mapping to determine more accurate statistics on tree numbers and production
- Introduction of DNA analysis for improved pest and disease management
- Export development strategy for Keo Romeat mangoes, incorporating use of Good Agricultural Practices (GAP)

10 References

10.1 References cited in report

Cambodian Marketing of Mangoes: An Estimate of Volume (Bo, Theang, Kay, Pann, & Newman, 2015)

Value chain analysis of the Cambodian mango industry , ACIAR Project AGB/2014/020 Final Report, June 2016 Vinning G

Regional Market Study – Cambodia as part of the ACIAR project AGB/2015/015 *Analysis of mango markets, trade and strategic research issues in the Asia Pacific.* Roat, M CARDI, 2016

"*Dry matter testing of mangoes" NT DPIR Factsheet, Owens, G and Moore C., 2014.*

Determination of Optimum Maturity Stages of Mangoes Using Fruit Spectral Signatures, Subedi, P, Walsh, K and Purdy, P.

*"Mango gall midges on Australia's doorstep" P. Kolesik, A. Rice**, B. Thistleton**, D. Tenakanai, V. Quintao, C.D.R. Medina, M.M. Thein, C.H. Heng, L.A. Halling, F. Tsatsia and G.A. Bellis*

Census of Agriculture in Cambodia 2013: National Report on Final Census Results. National Institute of Statistics, Ministry of Planning and Ministry of Agriculture, Forestry and Fisheries, Phnom Penh. Available from: http://www.fao.org/fileadmin/templates/ess/ess_test_folder/World_Census_Agriculture/Country_info_2010/Reports/Reports_5/KHM_ENG_REP_2013.pdf.

Mango plantations by district in Kampong Speu in 2016. Unpublished data. Provincial Department of Agriculture, Kampong Speu, Cambodia.

Final report: Eastern Indonesia Agribusiness Development Opportunities - Mango Value Chain Wandschneider, T. Baker, I., Natawidjaja, R. (2014).. Report prepared by Collins Higgins Consulting Group for the Australian Centre for International Agricultural Research Project AGB/2012/006. ACIAR, Canberra, Australia. Available from: http://ei-ado.aciar.gov.au/sites/default/files/docs/mango_value_chain_studies.pdf.

10.2 List of publications produced by project

"Effect of temperature, potassium nitrate and thiourea on expression of flowering related genes in Magnifera indica" by Stacey Cook 12 International Mango Symposium in Guangxi, China in July 2017

Chemical control of mango flower initiation in Darwin (Australia)" by Cameron McConchie, Amy Dobell and Robert Williams 12 International Mango Symposium in Guangxi, China in July 2017

Managing mango flowering and implications for growers" by Cameron McConchie 11th Australian Mango Conference Bowen My 2017. .

"An emerging industry – Cambodian mango supply chains" by Suzie Newman and Bo Sokun, 11th International Mango Symposium, Darwin September 2015 (oral presentation).

HORT/2012/003 Annual Report 2013/14 – Hickey M, Newman S, McConchie C, Williams B, Thistleton B, Daly A, Heng C, Vang S, Chuong S

HORT/2012/003 Annual Report 2014/15 - Hickey M, Newman, Bright J, S, McConchie C, Williams B, Thistleton B, Daly A, Heng C, Vang S, Chuong S

HORT/2012/003 Annual Report 2015/16 - Hickey M, Bright J, Newman S, McConchie C, Williams B, Thistleton B, Tran Nguyen L,Heng C, Vang S, Chuong S

HORT/2012/003 Annual Report 2016/17 - Hickey M, Bright J, McConchie C, Thistleton B, Tran Nguyen L, Heng C, Vang S, Chuong S

Development of Pest and Disease Lists for Mangoes in Cambodia - Heng Chhun Hy, So Thavrith, Khay Sathya, Seyla Sem, Chuong Sophal, Andrew Daly, Jeremy Bright, Mary Finlay-Doney and Brian Thistleton. Paper presented at the International Mango Symposium, Darwin, September 2015.

11 Appendixes

11.1 Appendix 1:

ABSTRACT – 11th ISHS International Mango Conference, Darwin, 2015.

An emerging industry – Cambodian mango supply chains

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The emerging Cambodian mango industry faces many challenges including dependency on a single variety (Keo Romeat), lack of export protocols, competition with regional neighbours (Thailand and Vietnam), variable quality and declines in productivity. Despite this demand from regional neighbours, particularly in the off-season remains strong. As part of an Australian Centre for International Agricultural Research (ACIAR) project, this study seeks to identify and prioritise the key supply chain constraints, including postharvest losses, packaging, storage and transport to deliver mangoes into selected markets. To that end, 2 surveys have been undertaken to better understand Cambodian mango supply chains and identify opportunities for intervention. In 2012, one hundred mango farmers across 5 provinces (Kandal, Kompong Speu, Kompong Cham, Battambang and Siem Reap), stratified by farm size, were surveyed to understand current production practices and constraints. In 2014, a semi-structured survey of supply chain actors was undertaken in Battambang province to gain insight into mango flows, supply chain constraints and opportunities for intervention. In this paper we present, a situational-analysis of the Cambodian mango industry, a description of the state-of-play for current supply chains and propose some intervention opportunities that will enable the industry to better meet both domestic and export demand.

11.2 Appendix 2

ABSTRACT: 11th ISHS International Mango Conference, Darwin, 2015

Development of Pest and Disease Lists for Mangoes in Cambodia

Heng Chhun Hy (GDA), So Thavrith (GDA), Khay Sathya (CARDI), Seyla Sem (CARDI) Chuong Sophal (RUA), Andrew Daly (NT DPIF), Jeremy Bright (NSW DPI), Mary Finlay-Doney (NT DPIF) and Brian Thistleton (NT DPIF)

To trade a particular crop commodity, the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) requires that countries provide trading partners with complete lists of insects and pathogens found on that crop. These official lists should be maintained by National Plant Protection Organizations (NPPOs) and voucher specimens should be deposited in an official national collection. Additionally, for improving production, pest lists identify the most damaging insects and pathogens and help prioritise the development of techniques which minimise losses.

In Cambodia pest lists for mangoes are being developed as an activity within the project "Building a resilient mango industry in Cambodia and Australia through improved production and supply chain practices", funded by the Australian Centre for International Agricultural Research (ACIAR). This project involves collaboration between the Cambodian General Directorate of Agriculture (GDA), the Cambodian Agricultural Research and Development Institute (CARDI), the Cambodian Royal University of Agriculture (RUA), the NSW Department of Primary Industries (NSW DPI) and the NT Department of Primary Industry and Fisheries (NT DPIF).

This paper gives details of the survey methods being used to develop the pest lists and also presents some preliminary results, in particular for some of the more important insects and diseases.

11.3 Appendix 3 An emerging industry – Cambodian mango supply chains

Project team: Suzie Newman, Chea Sarith, Nicki Dumbrell, Bo Sokun, Lim Sophornthida, Men Pagnchak-Roa and enumerators from the CARDI SE Team and GDA.

7. Introduction

Mangoes are an important fruit crop for Cambodia. This is evidenced by: (1) an increasing area under mango production; (2) capacity to sell Cambodia's most common mango variety, Keo Romeat, when green or ripe; and (3) increasing demand for the Keo Romeat in the export market, particularly to Vietnam, China and Thailand for consumption or on-sale to other markets such as the European Union. At the time of the Census of Agriculture in Cambodia 2013 the area planted to mango orchards was reported to be 42,000 hectares (MoP and MAFF, 2015). Recent news reports also detail growing and new export markets for fresh mangoes. For example, in 2015 the Cambodian government signed a memorandum of understanding with South Korea to formalize the direct export of fresh mangoes (Kang, 2015) and in 2016 fresh mangoes were shipped direct to the European Union for the first time (Cheng, 2016). With this growth come opportunities to contribute to regional development. To leverage on economic and development opportunities associated with mango production in Cambodia and to build competitiveness and resilience in the industry we require an understanding of on-farm production practices and the key stakeholders in and spatial flows of the supply chain.

The emerging Cambodian mango industry faces many challenges including dependency on a single variety (Keo Romeat), lack of export protocols, competition with regional neighbours (Thailand and Vietnam), variable quality and declines in productivity. Despite this demand from regional neighbours, particularly in the off-season remains strong. As part of an Australian Centre for International Agricultural Research (ACIAR) project, this study seeks to identify and prioritise the key supply chain constraints, including postharvest losses, packaging, storage and transport to deliver mangoes into selected markets. To that end, 3 surveys have been undertaken to better understand Cambodian mango supply chains and identify opportunities for intervention:

- In 2014, a semi-structured survey of supply chain actors was undertaken in Battambang province to gain insight into mango flows, supply chain constraints and opportunities for intervention;
- In 2015, a trader survey was undertaken in the Neak Meas and Deum Kor wholesale markets in Phnom Penh to understand product flows for both domestic and imported fruit, quality requirements and collect some preliminary price data;
- In 2016, a semi-structured survey of supply chain actors was undertaken in Kampong Speu province.

As part of an earlier ACIAR SRA in 2012, one hundred mango farmers across 5 provinces (Kandal, Kompong Speu, Kompong Cham, Battambang and Siem Reap), stratified by farm size, were surveyed to understand current production practices and constraints. This provided us with a good understanding of at the farm end of the chain and so this was not included in this study.

8. Approach and Methodology

a. Pilot study (March 2014)

Survey checklists from AGB/2012/006 *Eastern Indonesia Agribusiness Development Opportunities – Mango Value Chains* (developed by Tiago Wandschneider) were obtained, discussed and refined to tailor them for use in Cambodia. These checklists were also translated ready for pre-testing in Battambang and Siem Reap. Much discussion centred on the selection of collectors and other supply chain partners, with the core difficulty being able to locate collectors and aggregators. This is particularly problematic for mango supply chains where limited provincial resources have been invested in fruit production.

Pre-testing of the check-lists was undertaken in both Battambang (4 interviews) and Siem Reap (3 interviews) – interview transcript reports were prepared by Lim Sophornthida and used to review and refine the check lists. Meetings with Provincial Departments of Agriculture (PDA) were also held in both provinces to get a broad overview of the mango industry and to facilitate contacting contractors, collectors and wholesalers.

Following the pre-testing significant re-working of the check lists was needed to make them more suited to Cambodian conditions. To determine an effective sampling strategy we needed to: 1) identify major production districts in Battambang and sample contractors within this; 2) identify collectors/aggregators as these are the key people to understanding product flows and 3) visit key border posts to see first hand the cross-border trade and identify traders. The pilot study enabled us to gain sufficient insight into mango supply chains to determine the approach needed for the design of our Battambang study, we then refined this approach further for the Kampong Speu and Phnom Penh wholesale market studies.

b. Battambang supply chain study (2014)

Our overall objective was to gain insight into mango flows, supply chain constraints and opportunities for intervention. To that end we used the following approach:

- Direct observation – market/field/export gate visits during the main production season – enabling us to follow the mango chain from farm to market
- Visits and interviews of exporters/importers at 3 border gates - Prum (Pailin province), Liem, and Phnom Deiy (Battambang province).
- Conducted a semi-structured survey in Battambang province of wholesalers, collectors and contractors using refined checklists from *AGB/2012/006 Eastern Indonesia Agribusiness Development Opportunities – Mango Value Chains.*

Full interview transcripts were then prepared (available upon request) and this was used to analyse the supply chains. The approach used is qualitative in nature and primarily designed to gain insight into the supply chains understudy. Further quantitative work would be recommended to gain an understanding about marketing margins etc. – however our attempts to include this in the semi-structured surveys overcomplicated the surveys resulting in data that was difficult to use in an effective way.

The contractor/farmer interviews were conducted in three main parts. Firstly, information was collected on contractors'/farmers' experience, sources of information for management practices, and relationships with the tree owners. Secondly, information was collected on crop management including type and timing of fertiliser and chemical application and the associated input costs. Thirdly, they were asked about mango yield and price information and information regarding mango buyers. The collector interviews were separated into two main parts: (1) detailing the collectors' relationship with contractors/farmers and wholesalers (in the domestic or export market) and (2) establishing the collectors' scale of operations and margins.

c. Phnom Penh Wholesale market survey (2015)

In Phnom Penh, there are two major wholesale markets where mango trade is conducted – they are Neak Meas and Deum Kor markets. To enable us to obtain representative data we first needed to estimate the population of wholesalers (large, medium, small) in these two major wholesale markets. This then enabled us to determine the number of interviews that would provide us with a representative sample. These population estimates were undertaken during the height of the mango season (April 2015) with the actual survey then undertaken post-mango season as we felt that wholesalers would be better placed to answer questions at this time. Also undertaken at the height of the mango season was an observational study at the markets to enable us to document postharvest handling practices, estimate traded volumes and obtain wholesaler contact details. Following the mango season we then undertook semi-structured interviews with 15 wholesalers in Neak Meas and 10 wholesalers in Deum Kor markets.

d. Kampong Speu supply chain study

A similar approach was used to that detailed in (4) above.

9. Key results

a. Battambang supply chains

In Battambang, mango occupies 2224 ha, comprising 889,600 mango trees (400 mango trees/ha). Mangoes are produced in 6 districts including Rattanak Mondol, Banan, Samlot, Bavil, Kam Reang and Phnom Prek districts. The districts of Kam Reang and Phnom Prek, are recent additions with production in this region only starting a few years ago. There are 5 main retail markets including Toul Ta Ek, Leur, Thmey, Nat and Chrey market.

Fig. 1 shows the number of semi-structured interviews undertaken with different actors in the chain.

Details	Battambang province					Pailin province		Siem Reap province		Total
	Sangkei and Banan districts	Boeung Chouk market	Theiy market	Phnom Deiy market	Liem market	Pailin Town	Prum market	Banteays rey district	Samaky market	
collector	5						2			7
collector/ grower/ producer off season	2					1				3
collector & wholesaler				1	2	1				4
Contract farming	9							1		10
Wholesaler		6	2		1		1		2	12
Total	16	6	2	1	3	2	3	1	2	36

Fig. 1 Semi-structured interview summary by function and location

Fig 2 shows pictorially our current understanding of the mango supply chain in Battambang. The supply chain on the right represents the export supply chain with limited supply going on to the domestic market. The chain on the left represents the domestic mango supply chain in Battambang with product largely supplied from outside the region. Additionally some of this product is also sold to Thai retailers.

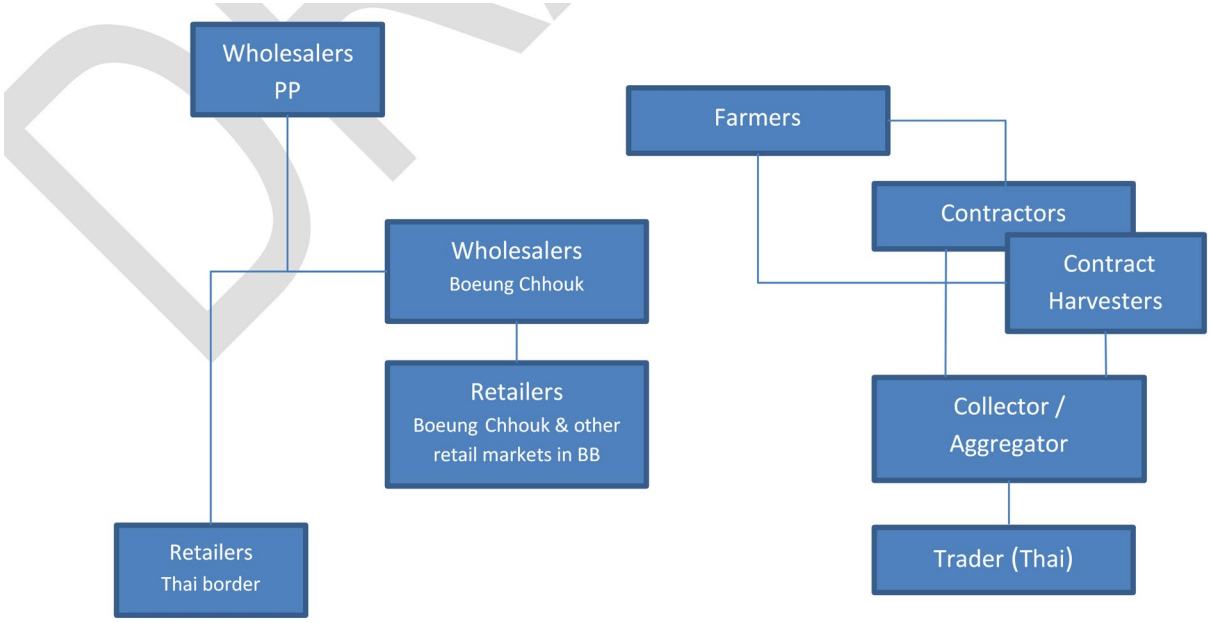

Fig. 2 Battambang mango supply chain

i. Variety availability

Keo Romeat is the main variety exported to Thailand and Vietnam. In the off-season (December) collectors typically sell the fruit for R3000/kg, whereas on-season (April) prices are typically R1000/kg. Fig. 3 shows the availability of the two key varieties – Keo Romeat and Keo Chen.

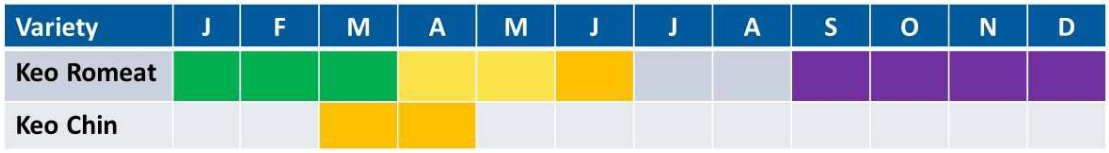

Fig. 3 Availability of key mango varieties (note: other varieties are sold in small quantities on the local market including: Khiev Savoeu, T Chau Anant, and Keo Morokot.)

ii. Contractors

In Battambang, contractors play an increasingly important role, with more farmers relinquishing their trees to contractor management. Contractors typically undertake production, flower manipulation and harvesting with each contractor managing 2-5 farms (415 -1450 trees). Depending on tree age, contractors typically pay $15 per tree/year for a 7-9 year old tree. Contracts may be entered into on an annual or triannual basis. Typically mangoes are produced in 2 main seasons: 1) on-season – April/May and 2) off-season – October-January.

Flower manipulation for off-season production is critical to ensure a profitable business, however from our discussions with contractors there appears to be a wide variety of techniques employed. Given the strong returns for off-season production, contractors tend to try and produce fruit predominantly in the off-season. For example they may look to produce 100 tonnes during the on-season and 130 tonnes in the off-season. Contractors appear to have learnt techniques from Thai or Vietnamese traders and largely do not understand the phenological cycle of mango and how they might get the best off-season performance. Indications are that they are also stressing the tree resulting through over-production and limited nutrient replenishment. Part of this is likely to be due to unsuccessful attempts to induce flowering.

Contractors are likely to be a strong intervention point – given their diverse role and relationships with other key players in the chain.

iii. Traders/collectors/exporters

Battambang wholesalers in Boeung Chhouk wholesale market (and the smaller Thmei wholesale market) are small scale (500 kg/day) and therefore need to source mangoes from PP wholesalers (Neak Meas) rather than from Battambang where mangoes are exported directly to Thailand.

Mangoes are typically sorted into 2 grades. Grade 1 mangoes are large (3-4 fruit/kg), well coloured and no pest or disease damage. Grade 2 fruit are typically smaller (5-6 fruit/kg) and small defections are permitted. Mangoes for export are packed in 25 kg plastic crates. Whereas product for the local market tends to be packed into plastic bags. Typically Khmer traders purchase the product following a visit to the farm. They are often responsible for harvesting and packing. Transport to the Thai border by ute (3-4 T) is USD100/trip, whilst transport by truck (8T) costs around USD200/trip. At Leim gate/border - 3 large collectors/traders selling directly to Thai traders - 100-200 t/day during on-season, 20-40 t/day during off-season; many small collectors/traders. A similar situation exists at other border gates. Exported produce is sold into both the fresh and processing sector. On-season production is mainly used by the Thai processing sector 650 riel/kg (US16c/kg), whereas off-season production is mainly sold into the fresh sector 1200-1300 riel/kg (US30c/kg) Product is sourced from throughout Cambodia, traders/collectors at border supplied by network of collectors from other provinces. Most medium- high quality Battambang produced mangoes are being directly exported (limited locally produced fruit in Battambang markets).

Full interview transcripts prepared by the CARDI Socio-economic team have been provided in earlier reports.

b. Kampong Speu supply chain

The following descriptions of mango production, inputs, and prices and geographical spread of buyers are based on interviews with 10 contractors and 15 mango collectors in Kampong Speu Province, Cambodia.

i. Contractors

The majority of the contractors interviewed were relatively new to the industry with a maximum of 10 years of experience as a contractor. However, half of the contractors interviewed (five) also owned trees that they manage alongside the trees they contract. The average number of trees managed by each contractor was 6,000 (range: 800 – 23,300). The contracted trees ranged from three to 15 years old. The older trees were commonly more expensive to rent however, tree rents were also dependent on the relationship between the farm owner and the contractor. One contractor was able to rent 10 year old trees for only $2.58[2] per tree because he worked for the farm (and tree) owner for a long time. Other contractors in the sample paid an average of $11.15 for a tree. The range of prices paid across trees that were 3 years old up to 15 years old was $2.58 to $20 per tree. Higher prices are paid for older trees (10-15 years old) and lower prices were paid for younger trees, e.g. $3 per tree for a 3 year old trees.

All contractors interviewed were growing the Keo Romeat variety to harvest and sell in the on-season, typically in April, and off-season which typically runs from October to March. No contractors were growing other mango varieties or targeting other harvest/sale times.

[2] *All dollar figures are in 2016 US Dollars.*

Important inputs to mango production include fertiliser, chemicals to manipulate flowering, fungicides, pesticides, fuel and labour. Inputs such as fertiliser and chemicals were sourced locally or from as far away as Phnom Penh. Fuel was commonly sourced from within the Kampong Speu province. The contractor typically supplements their own labour with local sources of labour for tree management activities that are time critical. Harvest labour was commonly described as the responsibility of either the contractor or the buyer, depending on the buyer and mango price. Information inputs especially related to chemical use decisions tend to be sourced from the chemical sellers or the contractors' previous experiences.

Without specifying the split between on- and off- season production, the annual production of each interviewed contractor for 2015 is listed in Table 1, with quantities sold separated by quality grades. Fruit are classified as Grade 1 if they are large (3 fruit per kilogram) and have no skin blemishes and classified as Grade 2 if they are smaller (5-6 fruit per kilogram) with few skin blemishes. The largest producer was able to sell 610 tonnes of mangoes in 2015 however, the large majority of these were sold as Grade 2 (Table 1). Alternatively some of the smaller producers were able to sell 100% of their mangoes as Grade 1 (Table 1).

Table 1. Tonnes of mangos sold in 2015 as separated by quality grades for each contractor interviewed.

Contractor	Grade 1	Grade 2	Ungraded	Total
1	60	20		80
2	150	460		610
3	400		100	500
4	30			30
5			45	45
6			40	40
7	40			40
8	260	60		320
9	96		144	240
10	200		200	400

Maximum, minimum and average farm-gate prices for 2015 as reported by the interviewed contractors are provided in Table 2. Grade 1 mangoes attract higher prices than Grade 2, small and ungraded fruit whereas ungraded mangoes attract higher prices than Grade 2 and small fruit (Table 2). This indicates that unless the fruit is Grade 1 it may not be worth grading before sale. Packaged mangoes attract the highest price at the farm-gate as this

reduces packaging costs further down the supply chain. All interviewed contractors reported that their mangoes sold on farm to a collector or large export traders. For the 2014 and 2015 season there was a 50:50 split in mangoes sold at the farm-gate to Cambodian collectors and Vietnamese traders.

Table 2. Maximum, minimum and average mango prices (USD/kilogram) at the farm-gate in the on-season and off-season for 2015 as separated by quality grades.

	On-season			Off-season		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Grade 1	0.43	0.33	0.38	0.55	0.38	0.46
Grade 2	0.20	0.20	0.20	0.33	0.20	0.25
Small	0.15	0.08	0.11	0.15	0.08	0.11
Ungraded	0.50	0.18	0.30	0.58	0.35	0.44
Packaged	0.95	0.50	0.66	-	-	-

ii. Collectors

Of the 15 mango collectors interviewed four were classified as small, five as medium and six as large. These classifications were based on the scale of mango collecting operations and the capital owned and operated by the collector. For example, a small collector typically collects 600 tonnes per year and sells on to a larger collector or trader whereas a large collector typically collects more than 7000 tonnes per year and will collect from smaller collectors and farms. Larger collectors also tend to own a truck to transport mangoes to the Cambodian border to access export markets.

The collectors interviewed had a range of relationships and experiences with traders and wholesalers. All collectors on-sold mangoes to a larger collector or trader, i.e. no collectors sell directly to consumers. Based on on-sale information from collectors, Thailand is the key market for mangoes grown in Kampong Speu Province (Table 3). The Thai market is followed by Vietnam, local sales to larger collectors and China (Table 3) however there is anecdotal evidence for much of the product entering the Vietnamese market continuing on to China. The different markets also tend to purchase different products. The Vietnamese tend to select good quality fruit (e.g. grade 1) and pay different prices for different quality grades. Whereas much of the fruit that collectors send to Thailand is ungraded with one price.

Table 3. Average percentage of mangoes, acquired by interviewed collectors, that is on-sold to different buyers for 2013-2015.

Buyers	2013		2014		2015	
	On-season	Off-season	On-season	Off-season	On-season	Off-season
Collector at Thailand border	63%	63%	66%	66%	48%	43%
Vietnamese trader	29%	29%	34%	34%	32%	36%
Chinese trader	-	-	-	-	9%	9%

Cambodian collector	8%	8%	-	-	11%	12%

iii. Chain structure and product flows

Mango producers in Kampong Speu Province typically have two options to sell their fresh fruit. The first option could be to sell direct to a large-scale international trader (Figure 2). This arrangement is most common with Vietnamese traders. The arrangement is established at the beginning of the season with decisions on provision of inputs such as paper bags to cover fruit to protect against fruit fly and the provision of labour for bagging and harvest activities. Traders working directly with producers tend to seek the best quality fruit and thus are engaged throughout the season to ensure they maximise the access they have to high quality fruit.

The alternative option is to sell to a collector (Fig. 4). The collector will approach the grower toward the end of the season when the mangoes need to be harvested. There is rarely a pre-arranged contract to buy from a grower at the beginning of the season. The collectors vary in their scale of operations. Once a collector aggregates the products from multiple farms they have two options, one is to sell to a larger collector (this is common if the collector is small scale and collects relatively small quantities e.g. 600 tonne per year) the alternative is to sell direct to a trader. The collectors do not sell to retailers or consumers. If the trader is based at the border, it is the responsibility of the collector to transport the fruit to the border. For this reason it is often the large collectors that sell fruit to traders based at the border. Much of the fruit collected by the interview sample was sold on to Thailand or Vietnam. Though, anecdotal evidence was provided to indicate that fruit sent to Vietnam may eventually be on-sold to retailers and consumers in China.

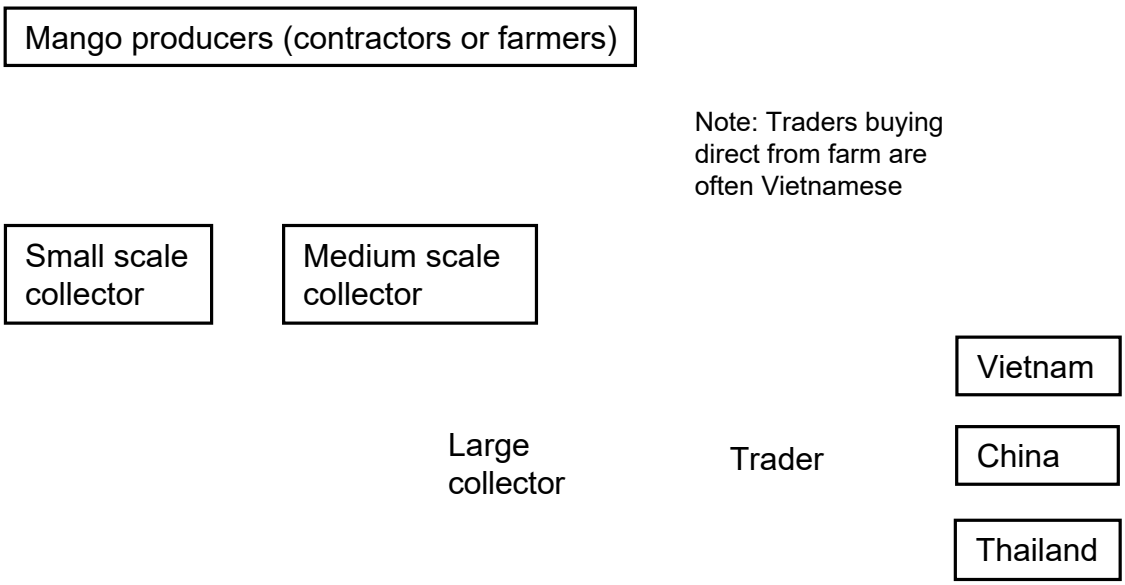

Fig. 4 Simplified supply chain for fresh mangoes produced in Kampong Speu Province, Cambodia.

iv. Supply chain issues

Key issues that arose during interviews with contractors and collectors include: mango price variability, relationships between supply chain actors and information flows. When respondents were given the opportunity to express concerns or future opportunities for the industry price was mentioned most often. For the three-year recall period, 2013 to 2015, many contractors described the mango price as a concern as it had been very variable. On the contrary, a small sample of the collectors described the mango price as increasing with increasing opportunities to enter the export market. This may indicate that any increase in price is not being passed on to the grower.

Contractors are motivated to manage leased mango orchards in a sustainable manner. They are aware that their opportunity to renew leases depends on the owners perceptions of their management. With one to three year leases being typical it is important for the contractor to renew their lease in order to learn about their environment, get returns on investments and build relationships upstream and downstream in the supply chain. The tree rent agreed is variable across farms with tree age the main driver of rent prices. Rents were lower for the contractors that knew the owners and were trusted by the owners. It was difficult to ascertain any other situations in which the relationship between owner and contractor was important. For example, none of the interviewed contractors were offered extended lease times or management advice or assistance from owners if they knew each other well. Contractors sell to collectors that offer the highest price. As a consequence it is rare to sell to the same collector for multiple consecutive harvests and thus difficult to establish trust, expectations and certainty in the market.

Information exchanges between contractors and collectors tend to be limited to price and harvest date; the collector sets the price and date and the contractor responds with whether they can deliver to those conditions. There appears to be little forward contracting or negotiation of price and timing of sale between the contractors and collectors. There is also little communication between the land-owner and the contractor outside of the negotiation of the lease. The contractor appears to get most information about farm management practices from their own experiences or input suppliers.

c. Phnom Penh wholesale markets

There are 2 main wholesale markets in Phnom Penh for mangoes – Deum Kor and Neak Meas. Deum Kor sources mangoes from Kandal (Kean Svay), Kampong Speu (Kirirom), Takeo, Vietnam and Thailand. Mangoes are then sold to other Phnom Penh markets and provincial markets such as Kampot, Kampong Chhnang, Kampong Cham, Sihanouk Ville, Prey Veng, Takeo, Kampong Speu and Kandal, The market has 15 mango wholesalers in and around the market and 50 mango retailers. The main selling times – early morning (3am) and late evening (9-10pm).

Neak Meas sources mangoes from provinces throughout Cambodia and is considered the primary wholesale market for mangoes. Within the market there are exporters marketing to Thailand, Vietnam and in-direct to China. Within the market there are
10-15 large wholesalers selling 30-100 tonnes/day, 20-30 small wholesalers selling <10 tonnes/day,

i. A Neak Meas wholesaler – one of the large wholesalers

For off-season trade (September – December) their focus is Thailand (50 tonnes/day), Vietnam (20 tonnes/day) and China (18 tonnes/day). They classify fruit into 2 grades: Grade 1 – 2-3 fruit/kg and Grade 2 – 4-5 fruit/kg. For Grade 1 fruit in 2015 they typically receive 1500-2000 riel/kg (Thailand), 2000 riel/kg (Vietnam) and 2800-3000 riel/kg (China). Each market has different packaging with Grade 1 fruit destined for Thailand packed in 25 kg plastic crate, whilst a 70 kg box is used for Vietnam and a 40 kg plastic crate for China.
For on-season trade (March-May) they typically export 100-180 tonnes/day to Thailand, 20 tonnes/day to Vietnam and 16 tonnes/day in-direct to China.

Note: Interview transcripts have been previously supplied in earlier reports.

10. Conclusion

The Cambodian mango industry is expanding rapidly and this is primarily been driven by regional export trade of Keo Romeat. Despite its popularity in regional markets there is limited domestic trade due to Khmer consumers strong preference for Keo Chen and other varieties. For future growth to be sustainable there is a need for a greater diversity of varieties. The strong regional trade networks into Thailand, Vietnam and China provide significant opportunities for the industry to capitalise on these. However with ASEAN trade agreements (AEC) this is likely to have implications for product standards, food safety standards and also result in greater official trade. Ultimately this will drive improvements across the sector but for Cambodia to remain competitive these changes need to be implemented now.

Information gained from interviews with mango contractors and collectors in Battambang and Kampong Speu Province, indicates that the mango industry is growing and has further growth potential if productivity an on-farm and post-farm management practices can be improved. Thailand and Vietnam are important and growing markets for fresh mangoes grown in both provinces. To capture the opportunities associated with these markets there is a need to improve on-farm management and information flows in the Cambodian mango industry.

There is much variability in the on-farm management practices and use of inputs across mango contractors. Information from the contractors interviewed also indicated that inputs are sourced from a number of markets and suppliers. Much information regarding the use of chemicals and fertilisers was said to come from the seller or previous experience. This raises a number of concerns. Overuse or inappropriate use of such inputs could represent a waste

of resources and drive up the cost of production for growers. Further, chemicals are essential to achieve commercial yields of mangoes in provinces however overuse or ignorance of maximum residue limits and withholding periods will increasingly threaten export opportunities as international consumers demand safe products. It will be important for extension agents or local government groups to provide information to contractors and farmers on the appropriate use of chemicals and the market access issues that are associated with produce that is contaminated with chemicals.

The relationships between key supply chain actors are short-term with many one-off interactions. For example, collectors often buy mangoes from different orchards each year. Without continued relationships with the same producers it is difficult to build rapport and trust. On-going relationships could improve the flow of information between buyers and sellers of mangoes and allow forward thinking or planning to meet changing market requirements. At present the information flow between contractors and collectors is restricted to price and timing of harvest for the current season. Frequent discussions about expected yields, quality, seasonal conditions and market demands between collectors and contractors could aid both to modify their operations to take advantage of market opportunities.

11. References

Cheng, S. (2016), 'Mangoes bound for European market', *The Phnom Penh Post,* 6 May 2016. Available from: http://www.phnompenhpost.com/business/mangoes-bound-european-market.

Kang, S. (2015), 'Gov't Signs Deals to Export Mangoes to S Korea', **The Cambodia Daily**, 9 December 2015. Available from: https://www.cambodiadaily.com/news/govt-signs-deal-to-export-mangoes-to-s-korea-102475/.

MoP and MAFF (2015). Census of Agriculture in Cambodia 2013: National Report on Final Census Results. National Institute of Statistics, Ministry of Planning and Ministry of Agriculture, Forestry and Fisheries, Phnom Penh. Available from: http://www.fao.org/fileadmin/templates/ess/ess_test_folder/World_Census_Agriculture/Country_info_2010/Reports/Reports_5/KHM_ENG_REP_2013.pdf.

PDA Kampong Speu (2016). Mango plantations by district in Kampong Speu in 2016. Unpublished data. Provincial Department of Agriculture, Kampong Speu, Cambodia.

Wandschneider, T. Baker, I., Natawidjaja, R. (2014). Final report: Eastern Indonesia Agribusiness Development Opportunities - Mango Value Chain. Report prepared by Collins Higgins Consulting Group for the Australian Centre for International Agricultural Research Project AGB/2012/006. ACIAR, Canberra, Australia. Available from: http://ei-ado.aciar.gov.au/sites/default/files/docs/mango_value_chain_studies.pdf.

11.4 Appendix 4 Mango Potting Mix Trial in 2015

Ms Srey Nech Ouch, Research Assistant, Plant Breeding Group, CARDI.

A nursery for mango seedling rootstock production was established at CARDI. The aim of CARDI nursery is to maintain good practice for mango seedling production, and develop improved tree nursery management practices for Cambodian farmers. To accomplish this objective one trial of Growth Performance of mango seedling in different potting media mixtures under net house conditions was conducted to gain useful information for using the appropriate potting mix for mango seedling.

Two cultivars of mango, Keo Romeat, which is popular between Cambodian market and exportation, and wild mango (Svay Prey), which is a valuable for rootstock, were used in this trial under seven different potting mix media. A range of data was collected and subjected to analysis of variance (ANOVA). Analysis of the data shows that there is no interaction of different potting mix on mango cultivar seedling. Both cultivars (Keo Romeat and wild mango) grew well and vigorously in potting mix number 5: Cow manure+ rice husk burned+ soil (1:2:1). Wild mango grows more vigorously than Keo Romeat in all tested potting mixes.

Table 1: Seedling high, seedling girth, and Number of leaves preferment among each cultivar and potting mix.

Potting Mix*	Seedling Height (cm)		Seedling Girth (cm)		No of Leaves	
	V1	V2	V1	V2	V1	V2
P1	29.3	29.7	1.9	2.0	17	14
P2	28.3	33.3	1.6	2.3	13	17
P3	28.1	29.4	1.8	2.2	14	16
P4	28.7	35.9	1.8	2.3	17	18
P5	34.4	39.5	1.8	2.5	22	20
P6	26.1	28.6	1.7	2.2	14	14
P7	29.4	32.7	1.8	2.1	16	16
Mean	29.2	32.7	1.8	2.4	16.0	16

Variety (V)	2.9*	0.3**	ns
Potting Mix (P)	ns	ns	ns
VxP	ns	ns	ns

* Potting mix media were as follows:

- P1: Soil (control)
- P2: Cow manure+ rice husk+ soil (1:2:1)
- P3: Cow manure+ rice husk+ sand (1:1:1)
- P4: Cow manure+ rice husk+ soil (1:1:1)
- P5: Cow manure+ rice husk burned+ soil (1:2:1)
- P6: Cow manure+ rice husk burned+ sand (1:1:1)
- P7: Cow manure+ compost+ soil (1:1:1)

Conclusion and Recommendations: Although none of the soil mixes performed significantly better than the others in terms of plant growth, the better drained mixes, such as Potting Mix No 5 Cow manure+ rice husk burned+ soil (1:2:1) generally produced healthier plants. This experiment would need to be repeated, maybe including commercial mixes as a comparison to locally sourced potting materials.

11.5 Appendix 5

The use of tip-pruning to manipulate flowering, yield, and harvest maturity in two Australian mango cvs. Honey Gold and Calypso (B74) in subtropical climate of Northern Territory (Katherine region).

Summary

This study aimed to takes advantage of the progressively cooler weather in Katherine regions (Northern Australia) that occurs from April to July to investigate the effects of night temperatures on developing mango buds. The experiment was conducted using seven-year-old commercial mango cultivars Honey Gold and Calypso (B74) grown on separate properties. Tip-pruning treatments were applied to three replicate trees at four weekly intervals for five months. All stems (branches) around the canopy were pruned 10cm approximately above the last internode for each branch on each tree. The length of new flush growth for 20 randomly selected pruned branches on each tree was recorded on a weekly basis. During experiment from April to November (harvesting time) climate data including; temperature (mean, max, min), chill and heat sums, chill and heat cumulative (cum), and relative humidity (RH %) were recorded hourly to find their impact on growth characteristics such as; vegetative bud growth, flowering time, canopy flowering (%),inflorescence length, number of fruit/tree, and fruit maturity. Results revealed that the pruned trees produced more inflorescence significantly when the axillary bud below cutting point received over 300 hrs chill cum<20˚C over first three to four weeks after tip pruning performed. Dry matter data indicated that tip pruning at the right time could be used as an agro-technical tool to delay harvesting time for the studied mango cultivars. The result showed that combination of cool weather (<20˚C) and tip-pruning is promising alternative strategies to the use of paclobutrazol for sustainable mangoes production in the region, when applied in May, June, and first two weeks of July.

Introduction

Controlling growth and stimulating the formation of vegetative and reproductive buds is a common task in fruit trees by pruning performance. The aim of this study was to evaluate six different times of tip-pruning from April to July on two commercial mango cultivars Honey Gold and Calypso applying every four weeks. Taking advantage of cold weather from April to July during the dry season in Katherine region with a combination of tip-pruning is promising to develop agro-technical alternative strategies to the use of paclobutrazol for growing mangoes in the Katherine region to maintain the production sustainably.

Plant materials

The experiment was conducted on cv. Honey Gold at Piñata Farm (Fox Road, 14°32'44.2"S 132°28'21.9"E) and cv. B74 at NT Land Development Farm (Florina Road, 14°35'31.0"S 131°58'53.4"E) in Katherine region. A total of 24 trees of each cultivar on Kensington Pride (KP) rootstock was selected from 7-year-old trees for each cultivar. All trees were subjected to pruning after fruit harvesting in first January. A randomised complete block with sex pruning times was used for each cultivar to prune three trees every four weeks for each pruning time based on week of the year; time 1 (week 13), time 2 (week 17), time 3 (week 21), time 4 (week 25), time 5 (week 29), and time 6 (week 33). All terminal stems around the canopy were pruned by scissor pruner above 10cm of the last internode for each branch on each tree for each pruning time, and 20 branches were randomly selected and tagged around the canopy on each tree.

Measuring of variables

For each experiment site two data loggers (Tinytag Plus 2, Hastings Data Loggers, NSW, Australia) were installed to measure temperature (mean, max, min) and relative humidity (RH %) hourly from week 13 to week 46 (harvesting time) of the year. During this period temperature <20˚C was calculated as chill sums as well as heat sums calculated as follow; [(max temp + min temp)/2] – 12 (Moore, 2013). The heat sums calculator is a tool for

predicting fruit maturity in mango in Australia. Different mango varieties have slightly different heat units, generally growers count heat sums from flowering at stage 6 of panicle emergence (the first time the bud can be seen) to the time that dry matter reaches to certain percentage (DM%) for each variety e.g. the DM% for both cultivar Honey Gold and B74 is approximately 18% percentage as harvesting index.

After one week of pruning, axillary flushes length below the point of cutting for each new 20 selected was recorded on weekly vegetative growth based on mm. For all treatments, flowering time (week of the year), canopy flowering (%), the number of panicle emerging below of cutting point, and the number of fruit/tree were recorded. For each pruning times, 20 fruit/tree (60/treatment) were harvested in week 46 of the year (second week of November) for both cultivars. Fruit transferred to the lab for measuring following variable; Brix˚ (digital reflectometer, two times/fruit from both side), fruit weight (g), and dry matter (DM%). The dry matter has been used as maturity indicator by most of the growers for harvesting mango in Australia, therefore in this study were measured the maturity using traditional and infrared spectroscopy methods as either of them often are used by farmers. To measure DM% based on destructive prediction (traditional method) for each harvested fruit, a sample from both sides (center) of the fruit were taken using the apple corer (1 cm thick). The samples then were peeled and weighed. Samples then were dried in the oven at 65˚C for 48 hrs and weighed. Dry matter was calculated by following formula; DM%= (Dry weight/Wet weight) × 100%. Non-destructive prediction of DM% also performed using pre-calibrated near infrared spectroscopy F-750 NIR unit (Felix Instruments, WA, USA) for both cultivars.

Statistical analysis

The experiment was arranged as a randomized complete block design (RCBD) with three replications. Data were subjected to ANOVA analysis using SAS version 9.3 statistical

software (SAS Institute, Cary, NC, USA) and Excel (2013). However, data were analyzed separately for each farm (each cultivar).

Results and Discussion

As in many studies whether condition have been reported to have a key role on mango vegetative and flower buds induction the results of climate data collected from both mango plantation site in this study including; temperature (mean, max, min), day/night temperature fluctuation, chill and heat sums, chill and heat cumulative (cum), and RH% was presented for 'Honey Gold'(Piñata Farm, Fox Road, 14°32'44.2"S 132°28'21.9"E) and 'B74' (NT Land Development Farm, Florina Road, 14°35'31.0"S 131°58'53.4"E) in Fig. 1 and Table 1 and in Fig. 2 and Table 2 respectively.

Growth characteristics affected by different pruning times is presented in Table 1 and 2. First growth was observed 2-3 weeks after tip pruning for the buds close to the cutting point regardless of treatments, For the tip-pruning times 1 and 6, the vigor (length) of the first and second growth cycle were not statistically different to the unpruned tree (control) in both CVS. Honey Gold and B74. Fig. 3 shows the flushing pattern in both cultivars that obtained based on data collected for tip-pruning times 1 (pruning performed in 13 weeks of the year). As most of the bud (>80%) below the cutting point in tip-pruning times 2, 3, 4, and 5 were flower buds, there were not enough vegetative buds available for these pruning times to be analyzed for vegetative flush growth pattern (Table 1, canopy flowering %). When the pruning was conducted in weeks 13 (April) and 33 (August) of the year, the number of vegetative growths below cutting point was varied between 2 to 4 (Fig 4). Although, about 50% these vegetative buds in tip-pruning times 1 in both studied cultivars produced terminal inflorescences when the vegetative flush became mature (length >100mm, and hard and dark green leaves) and receiving around 400 hrs chill sums (Table 1 and 2). However, in both cultivars vegetative buds with same characteristics did not produce terminal inflorescences when tip-pruning performed in August (week 33) due to not receiving not enough chill sums, where the chill sums below 20˚C was recorded >125 hrs for Honey Gold

and > 95hrs in B74 below). In mango irregular or recurring flushing are commonly happen, as results apical or axillary buds are released and the new shoots enlarge constantly through several nodes and then become mature. To have a successful flowering rate in mango the timing of flush development is essential because bud release as vegetative or reproductive growth, can only happen from mature flush. In addition, buds seem to be approachable to the floral inducement for only a slight portion of the flush development cycle, because floral induction seems to require inductive temperatures approximately to occur at the same time with bud release.

Canopy flowering (%) in both cultivars reacted slightly similar to different pruning times. The highest canopy flowering percentage in both cultivars was observed when pruning was performed in week 17, 21, 25, and 29 of the year, where the rate of flowering was observed 86% to 95% for ‘Honey Gold’ and 63% to 83% for ‘B74’ (Table 1, 2). However, flowering reduced to 63% in ‘B74’ when tree pruned in week 29, and eliminated in both cultivars when tip-pruning performed in week 33 (time 6). Canopy flowering percentage for control and pruning times 1 (week 13) for both cultivars was recorded from 51% to 58% for ‘Honey Gold’ and ‘B74’ respectively. Based on Table 1 and 2 data different chill sums (from pruning time to flowering time) was recorded for each different pruning times for both cultivars plantation sites. In ‘Honey Gold’ more than 420hrs chill sums (>20˚C) was recorded for unpruned tree and pruning times 1, 2, 3, and 4, chill sums was 354hrs for pruning times 5 and 122hrs for pruning times 6. In ‘B74’ more than 340hrs chill sums (>20˚C) was recorded for unpruned tree and pruning times 1, 2, 3, and 4, chill sums was 310hrs for pruning times 5 and 93hrs for pruning times 6. The highest fluctuation in day/night temperatures was recorded at 25.39 ˚C in week 25 and 26.92 ˚C in week 31 for ‘B74’. The highest fluctuation in day/night temperatures was recorded at 26.65 ˚C in week 25 and 27.74 ˚C in week 31 for ‘B74’ (Fig 1 and 2). The canopy flowering was recorded 0% for the pruning times 6 in both cultivars (Fig 4). The results also confirmed that in mango a combination of cold temperatures and hard dark green mature leaves are necessary for flower.

Despite performing different tip-pruning times, flowering occurred by maximum four weeks delay among treatments in both cultivars (Table 1, 2). Flowering occurred in control plants on August 10 (2014) and in tip-pruned trees on September 02 (2014). In both cultivars, vegetative buds, floral buds, developing panicles, and panicles with floral opening in the same was observed at the same times on the canopy of control tree and trees submitted to tip-pruning times 1 (week 13), while tree submitted to tip pruning in time 2, 3, 4, and 5 panicles happened consistently in the whole canopy. Also, in both cultivar number of panicle below cutting point was varied from 2 to 5 in trees submitted to tip-pruning time 2, 3, 4, and 5 resulted in a higher percentage of flowering than control and time pruned trees (Fig 4). ,

In both studied cultivars, tip pruning increased the number of panicle/tree significantly but not the number of fruit/tree (Table 1 and 2). As the number of panicles increased in tip-pruned branches (time 2, 3, 4, and 5) the panicle size reduced in comparison to control and times pruning 1 (Fig 3). Slightly differences were observed among treatments for the fruit characteristics such as dry matter (DM%), Brix˚, and weight, however they were not statistically significant. A high correlation (r=0.76) was observed between destructive prediction (traditional method) and Non-destructive prediction (near infrared spectroscopy), as both approaches were used for DM% measuring. Differences in DM% affected by tip-pruning indicating that tip pruning at the right time could be used as an agro-technical tool to advance or delay harvesting time for both cultivars. The heating unit cumulative from flowering time to harvesting recorded in the current study, which varies from 1562 to 2028 in both cultivars (Table 1 and 2), this might explain that heating unit has a pivotal role on mango maturity than timing. The current study shows promising results in the flowering induction and improving a number of panicle without affecting the quality of fruit in both cultivars Honey Gold and B74 by tip pruning, however further study should be carried out to use tip-pruning in commercial scale for better fruit production, harvesting management, and postharvest.

Conclusions

Cool conditions in mango are detected in mature hard green leaves and transmitted to growing shoots where flower development occurs. In this study, we showed that axillary bud development could be triggered by tip-pruning of branches in both mango cultivars Honey Gold and B74 in Katherine region with having a subtropical climate. This experiment also proved that cool weather/chill sums<20 ˚C over 300 hrs (unit) during axillary buds development are needed for flower bud initiation and differentiation, especially in early weeks after tip pruning performed. Therefore, future studies are certainly warranted to use the tip pruning as agro-technical skill in the region. Pruner scissors were used in this study to perform tip pruning, it should be considered that labour in Australia cost somthing around $20 AUD/hr, therefore to use tip pruning in commercial scale also further study needed to develop commercial cutter-bar mounted in front or back of tractor with high precision to be able cut all branches around the canopy. Some commercials mango cultivars in Australia such as Kensington Pride (KP) are very low production. As in this study, we also showed that tip pruning could improve canopy flowering significantly when performed at the right time, thus applying tip pruning on KP cultivar to increase the number of inflorescence and number of fruit/tree as a consequence is recommended to be studied in the region.

Acknowledgments

Authors are thankful to the mango growers both Piñata Farm (Honey Gold) and NT Land Development Farm (B74) in Katherine region for providing accesses to their farms and trees during conducting to this study.

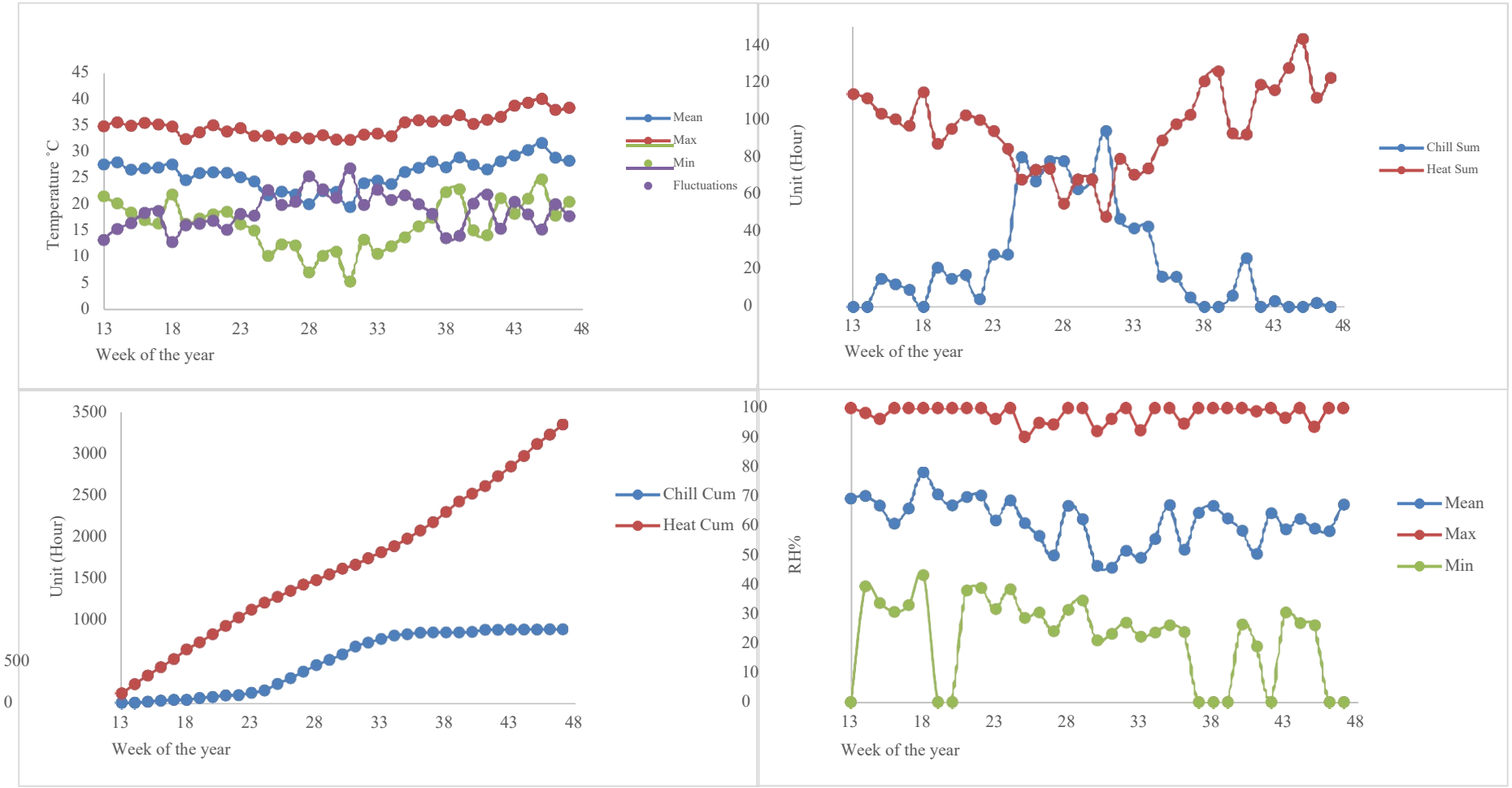

Fig 1. Hourly climacteric data indulging; temperature(mean, max. min), chill and heat sums, chill and heat cumulative, and relative humidity (mean, max, min) for 'Honey Gold' plantation site at Piñata Farm in Katherine region (Fox Road, 14°32'44.2"S 132°28'21.9"E), during week 13 to week 46 of the year.

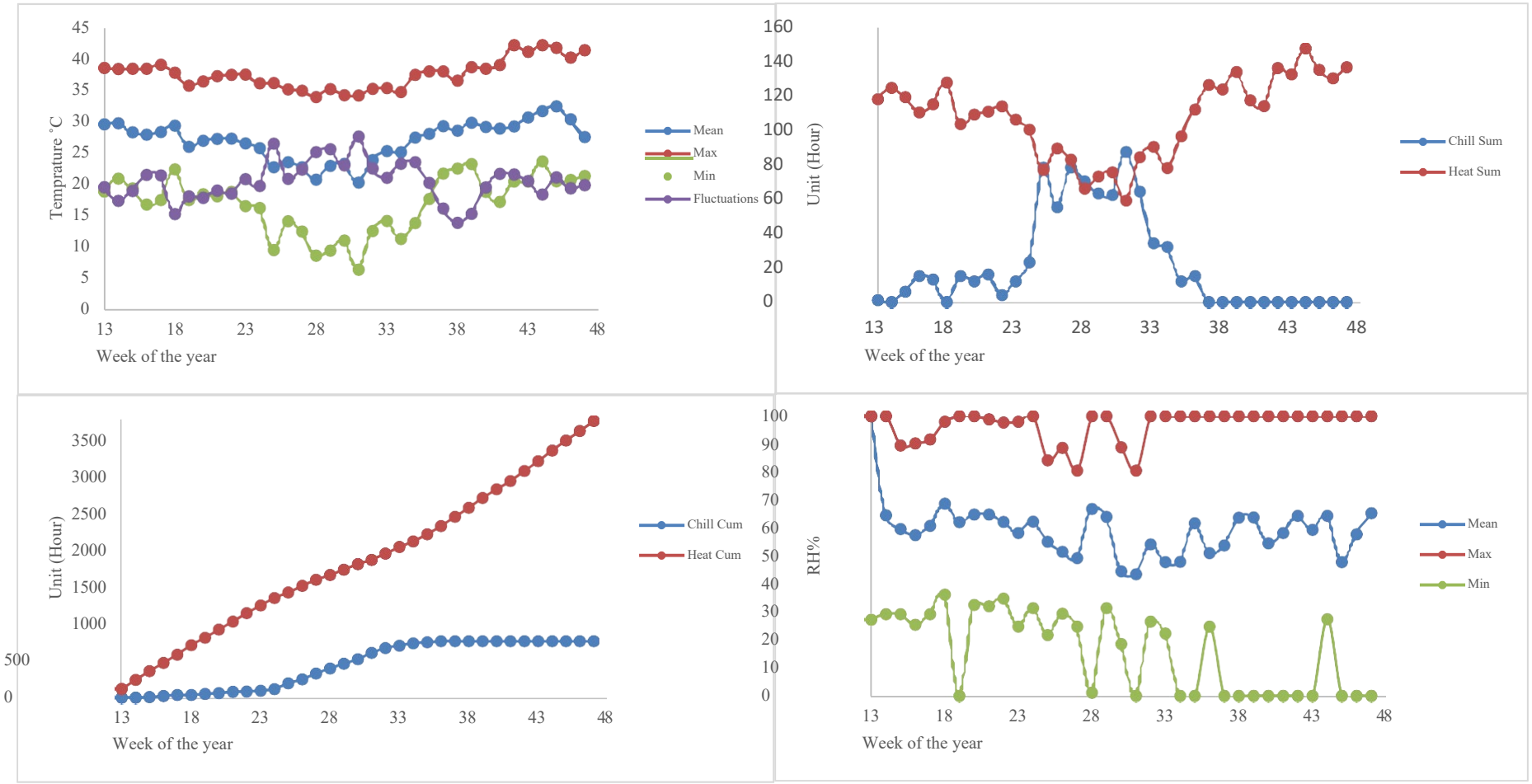

Fig 2. Hourly climacteric data indulging; temperature(mean, max. min), chill and heat sums, chill and heat cumulative, and relative humidity (mean, max, min) for 'B74' plantation site at NT Land Development Farm in Katherine region (Florina Road, 14°32'44.2"S 132°28'21.9"E), during week 13 to week 46 of the year.

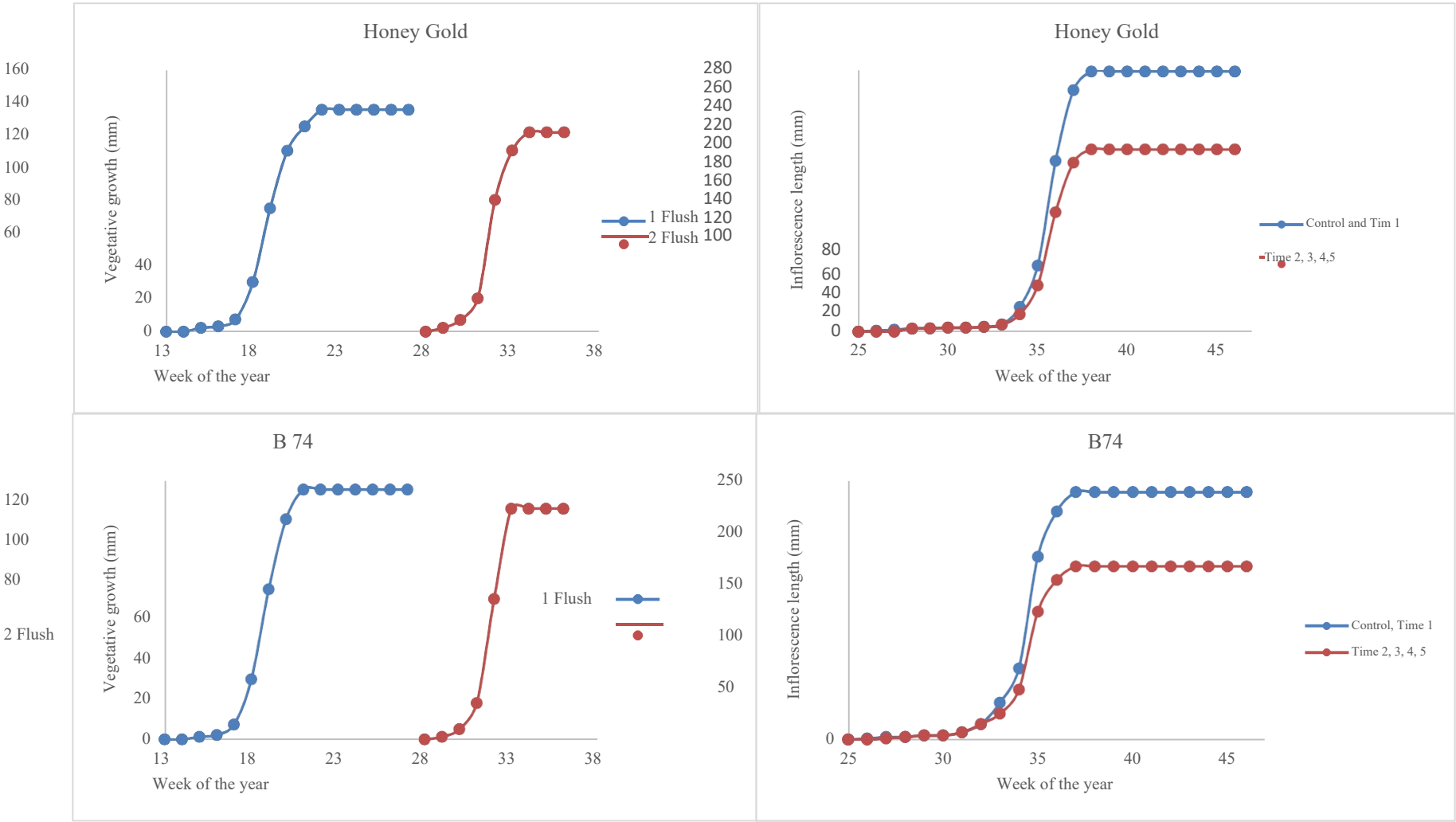

Fig 3. Effect of temperature on the pattern of vegetative growth (pruning times one) and inflorescence elongation (all treatments) for both cultivar mango cultivars during week 13 to week 46 of the year based on climatic data provided in Fig. 1 and 2 for both plantation sites in Katherine region.

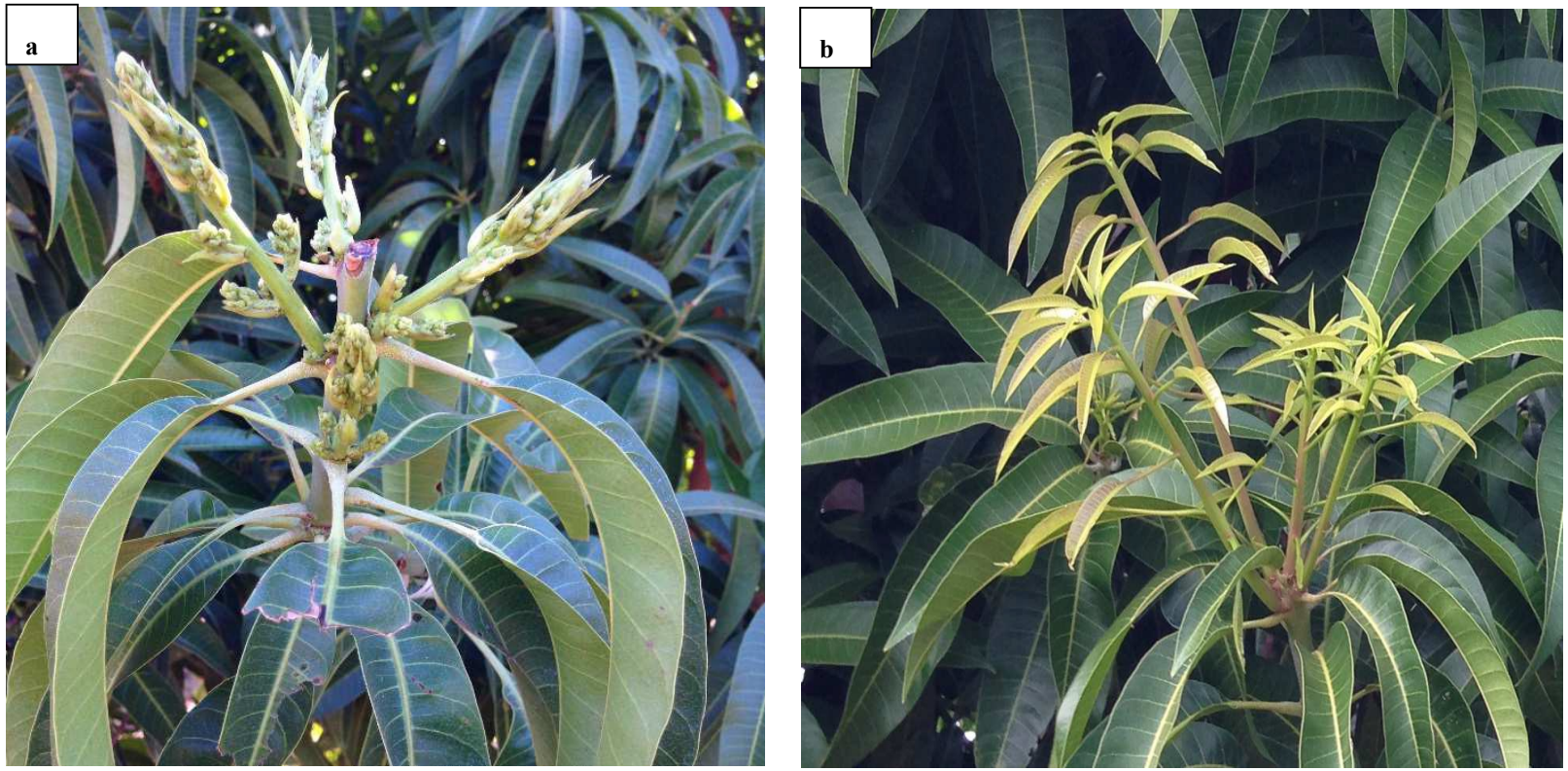

Fig 4. Inflorescence and vegetative growth below cutting point in both studied cultivars (Honey Gold and B74): a) when tip pruning was performed in week 17, 21, 25, 29 of the year, 63% to 100% of shoots in both cultivar have similar growth behaviour producing 2 to 5 inflorescences below cutting point, b) when tip pruning was performed in week 13 and week 33 of the year in both cultivar have similar growth producing 2 to 5 vegetative flushes below cutting point (photo was taken from Honey Gold Cultivar).

Pruning time	Week of the year	Canopy Flowering %	Number of fruit/tree	NIR dry matter	Traditional Dry matter	Fruit weight (g)	Brix°	Flowering time (week of the year)	heat unit Cum	Chill cum<20
Control	-	51±2.2	62±1.45	17.3±0.4	18.2±0.2	479±19.0	7.1±0.2	28	1869	452
Times 1	13	51.2±1.2	61±1.45	17.2±0.4	18.4±0.2	469±19.0	7.2±0.2	28	1869	452
Times 2	17	95±1.5	67.7±1.2	17.3±0.3	18.5±0.30	497±21.0	7.1±0.1	28	1869	425
Times 3	21	86.7±1.7	66.7±0.9	17.1±0.3	18.2±0.3	504.7±20.2	6.7±0.1	29	1799	443
Times 4	25	93.3±1.4	69.3±1.3	17.1±0.4	18.2±0.4	478.2±21.0	6.6±0.1	30	1743	434
Times 5	29	93.3±1.7	67±1.24	17.1±0.4	18.1±0.4	473.2±21.0	6.5±0.1	32	1561	354
Times 6	33	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	122

Table 1. Impact of six different tip-pruning times on vegetative and floral buds, and fruit some characteristics in mango cv. Honey Gold in Katherine region.

Pruning time	Week of the year	Canopy Flowering %	Number of fruit/tree	NIR dry mater	Traditional Dry matter	Fruit weight (g)	Brix	Flowering time (wee of the year)	heat Unit Cum	Chill cum <20
Control	-	57.2±1.2	112±1.5	17.5±0.4	18.2±0.2	399.58±15.2	9.2±0.2	28	2028.8	398
Times 1	13	58.12±1.3	114±1.3	17.5±0.4	18.2±0.2	399.58±15.2	9.2±0.2	28	2028.8	398
Times 2	17	83.23±1.6	113±1.3	17.41±0.2	18.13±0.3	415.23±10.8	9.1±0.2	28	2028.8	376
Times 3	21	90.13±1.7	111±1.2	17.26±0.2	18.10±0.3	421.15±16.3	9.1±0.2	29	1958.8	399
Times 4	25	100±0.0	121±1.7	17.29±0.2	18.13±0.3	395.28±10.4	9.1±0.2	29	1958.8	344
Times 5	29	63.1±1.6	116±1.7	16.20±0.2	16.90±0.3	3440.25±12.2	7.6±0.2	32	1752.0	310
Time	33	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0± 0.0	93

Table 2. Impact of six different tip-pruning times on vegetative and floral buds, and some fruit characteristics in mango cv. B74 (Calypso) in Katherine region.

11.6 Appendix 6 Nursery manual draft contents (See attachment for full version)

Mango Nursery Best Practice Guide

Content

Selecting a site

Surrounding environment

Ideally the nursery should be positioned in an open sunny location and separated (more than 100 metres) from existing orchard trees. Keeping the nursery away from existing orchards reduces the risk of diseases being introduced to the nursery by insects, winds or storms. High traffic areas where dust may regularly occur should also be avoided. If possible the nursery should not be placed in a windy area. Windbreaks may be installed in areas that experience frequent or strong winds to protect young foliage from damage.

(Photo – nursery trees under old mango tree)

Topography and drainage

The nursery should be placed in a well-drained position which is not prone to flooding or water saturation. Encourage drainage and prevent water accumulating on the nursery site by building the nursery on a slight slope (up to 2%) on free draining soil. Land can be levelled to the correct slope by grading. If possible a drainage system should be installed; this might include a gravel layer placed on the ground surface.

Area of land

Sufficient space must be available to construct plant houses, hardening off areas, work areas (for preparing potting mix, potting-up, grafting), storage sheds (chemical storage), water storage and treatment, fencing and pathways, offices, amenities and parking for vehicles.

Vehicle access

The nursery site needs good access for vehicles delivering raw materials and transporting nursery trees to customers. Vehicles should not enter the nursery unless they are decontaminated. Any raw materials arriving at the nursery should be deposited in separate areas (screened off and covered) away from the growing plants.

Water supply

The nursery site should have access to a reliable source of high quality water that is adequate for current and potential future needs. Chemical water treatment may be needed to manage water-borne soil diseases (For more information see Section 3 —Water supply).

Power supply

Ideally the nursery should have a reliable supply of electricity. Electricity interruptions can interfere with operations if automated irrigation scheduling and other powered functions are being used.

Nursery layout/ operation logistics

Access

It is good for the nursery to be fenced and only have one or two controlled access points. This is a good hygiene measure as it prevents visitors and animals wandering into the nursery accidentally and potentially transferring disease (see Section 12). A sterilisation footbath and hand wash facility should be placed at nursery entrances to further prevent any diseases entering the nursery.

Lockable storage

Chemicals and fertilisers should be stored in lockable cabinets and/or sheds to prevent unauthorised access. Similarly valuable tools are best secured over-night or when not in use in a lockable storage area.

Raised work station

It is desirable to have at least a small shaded work station with raised benches for detailed work. This facility will be very useful for careful plant propagation work (e.g. grafting) which requires a steady hand and detail to attention. The work station can be designed to be portable if required (on a wheeled base) to allow movement to different planting blocks.

Waste control

Cuttings, green waste and potting mix spills should be swept up, collected and removed from growing areas to be disposed of in separate bins outside the growing area. Diseased materials should be incinerated or solarised. They should not be let to fall on the ground in the nursery to decay and/or attract insects or spread disease. If at all possible, runoff water from the nursery should also be directed towards a treatment/filtration area (e.g. vegetated filtration wetland) before being allowed to return to waterways.

Potting mix bays

The potting area containing raw materials should kept separate (and covered), away from the growing areas to reduce dust and/or cross contamination. Materials for potting should be sterilised/treated before being brought into the nursery (refer to Section 6). Clean pots should be kept separate in a tidy location nearby to the potting mix bays and only filled with clean, sterilised material.

Growing bays

The areas designated for germinating or growing young plants should ideally be well drained, shaded and screened from dust and wind to avoid contamination and damage (refer to Section 7). Plants should not be placed directly on the soil, which may result in contamination, but rather on protective ground coverings or on raised benches (refer to Section 8).

Figure 1: Overcrowding of trees in growing bays should be avoided as poor air circulation can result in leaf disease

Hardening off area

Some areas with exposure to natural light and rainfall are needed to harden off plants being prepared for sale. Once again the ground should be well drained, plants should not be placed directly on the soil and the plants should be at least 100m away from existing mature plantations to minimise disease contamination. Refer to Section 9 for further details.

Further logistics of water supply, plant houses, nursery floors/benches, plant control/potting mix details are covered in more detail in sections below.

Water supply

Clean Water

Growing nursery trees successfully depends critically on having access to a secure and reliable water supply. All the water used for irrigation, cleaning and cooling should be clean and not contain disease-causing particles. The quality of the water supply can change over time; it may be good quality initially, but can change to borderline quality and then unusable in just a few months. Water may be initially obtained through a number of sources, for example from a channel, river, well, bore, dam or in rainwater storage tanks.

The water supply may need to be filtered or treated for diseases depending on the source. If there is a chance that the irrigation water could be contaminated by disease (either at the source or through storage), it should be treated and held in a separate holding tank before use in the nursery by either:

- Adding chlorine (2 ppm)
- Ultra-violet radiation
- Filtering through sand (e.g. slow flow sand filtration)

Water chemistry

In addition to treatment for disease, water also needs to be checked for appropriate pH and salinity.

Potting mix

Potted nursery trees need a media to grow in. Potting mix is simply a mix of different ingredients that provide the most beneficial environment for trees to develop in. An ideal potting mix has a balance of ingredients that creates good aeration, water-holding capacity, drainage, nutrients and pH level for root development. Potting mixes should be easy to wet, but not prone to waterlogging.

Ingredients

There are many different ingredients that can be combined to make a potting mix. These could include local materials (compost, soil, sand, manure, rice hulls) and/or outsourced materials (e.g. coconut fibre, vermiculite, perlite and peat moss) but it must be ensured that all materials are clean and disease free and mixed in appropriate ratios. An example of a potting mixture recipe suitable for mangoes is (mix the ingredients in these proportions by volume) is: Cow manure + burnt rice husk + soil (1:2:1).

Figure?: Poorly aerated potting mix can restrict root growth. Note multiple shoots from polyembryonic seed

Characteristics

Ideally an air-filled porosity of 10 -20% and water holding capacity of 40-50% is to be aimed for. Methods to calculate air filled porosity and water holding capacity of a given potting mix are given in appendices A and B. Avoid materials that reduce air-filled porosity, including mud, fine silts and heavy clays or sawdust which may compact as it decomposes. A slightly acidic to neutral pH is ideal (5.5 to 7.0) and materials must be low in salts. It is better if possible for the potting mix to be light weight so it is easy to move around and work with.

Figure ? : Testing the pH of the potting mix using a simple colourmetric test

Pasteurisation

Potting media may be a source of disease organisms, so it is important to reduce the potential for harm by pasteurising the potting mix before use. Pasteurisation can be achieved with steam or with solar radiation (solarisation). To pasteurise with steam, thoroughly mix and moisten all ingredients for 4 hours. Introduce the steam from the bottom of the potting mix and treat for 30 minutes after the surface temperature has reached 60°C. To pasteurise by solarisation, 25cm high mounds of moist potting mix are wrapped in two layers of clear (not black), thin, plastic sheeting and left in the sun during periods of high temperature for longer periods of time (5-15 days). The generated head effectively kills many annual and perennial weed seeds.

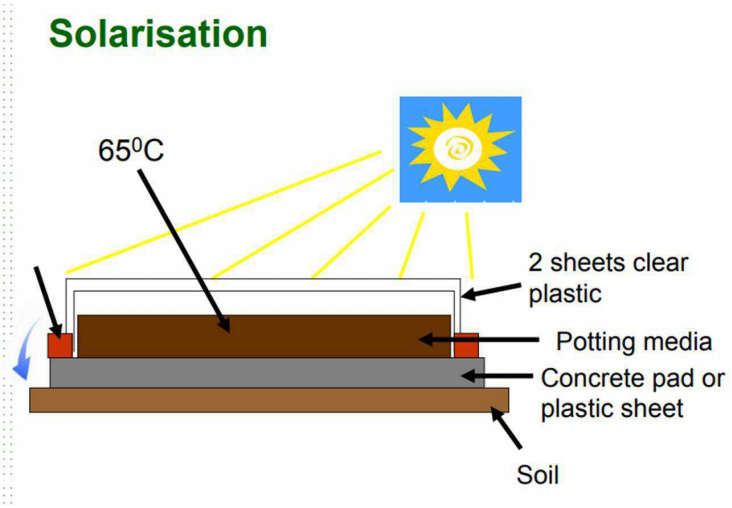

Image 4. Solarisation is an easy method using the power of the sun to kill diseases in potting mix before use.

11.7 Appendix 7: Sample pages from the pest and disease guide (under development)

Mango leafhopper

Hosts:
Mango

Description:
Immatures: Nymphs are pear-shaped, dull grey in colour with pale yellow and black legs and are covered with a light dusting of wax.

Eggs: Cigar-shaped, creamy-yellow in colour. Size: 0.9–1 mm in length.

Immatures: Nymphs greenish with black or brown markings, resemble small adults but without wings.

Adults: Usually golden-brown or dark brown in colour, wedge-shaped similar to a very small cicada. Size: 4–5 mm in length.

Life cycle and biology:

Damage:
Adults and nymphs feed on vegetative flush tissue by sucking the sap. Feeding and egg laying cause curling and distortion of new flush and young leaves. When populations are high, leaves and flowers may have a sticky wet appearance which is from the copious amount of honeydew the leafhoppers produce. Presence of sooty mould which grows on honeydew may also be an indication of leafhopper infestation.

Natural enemies:
There are various natural enemies including predatory ladybirds, lacewing larvae, hover fly larvae, parasitic wasps, big-eyed bugs and minute pirate bugs. Ants tending the colony may protect the aphids from predators.

Control

Monitoring

Flatid

Hosts:
Mango

Description:
Immatures:

Eggs:

Immatures:

Adults:

Life cycle and biology:

Damage:

Natural enemies:

Control

Monitoring

Red banded mango caterpillar

Hosts:
Mango

Description:
Immatures:

Egg:

Immatures:

Adults:

Life cycle and biology:

Damage:

Natural enemies:

Control

Monitoring

Longicorn

Hosts:
Mango

Description:
Immatures:

Egg:

Immatures:

Adults:

Life cycle and biology:

Damage:

Natural enemies:

Control

Monitoring

Gall midge

Hosts:
Mango

Description:
Immatures:

Eggs:

Immatures:

Adults:

Life cycle and biology:

Damage:

Natural enemies:

Control

Monitoring

11.8 Appendix 8 Mango Matters Articles Spring 2015 & July 2016

Industry Biosecurity

Being prepared is critical in minimising the impact of any exotic pest or disease incursion. Our industry has been fortunate to have our leading researchers working in countries which some of these exotics pests and diseases call home. Through funding from ACIAR, QDAF entomologist Ian Newton and NSW DPI project leader Mark Hickey have been conducting research on Cecid flies (Gall midges) in the Philippines and Cambodia. This research will ensure we are better prepared in the event of an incursion.

RESEARCHERS LOOKING TO ASIA TO PREPARE FOR POSSIBLE CECID FLY INCURSION

Cecid flies, also known as Gall Midges, are considered a high risk pest to the Australian mango industry. The damaging fly species attacks the leaves of mango trees, impacting flowering, fruit set and fruit quality. A number of Cecid fly species are found across South East Asia. In the past, one species has been detected in the Torres Strait and the northern tip of Cape York.

Researchers have been working in the Philippines and Cambodia where the Cecid fly is widespread, to develop management practices to limit the risk to the Australian mango industry. Dr Ian Newton is Project Leader of the Filipino project and said that it makes sense to study the pest in countries where it is already established.

"Cecid fly is a high priority emergency pest for the Australian mango industry. We want to learn as much about this pest as we can so we know the best way to manage it if it ever makes its way to the mango production regions of Australia. This includes looking at existing biological controls in the Philippines and developing management practices so we will be well equipped to manage an incursion," he said.

"Although the project is in the early stages, the results have already uncovered some interesting findings. The species of Cecid fly that was found in the Torres Strait appears to be the same species that is found in the Philippines. We have identified some of the pest's natural enemies and documented the population dynamics of the species that show patterns of annual occurrence. This information will help time insecticide applications and the design of integrated pest management programs," Dr Newton said.

The Cecid fly is responsible for substantially decreased mango yields in many areas of the Philippines. Cecid flies lay their eggs on young mango leaves, stems, flowers or fruit, causing mangoes to fall from the tree. Fruit that remains have brown scab-like spots, affecting their quality and return price. Wart-like galls form quickly on leaves, which can curl up and drop off prematurely, causing dieback of whole branches.

Cambodia is an ideal location to study Cecid fly, although the species found in this country are not as damaging as those found in the Philippines as they do not effect mango fruit. Leader of the Cambodian based project, Mark Hickey, said that the more that is known about all species of Cecid fly, the more robust management strategies will be.

"Researchers have a solid understanding of the species of Cecid fly found in Cambodia following extensive surveys of mango production regions. The work our team undertakes will add to the body of knowledge about Cecid fly and increase the ability to protect Australian mangoes from this pest. By understanding of the damage Cecid fly causes we can develop strategies to manage them," he said.

Current management tools include pruning after harvest to reduce infestations because Cecid flies are sensitive to sunlight. Cecid flies often reside on vegetation near mango orchards and clearing or spraying surrounding areas can reduce populations. Infested leaves and fallen fruits should be collected, burned, or sprayed with insecticide to prevent the spread.

Future research will look at the ecology of the Cecid fly to gain a greater understanding of the pests, their interactions with their environment and the most effective management and control techniques.

These research projects are funded by the Australian Centre for International Agricultural Research (ACIAR) and managed by the Department of Agriculture and Fisheries Queensland (QDAF) and the New South Wales Department of Primary Industries. ACIAR fund international and domestic research to improve the productivity and profitability of agricultural systems in partner countries as well as providing benefits to Australian industries. Research and development of integrated crop management for mango production in the southern Philippines and Australia is managed by Dr Ian Newton from the Department of Agriculture and Fisheries, Queensland (QDAF). Building a resilient mango industry in Cambodia and Australia through improved production and supply chain practices is managed by Mark Hickey from the NSW Department of Primary Industries.

EXAMPLES OF GALLS ON LEAVES, CAUSED BY MANGO LEAF-GALL MIDGE
(supplied by Plant Health Australia - www.planthealthaustralia.com.au)

Source:
Cambodia 2005, Brian Thistleton

Source:
Vietnam 2005, Brian Thistleton

Source:
SPS Capacity Building Workshop Thailand 2005

Extending the harvest window

Advancing or delaying mango flowering has the potential to improve the efficiencies and profitability of the Australian mango industry. Spreading mango production will create a more regulated supply of fruit from the start until the end of the season. This control will assist in managing the overlap between production regions and expand the duration of cropping, ideal for growing export demand.

A deeper understanding of the mechanisms within a mango tree that control flowering and fruiting is being studied by two complementary projects. Through the knowledge of what stimulates the genes associated with flowering, more targeted practices to effectively manipulate mango crops will be developed. The information will be used to develop tools and methodologies to monitor and manipulate flowering to maximise profit.

The process of mango gene expression is thought to be a key factor in expanding the harvest window. Researchers will identify treatments, such as growth regulators, that inhibit or stimulate the activity of these genes and understand more about their role in flower initiation and subsequent effects on harvest timing. To enable screening of chemical uptake and precise monitoring of tree responses, an 'Aeroponics'* system to monitor root and vegetative growth has been developed. This enables rapid screening of existing and new cultivars without the need to plant and wait for the new orchards to develop.

Project Leader Mark Hickey said that he estimates this technology could extend the harvest window by three to five weeks on individual properties.

"Improved practices will give mango growers more tools to know if and when their trees will flower and whether treatments to induce mango flowering were successful before flowers appear. Being able to shift the time that flowering occurs will expand export opportunities. It will also increases the return on investment for equipment that can be utilised for a longer period of time and will reduce reluctance by producers to invest in new technology which potentially is only utilised for a few weeks of the year," he said.

The success of these technologies relies on their effectiveness under commercial orchard conditions and integration with existing on-farm practices. The potential benefits of crop manipulation for the Australian industry are considerable. A recently conducted benefit/cost analysis demonstrated that these projects would yield a return for the Northern Territory industry of up to $2 million per season from 2019/20 onwards. This additional value is due to an extended harvest window and reduction in losses due to over concentration of the harvest period.

**editors note: Aeroponics is the process of growing plants in an air or mist environment without the use of soil or an aggregate medium*

"The key to managing flowering is to understand when buds start developing. In the early stages of development, old mature leaves detect and are responsive to temperature and inductive treatments. Early season flowering in the Darwin region can be triggered by evening temperatures below 20°C. These conditions are produced by high pressure systems travelling easterly across Australia from the Western Australian coast. Growers can promote bud growth in anticipation of these conditions by applying timed sprays of potassium nitrate," Mr Hickey said.

"Promoting early flowering and cropping is practiced by some growers in Australia to access higher priced market windows. A number of methods are used including chemical treatments, selective pruning, restricting irrigation and organic amendments. The success of these programs has been limited by a misunderstanding of the role weather conditions has on the process and an incomplete suite of tools to redress the situation if orchards become out of synchrony with environmental conditions.

The first stage of the research has involved optimising chemicals used worldwide that can potentially control flush growth and initiate and regulate flowering. Ethephon was thought to mature vegetative flush but Australian research has shown that it can reset flushing patterns and remove insensitive immature leaves. This treatment increases the likelihood of trees being in a prime condition to receive the triggers to flower. Similarly potassium nitrate has been widely used to give flowering more uniformity in Australia and work in the NT has shown that it can induce flowering in Kensington Pride trees in the absence of inductive temperatures. A better understanding of the long term impacts and environmental effects of these chemicals on mango trees and pesticide residues need to be understood and will be a component of this research.

"We have been investigating flower manipulation techniques used by Cambodian mango farmers to induce out of season flowering and double crop their mango trees. While double cropping may not be feasible in Australia, the techniques used to manipulate flowering and long term impacts on tree health are of interest to our team," Mr Hickey said.

The project Building a resilient mango industry in Cambodia and Australia through improved production and supply chain practices is funded by the Australian Centre for International Agricultural Research and managed by the New South Wales Department of Primary Industries. ACIAR fund international and domestic research to improve the productivity and profitability of agricultural systems in partner countries as well as providing benefits to Australian industries. The projects is supported by project Manipulating mango flowering to extend harvest window which is co-funded by the Northern Territory Department of Primary Industries and Fisheries and Horticulture Innovation Australia.

11.9 Appendix 9

Investigating the molecular mechanisms behind flowering in *Mangifera indica*

PhD Progress Report

Stacey Cook

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

ACIAR
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1 Background

Research into the mechanisms behind flowering began more than a hundred years ago. With the earliest work revealing that photoperiod had a role in flowering (Tournois, 1914, Klebs, 1918, Garner and Allard, 1920). This was furthered by the finding that day length is perceived in the leaves even though flowering occurred in the shoot apical meristem (Knott, 1934). This led to the theory of a mobile floral stimulus termed 'florigen' (Chailakhyan, 1936). Years later, temperature is shown to also play a role in flowering (Chouard, 1960). Decades after the development of the theory of 'florigen', molecular biology and genetics reveals the FT gene to be the precursor of the 'florigen' signal (Kardailsky et al., 1999, Kobayashi et al., 1999). Mutation studies in *Arabidopsis* was able to lead to the discovery of the autonomous flowering pathway (Amasino, 2010). This early flowering work was complemented by advances in genetics that saw the sequencing and annotation of the *Arabidopsis* genome. This led to the discovery of over 150 genes involved in flowering in *Arabidopsis*, this incudes genes such as FT, FLC, CO, LFY and AP1.

All early flowering work was mainly conducted in herbaceous annual/biennial (a/b) plants. It has been shown that perennial trees differ greatly in how flowering is regulated (Bangerth, 2009, Wilkie et al., 2008). Research into flowering mechanisms in perennial trees is lagging behind the same research into a/b species, this could be due to the complexity and time requirements of studying trees(Wilkie et al., 2008, Bangerth, 2009). Several homologs of *Arabidopsis* flowering genes have been found in a number of tree species and have been shown to function in similar ways (Tränkner et al., 2010, Zhang et al., 2014, Endo et al., 2005).

In mango dormant buds must be initiated before floral induction can occur. Initiation can be the result of a number of factors; including, irrigation after periods of drought, pruning and application of nitrogen fertiliser. Floral induction in mango is caused by low temperatures (<20°C), the low temperatures are perceived by the mature leaves and a signal is sent via the phloem to the bud. If buds are in the early stages of initiated and inductive conditions are present, floral shoots will occur (Davenport, 2009). Some molecular work has been done in mango to identify flowering genes and so far, FT, CO, LFY and AP1 homologs have been identified (Nakagawa et al., 2012, Hu et al., 2003, Davenport et al., 2006a, Luo et al., 2009, de los Santos-Villalobos et al., 2012). At present the MiFT genes is the strongest candidate for the genetic precursor of the mango FP, it's expression increases in response to cooling, the increased expression occurs in mature green leaves and the signal appears to be transported to the buds to stimulate expression of MAP1-1 (Nakagawa et al., 2012).

Despite the recent advances in the research into mango flowering there is still much more to be done. Only a small handful of homologs of a vast number of *Arabidopsis* flowering genes have been identified, and only in a few cultivars of no significance to the Australian market.

2 Aim of this research

The aim of this work is to further the research of flowering in trees, specifically, mango. Next generation sequencing can be used to identify candidate genes that may be involved in mango floral regulation. Real-time PCR will be used to gain an understanding of the expression patterns of these genes during exposure to different conditions. This information can be used in conjunction with phenotypic observations to design further studies and determine genes that play a significant role in floral induction in Mango and possibly propose a new model of floral regulation in mango.

3 Identification of flowering gene homologs in Mango transcriptome

3.1 Aim

To identify flowering gene homologs in Mango and study the differences and similarities between genes in Mango and other species

3.2 Methods

3.2.1 Bioinformatic analysis

A cDNA library was assembled using RNA extracted from a variety of tissues sampled from mango cultivar NMBP1243. This library was sequenced using Illumina next generation sequencing. The reads from the sequencing were assembled into a transcriptome.

Amino acid sequences for a number of flowering related genes (Table 3-1) were obtained from the TAIR (The *Arabidopsis* Information Resource www.Arabidopsis.org) database. Two databases were created using the BLAST+ suite of command line tools (Camacho et al., 2009). The mango transcriptome database was searched with the database of *Arabidopsis* amino acid sequences.

The resulting hits were uploaded to the Galaxy web platform, and were analysed using the public server at usegalaxy.org (Afgan et al., 2016).

Table 3-1 Arabidopsis Flowering Genes

Pathway	Gene Number	Gene Name	Phenotypic Information
Photoperiod	AT5G13790	AGL15 (AGAMOUS-LIKE 15)	*agl15 agl18 svp flc quadruple mutants are early flowering in LDs and SDs*
Gibberellin	AT2G22630	AGL17 (AGAMOUS-LIKE 17)	mutant late flowering in LDs
Photoperiod	AT3G57390	AGL18 (AGAMOUS-LIKE 18)	*agl15 agl18 svp flc quadruple mutants are early flowering in LDs and SDs*
Photoperiod	AT4G22950	AGL19 (AGAMOUS-LIKE 19)	
Photoperiod	AT4G24540	AGL24 (AGAMOUS-LIKE 24)	mutant late flowering in LDs and SDs
Photoperiod	AT1G69120	AP1 (APETALA1)	Abnormal flower development
Gibberellin	AT2G46830	CCA1 (CIRCADIAN CLOCK ASSOCIATED 1)	mutant early flowering in short days, lhy cca1 double mutant not responsive to photoperiod
Gibberellin	AT5G37260	CIR1 (CIRCADIAN 1)	mutant slightly early flowering, overexpressor late flowering
Meristem	AT5G15840	CO (CONSTANS)	mutant late flowering in LDs
Photoperiod	AT5G57660	COL5 (CONSTANS LIKE 5)	Overexpression promotes flowering in SDs
Vernalisation	AT4G08920	CRY1 (CRYPTOCHROME 1)	mutant early flowering in SD
Photoperiod	AT1G04400	CRY2 (CRYPTOCHROME 2)	mutant late flowering in LDs
Vernalisation	AT2G25930	ELF3 (EARLY FLOWERING 3)	mutant early flowering in SD, insensitive to photoperiod
Gibberellin	AT2G40080	ELF4 (EARLY FLOWERING 4)	mutant early flowering
Gibberellin	AT4G16280	FCA (FLOWERING TIME CONTROL PROTEIN ALPHA)	mutant late flowering
Gibberellin	AT4G35900	FD (FLOWERING LOCUS D)	mutant late flowering in LDs and SDs
Vernalisation	AT2G33835	FES (FRIGIDA ESSENTIAL 1)	mutant early flowering in winter annual accessions
Photoperiod	AT5G10140	FLC (FLOWERING LOCUS C) also called FLF (FLOWERING LOCUS F) and AGL25 (AGAMOUS LIKE 25)	mutant early flowering
Meristem	AT3G10390	FLD (FLOWERING LOCUS D)	mutant late flowering
Vernalisation	AT3G04610	FLK (FLOWERING LATE KH MOTIF)	mutant late flowering
	AT2G43410	FPA	mutant late flowering
	AT5G24860	FPF1 (FLOWERING PROMOTING FACTOR 1)	

Meristem	AT4G00650	FRI (FRIGIDA)	mutant early flowering in winter annual accessions
Photoperiod	AT5G16320	FRL1 (FRIGIDA LIKE 1)	
Vernalisation	AT1G65480	FT (FLOWERING LOCUS T)	mutant late flowering in LDs
Photoperiod	AT2G19520	FVE	mutant late flowering
Photoperiod	AT5G13480	FY	mutant late flowering
Meristem	AT1G22770	GI (GIGANTEA)	mutant late flowering in LDs
Gibberellin	AT5G61850	LFY (LEAFY)	Abnormal flower development
Vernalisation	AT1G01060	LHY (LATE ELONGATED HYPOCOTYL)	mutant early flowering in short days, lhy cca1 double mutant not responsive to photoperiod
Photoperiod	AT5G64813	LIP1 (LIGHT INSENSITIVE PERIOD 1)	
Vernalisation	AT3G26640	LWD2 (LIGHT-REGULATED WD 2)	*lwd1 lwd2 mutant early flowering in LD and SD*
Photoperiod	AT1G77080	MAF1 (MADS AFFECTING FLOWERING 1) also called FLM (FLOWERING LOCUS M) and AGL27 (AGAMOUS LIKE 27)	mutations cause early flowering, especially under short days
Gibberellin	AT1G09570	PHYA (PHYTOCHROME A)	mutant late flowering in LD, overexpressor early flowering in SD and LD
Vernalisation	AT2G18790	PHYB (PHYTOCHROME B)	mutant early flowering in LDs and SDs
Vernalisation	AT5G35840	PHYC (PHYTOCHROME C)	mutant early flowering in SDs
Meristem	AT4G16250	PHYD (PHYTOCHROME D)	*phyB phyD double mutant earlier flowering than phyB single*
Meristem	AT4G18130	PHYE (PHYTOCHROME E)	*phyB phyE double mutant earlier flowering than phyB single*
Vernalisation	AT3G12810	PIE1 (PHOTOPERIOD INDEPENDENT EARLY FLOWERING 1)	mutant early flowering
Meristem	AT5G61380	PRR1/TOC1	mutant early flowering in LD and DD
Photoperiod	AT2G45660	SOC1 (SUPPRESSOR OF OVEREXPRESSION OF CONSTANS)	mutant late flowering in LDs and SDs
Meristem	AT2G33810	SPL3 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3)	Overexpression promotes flowering
Photoperiod	AT2G22540	SVP (SHORT VEGETATIVE PHASE)	mutant early flowering in LDs and SDs
Vernalisation	AT5G03840	TFL1 (TERMINAL FLOWER 1)	mutant early flowering in LDs and SDs
	AT5G17690	TFL2 (TERMINAL FLOWER 2) also called LHP1 (LIKE HETEROCHROMATIN 1)	mutant early flowering in LDs and SDs

Photoperiod	AT4G20370	TSF (TWIN SISTER OF FT)	*ft tsf double mutants are late flowering in LDs*
Meristem	AT3G24440	VIL1 (VERNALIZATION INSENSTIVE 3 LIKE 1) also called VRN5 (VERNALIZATION 5)	
Vernalisation	AT5G57380	VIN3 (VERNALIZATION INSENSITIVE 3)	Mutants are insensitive to vernalisation.
Photoperiod	AT4G29830	VIP3 (VERNALIZATION INDEPENDENCE 3)	mutant early flowering
Meristem	AT5G61150	VIP4 (VERNALIZATION INDEPENDENCE 4)	mutant early flowering
Photoperiod	AT1G61040	VIP5 (VERNALIZATION INDEPENDENCE 5)	mutant early flowering
Photoperiod	AT2G06210	VIP6 (VERNALIZATION INDEPENDENCE 6) also called EARLY FLOWERING 8 (ELF8)	mutant early flowering
Vernalisation	AT3G18990	VRN1 (VERNALIZATION 1)	
Vernalisation	AT4G16845	VRN2 (VERNALIZATION 2)	

3.2.2 Primer design

The mango transcriptome sequences that had the highest similarity to the *Arabidopsis* amino acid sequences were chosen for primer design. For each mango transcriptome sequence, primer pairs were designed using the online tool Primer3 (Untergasser et al., 2012, Koressaar and Remm, 2007).

3.2.3 Phylogenetic Analysis

Some of the sequences used for primer design were also used for basic phylogenetic analysis. The program Geneious version R8 (http://www.geneious.com, Kearse et al., 2012) was used to generate phylogenetic trees of the translated transcriptome sequences and the *Arabidopsis* amino acid sequences,

3.3 Results and Outcomes

Table 3-2 and Figure 3-1 show that there is genetic similarity between *Arabidopsis* and mango. The mango sequences that have aligned to *Arabidopsis* flowering genes could potentially be functional in mango. This bioinformatics analysis does not show functionality of these genes in mango, only that similarities exist between mango and *Arabidopsis*, further work is needed to show functionality and activity in the regulation of flowering. This data was used to design primers for use in further work, see Table 3-3.

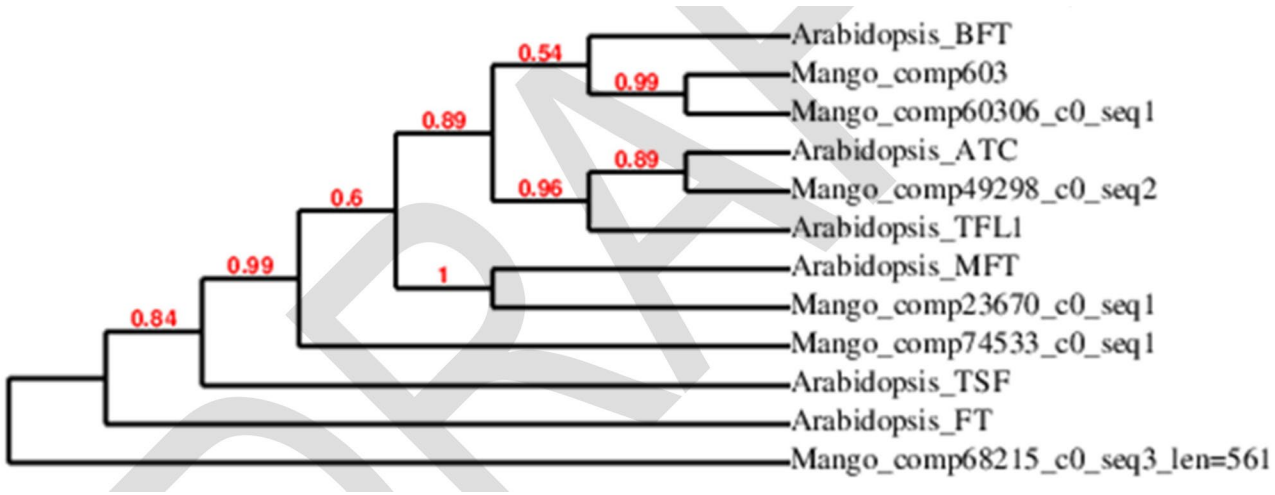

Figure 3-1Phylogenetic Tree of Mango and Arabidopsis Nucleotide sequences

Table 3-2 BLAST Results with lowest E-Values

Mango Sequence	*Arabidopsis* Sequence	% Ident.	E-Value	Mango Sequence	*Arabidopsis* Sequence	% Ident.	E-Value
comp68740_c0_seq4	AT5G64813||LIP1	78.655	4.9E-150	comp44672_c0_seq1	AT3G12810||PIE1	73.597	1.8E-121
comp70152_c0_seq2	AT5G61850||LFY	62.533	1.4E-138	comp62491_c0_seq2	AT3G10390||FLD	74.349	2.5E-140
comp73092_c0_seq1	AT5G61380||TOC1	82.222	6.5E-123	comp70322_c0_seq1	AT3G04610||FLK	73.798	1.7E-177
comp53674_c0_seq1	AT5G57660||COL5	53.209	3.9E-113	comp71305_c0_seq1	AT2G46830||CCA1	59.434	8.62E-55
comp65056_c0_seq2	AT5G57380||VIN3	49.673	1.3E-38	comp64891_c0_seq1	AT2G45660||SOC1	70.476	2.29E-78
comp72490_c0_seq2	AT5G37260||CIR1	59.394	2.7E-47	comp60652_c0_seq2	AT2G43410||FPA	40.426	3.14E-68
comp72568_c0_seq1	AT5G35840||PHYC	63.876	0	comp63001_c0_seq3	AT2G40080||ELF4	65.823	9.13E-33
comp23391_c0_seq1	AT5G24860||FPF1	59.259	5.53E-40	comp71960_c0_seq3	AT2G33835||FES1	38.489	1.88E-42
comp66538_c0_seq3	AT5G17690||TFL2	46.049	5.84E-70	comp59648_c0_seq2	AT2G33810||SPL3	70	2E-41
comp71865_c0_seq3	AT5G16320||FRL1	35.581	8.92E-59	comp73610_c0_seq7	AT2G25930||ELF3	43.327	2.22E-82
comp61209_c0_seq1	AT5G15840||CO	51.026	5E-83	comp57706_c0_seq2	AT2G22540||SVP	65.69	3.4E-99
comp65812_c0_seq4	AT5G13790||AGL15	56.746	2.98E-87	comp37047_c0_seq2	AT2G19520||FVE	77.586	0
comp60936_c0_seq1	AT5G13480||FY	86.667	1.7E-167	comp73619_c1_seq2	AT2G18790||PHYB	76.652	0
comp74950_c0_seq1	AT5G10140||FLC	40.789	9.82E-33	comp67891_c0_seq2	AT2G06210||VIP6	72.703	0
comp49298_c0_seq2	AT5G03840||TFL1	72.289	2.65E-88	comp19742_c0_seq2	AT1G77080||MAF1	46.386	1.61E-39
comp56384_c0_seq2	AT4G35900||FD	45.299	3.19E-29	comp49870_c0_seq2	AT1G69120||AP1	62.992	1.8E-106
comp43752_c0_seq2	AT4G29830||VIP3	83.178	0	comp63232_c0_seq2	AT1G68800||BRC2	72.5	4.29E-32
comp60306_c0_seq2	AT4G20370||TSF	58.788	2.2E-64	comp74533_c0_seq1	AT1G65480||FT	68	1.59E-76

comp73269_c0_seq8	AT4G18130||PHYE	64.625	0	comp67538_c0_seq5	AT1G61040||VIP5	62.887	2.1E-153
comp69141_c0_seq8	AT4G16845||VRN2	54.415	9.3E-129	comp59833_c1_seq1	AT1G22770||GI	77.053	0
comp63111_c0_seq2	AT4G16280||FCA	61.963	1.18E-68	comp131820_c0_seq1	AT1G09570||PHYA	91.429	4.6E-114
comp152319_c0_seq1	AT4G16250||PHYD	88.172	8.69E-54	comp51987_c0_seq1	AT1G04400||CRY2	69.464	0
comp69071_c0_seq1	AT4G08920||CRY1	82.857	1.11E-23	comp71305_c0_seq2	AT1G01060||LHY	45.063	9.2E-123
comp64619_c1_seq2	AT4G00650||FRI	43.116	1.99E-62	comp68114_c0_seq3	AT3G24440||VIL1	46.018	3.26E-77
comp18356_c0_seq1	AT3G57390||AGL18	48.12	4.26E-64	comp71613_c0_seq12	AT3G18990||VRN1	62.464	5.3E-130
comp63541_c0_seq2	AT3G26640||LWD2	85.838	0	comp60015_c0_seq1	AT3G18550||BRC1	32.051	2.65E-24

Table 3-3 Primer Pairs Generated by Primer3

Gene	Sequence From Transcriptome	Forward primer	Reverse primer
AGL15	comp65812_c0_seq4_AT5G13790||AGL15_2.98e-087	ATGAGGGGATGCTACTGGTG	CTCCTCAACCTGTCTGCGTA
AGL18	comp18356_c0_seq1_AT3G57390||AGL18_4.26e-064	TGCTCCACCAAAACTTGCTC	CCCTGTCAAGAGCAGTCAGA
AP1	comp49870_c0_seq2_AT1G69120||AP1_1.77e-106	CAAAACCAGGGCCCCAATAC	CAAGCCTGTTACGCCTCATC
BRC1	comp60015_c0_seq1_AT3G18550||BRC1_2.65e-024	AAGTGGCATCCGAAACACAC	TTGTCCTCTCCCTTGCCTTT
BRC2	comp63232_c0_seq2_AT1G68800||BRC2_4.29e-032	CTGTCCCTTCAAATCGCTCG	CAGTGAGTTCCTGGATTGCG
CCA1	comp71305_c0_seq1_AT2G46830||CCA1_8.62e-055_193	TCCAGGGCGAGAAAACAGAT	AGTACCAGTTCCAAGGCTCC
CIR1	comp72490_c0_seq2_AT5G37260||CIR1_2.70e-047_162	TCAGCTGCTTCTTCCCTGAA	GCCAGTCCAAGATCCTTCCT
CO	comp61209_c0_seq1_AT5G15840||CO_5.00e-083_257	GTGTTCCGATTCTGGCCATC	TCATCTTCATCTCCCTCCGC
COL5	comp53674_c0_seq1_AT5G57660||COL5_3.93e-113_336	GACATTGAATTCCCGTCCGG	TCTGCCGATCCAAACTCACT

CRY1	comp69071_c0_seq1_AT4G08920||CRY1_0.0_892	ACCCAGCTCTTCTTCAACCA	GCATTAAACGAACGCACAGC
CRY2	comp51987_c0_seq1_AT1G04400||CRY2_0.0_825	CTGGGGTTGAGCTGGGATTA	GCAGCTTCCATTTCCCACAT
ELF3	comp73610_c0_seq7_AT2G25930||ELF3_2.22e-082_270	TGCAACAAAATGACCGTGGA	TGCCATGCTCTTCCCTTGTA
ELF4	comp63001_c0_seq3_AT2G40080||ELF4_9.13e-033_113	TCACCAGTCAAAGATTCCCGA	CACCATCAGCGCTCTTGTTA
FCA	comp63111_c0_seq2_AT4G16280||FCA_1.18e-068_224	GCAGGCTGCGATTAAAGTCA	CAACATGCCCAAACGAGGAA
FD	comp56384_c0_seq2_AT4G35900||FD_3.19e-029_109	CTTGCCACCATGTTGAGCTT	TATGGAGGGAACACAGTCGG
FES	comp71960_c0_seq3_AT2G33835||FES1_1.88e-042_153	CCCTTCTTCCCAGCGATTTG	CTCAGGAAGCAAGCTGTTCC
FLC	comp74950_c0_seq1_AT5G10140||FLC_9.82e-033_115	AAATGGGCCGAAAGAAGGTG	GCGGCTGGAAAAGATGATGA
FLD	comp62491_c0_seq2_AT3G10390||FLD_2.52e-140_417	GGTGGCGATCCTTTTAGCTT	AAGAAGAGTCGTCCATCCCC
FLK	comp70322_c0_seq1_AT3G04610||FLK_1.66e-177_514	GGTAGCATAATTGGCCGCAA	CTATGCGCTTGTGAACCCTC
FPA	comp60652_c0_seq2_AT2G43410||FPA_3.14e-068_230	GGCTCATCAAAGTGCACACA	ATGCACCACTCCATAGCCTT
FPF1	comp23391_c0_seq1_AT5G24860||FPF1_5.53e-040_129	CAAAGGTGGGGTTGTTCGTT	ATAGTACCTCTCCCAGCCCA
FRI	comp64619_c1_seq2_AT4G00650||FRI_1.99e-062_206	CATGATTTCGTCTCGCCAGG	TCCTTCTCGGCCTCTTGTTT
FRL1	comp71865_c0_seq3_AT5G16320||FRL1_8.92e-059_201	CAACTTCGCGAGGATGGAAG	GCGTTTTCTGTCTGCCTTCA
FT	comp74533_c0_seq1_AT1G65480||FT_1.59e-076_227	ACAGGAGCAACTTATGGCAA	AGCTCAGCAAAGTCTCTGGT
FVE	comp37047_c0_seq2_AT2G19520||FVE_0.0_656	ACATCGTGTCTGGTGGAGTT	CCTCTGCTGAACTGCCAAAG
FY	comp60936_c0_seq1_AT5G13480||FY_1.71e-167_427	GAATCATTCCGTGGACACCG	CAACCTGGGGAGTTTCATGC
GI	comp59833_c1_seq1_AT1G22770||GI_0.0_1326	GCCATCAACGAGCATTCCAT	GAGAGGGAATTGCAGCCTTG
LFY	comp70152_c0_seq2_AT5G61850||LFY_1.39e-138_401	AGGTTTGCAAAGAAGGCTGG	CCCCACGTTCTCTCCTCTTT
LHY	comp71305_c0_seq2_AT1G01060||LHY_9.24e-123_384	CTATGGACGAGCTTGGCAAC	GGAATGAGCCTCCTTCTCCA

LIP1	comp68740_c0_seq4_AT5G64813||LIP1_4.85e-150_432	TGAGCGGGAGAACAATAGCA	GTTTGGGGAGGGTGAACAAC
LWD2	comp63541_c0_seq2_AT3G26640||LWD2_0.0_625	TCGATCAGTACCCTAACCGC	TCGGGCTTCTGACACTCTTT
MAF1	comp19742_c0_seq2_AT1G77080||MAF1_1.61e-039_132	CGACAGCAAGGCCAACATTT	GGTCCCTCAAGTTGCCTTTG
PHYA	comp131820_c0_seq1_AT1G09570||PHYA_4.63e-114_345	GCTACCTGTTACTCGGTGGA	GCCCTAGGATTTGGTGAGGT
PHYB	comp73619_c1_seq2_AT2G18790||PHYB_0.0_1084	TTCTGGTTCCGGTCCCATAC	TTTTCCCATGGCAAACTCCG
PHYC	comp72568_c0_seq1_AT5G35840||PHYC_0.0_1119	CCCAAGCAGCCAAAGTTTCT	CTGCGAACTCAAACTGTGCT
PHYD	comp152319_c0_seq1_AT4G16250||PHYD_8.69e-054_174	GTGAGGCAGCTAACAGGGTA	GGCTCCAAATCAGGCCTTTT
PHYE	comp73269_c0_seq8_AT4G18130||PHYE_0.0_1083	CTTGCCTGGCCTAAAACTGA	AAACCCTCCTGTGAACTCCA
PIE1	comp44672_c0_seq1_AT3G12810||PIE1_1.79e-121_378	TGCTGATCAGAGTGGCTTGA	CATCAGCCAGCATGTCAACA
SOC1	comp64891_c0_seq1_AT2G45660||SOC1_2.29e-078_236	GCGAAATGGGCTGCTTAAGA	CGTCCTATCGTCTCCTGCAT
SPL3	comp59648_c0_seq2_AT2G33810||SPL3_2.00e-041_134	TCGGTTTCTTGTCAGGTGGA	TCCGACAGCTCATGAAACCT
SVP	comp57706_c0_seq2_AT2G22540||SVP_3.39e-099_290	TGCTTGAATCAGGACTTGCC	TCTCCTCCAACAGTTGTGCT
TFL1	comp49298_c0_seq2_AT5G03840||TFL1_2.65e-088_257	GACCCATACTTGAGGGAGCA	TGAAGCCGTCCCTTGAAGAT
TFL2	comp66538_c0_seq3_AT5G17690||TFL2_5.84e-070_229	CGGAAGGGTCAGCTTCAGTA	AGATTTGCCACTGGACCTCA
TOC1	comp73092_c0_seq1_AT5G61380||TOC1_6.50e-123_307	CGGCACAGGATGAAGTCTCT	ATCCGCCTTCTTCTCCACAT
TSF	comp60306_c0_seq2_AT4G20370||TSF_2.20e-064_196	TTCAGCCCCAGTGTGAAGAT	TGTAGGCAGCTCTCAAGTCC
VIL1	comp68114_c0_seq3_AT3G24440||VIL1_3.26e-077_248	CTGGGCTCGAGAACAAGGAT	GGCGGCATTGGATCTTCTTC
VIN3	comp65056_c0_seq2_AT5G57380||VIN3_5.64e-075_248	AGATGCTTTCCGGGACTTGA	AAGTTTCTGGCCCAAGGAGA
VIP3	comp43752_c0_seq2_AT4G29830||VIP3_0.0_528	CTCGACCCGCTCTCTTACTT	AACACACGAACGAAGCTGTC
VIP4	comp72731_c0_seq4_AT5G61150||VIP4_7.40e-139_420	CGTATGAAAGCAATGCACGC	AGGAAAAGGTGCGCATGATC

VIP5	comp67538_c0_seq5_AT1G61040||VIP5_2.12e-153_450	ATGGCTGACAGTGATGACGA	AAAACGGCTCCATGAACCAC
VIP6	comp67891_c0_seq2_AT2G06210||VIP6_0.0_1012	TGGTGCTGGAGTGGTCTTAG	CATCGGGCATCTGGACAAAG

4 Investigation of regulation of flowering in Mango

4.1 Aim

Identify when flowering occurs in Mango and correlate with gene expression data to determine possible genes responsible for floral induction.

4.2 Hypothesis

Flowering gene homologs will act in the same way; to regulate flowering in Mango; as they do in other species.

4.3 Methods

4.3.1 Year 1

Sampling was conducted on a commercial mango orchard in the Darwin Region. In March 2017 six groups of four trees were selected from various sites in the orchard and tagged. The new vegetative growth on each of these trees was also tagged. Sampling began on the 10th of May 2017 and was conducted weekly for eight weeks.

Two trees from each of the six groups were sampled each week. Samples were taken from the growth tagged in March to ensure maturity. Samples taken were, the terminal buds, leaves nearest the terminal bud and leaves at least 2 growth units from the terminal bud. Buds were photographed (See Figure 4-1Photograph of dormant terminal bud from mango. Small ticks on scale bar represent 1mm, large ticks represent 1cm.) and cut in half longitudinally, half was placed on dry ice for RNA extraction and the other half was placed in fixative for sectioning and microscopic analysis.

Figure 4-1Photograph of dormant terminal bud from mango. Small ticks on scale bar represent 1mm, large ticks represent 1cm.

Samples for RNA extraction were ground at cryogenic temperatures and extracted in plate form using a modified CTAB method. RNA was used to make cDNA and real-time PCR

was done on all samples using a subset of the primers in Table 3-3 Primer Pairs Generated by Primer3

4.3.2 Year 2

The work above will be repeated in 2018 following the same method but at different locations. In 2018 buds will not be harvested and instead the fate of the bud above sampled leaves will be recorded.

4.4 .Results

4.4.1 Year one gene expression results.

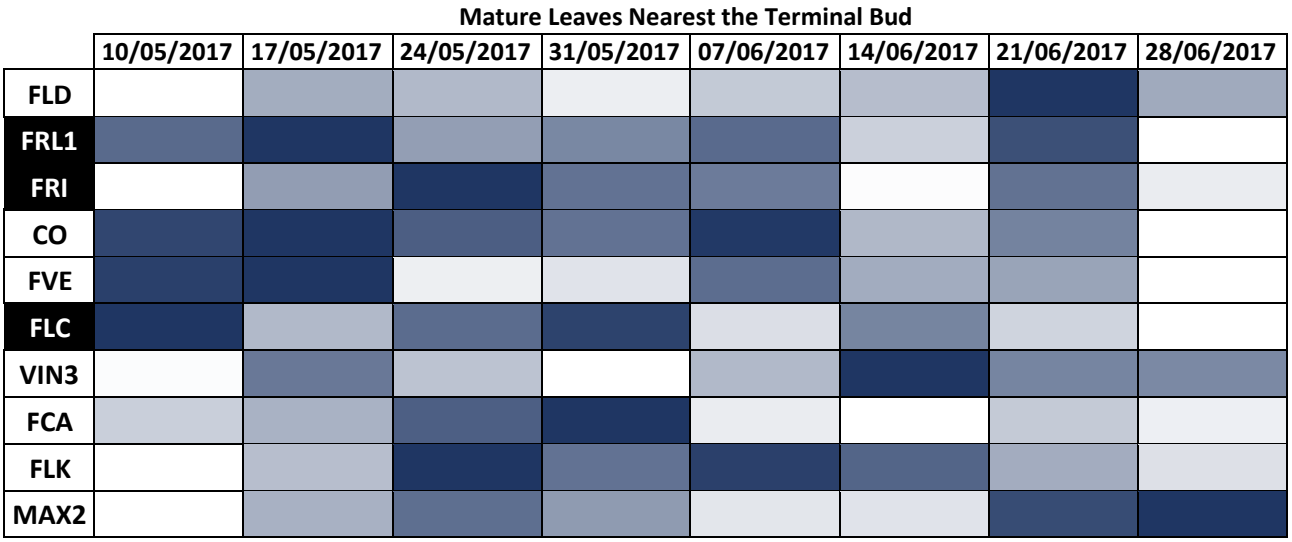

Figure 4-2 Heat map showing expression of various genes at different dates of sampling. The darkest colour represents the highest level of expression, white represents the lowest. Genes in black on white background are positive regulators. Genes in white on black background are negative regulators.

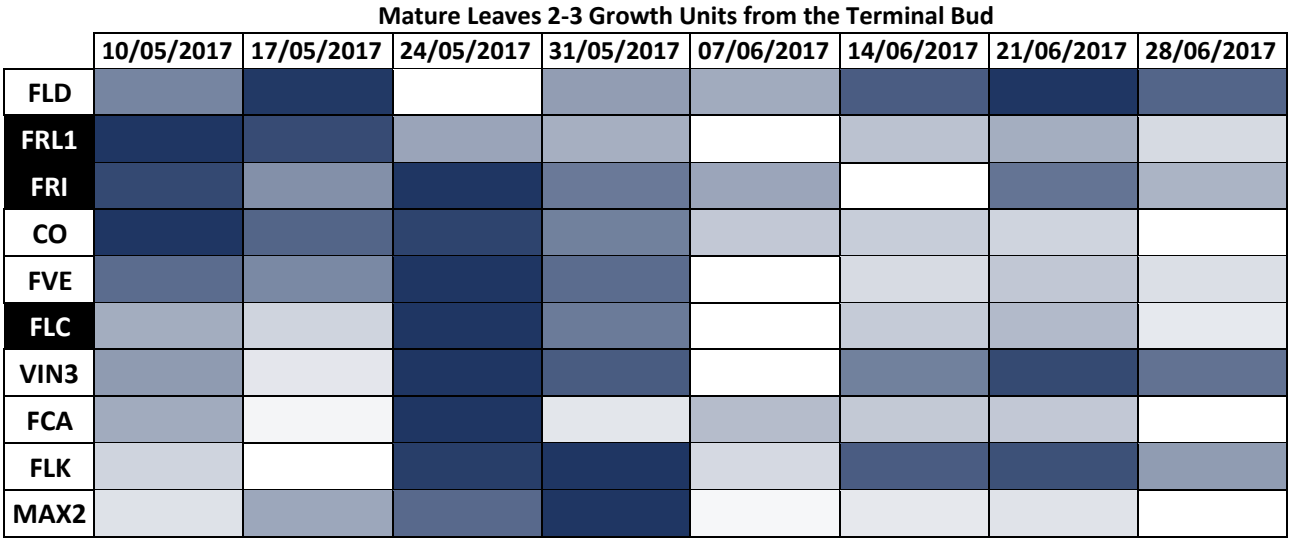

Figure 4-3 Heat map showing expression of various genes at different dates of sampling. The darkest colour represents the highest level of expression, white represents the lowest. Genes in black on white background are positive regulators. Genes in white on black background are negative regulators

4.5 Discussion

The gene expression results from year one of sampling (See Figure 4-2 Heat map showing expression of various genes at different dates of sampling.and Figure 4-3 Heat map showing expression of various genes at different dates of samplingshow variation in the expression levels of different genes across the eight weeks of sampling. There does not appear to be any clear pattern between the expression of negative regulators and positive regulators. There is a distinct difference in the pattern of expression between the leaves nearest the terminal bud and leaves further along the branch. This could indicate that there are different roles that mature leaves play in floral regulation in relation to their position relative to the bud.

The flowering seen on this orchard was extremely low for this season and could be a factor contributing to the weak results. The expression data also needs to be paired with the pictures of the bud cross sections. Expression data from the leaves can be divided into two groups, those with floral buds and those with vegetative buds, this could hopefully show stronger correlations between gene expression and flowering.

The currently results do not support the hypothesis that flowering gene homologs will act in the same way; to regulate flowering in Mango; as they do in other species.

5 Effect of temperature, potassium nitrate and Thiourea on expression of flowering related genes in Mango

5.1 Aim

To observe the effect of chilling and chemical sprays on the regulation of flowering in Mango

5.2 Hypothesis

Expression of flowering regulation genes is influenced by chilling and application of KNO3 and Thiourea

5.3 Methods

5.3.1 Plant material and treatments

Two mango cultivars were used for this work, the common commercial variety Kensington Pride and a new variety NMBP1243. Mature scions of each variety were grafted to seedlings of a common rootstock. Plants were grown in small pots and kept as small trees. The trees were subjected to six experimental conditions; cold temperature (<20°C), 4% KNO_3, 1% Thiourea, Cold with 4% KNO_3 and cold with 1% Thiourea. The control treatment was temperature above 20°C with no chemical application.

5.3.2 Sample collection

Samples were taken at the time of chemical application and movement into temperature controlled glasshouses (0h), then 1, 3, 6, 18 and 36 hours post treatment. Samples were

taken from mature leaves in the form of 12 hole punch discs. Samples were immediately dropped into liquid nitrogen and stored at -80°C until processing.

5.3.3 Sample processing

Samples were ground at cryogenic temperatures and RNA was extracted in plate form using a modified CTAB method. RNA was used to make cDNA and real-time PCR was done on all samples using a subset of the primers in Table 3-3 Primer Pairs Generated by Primer3

5.4 Results

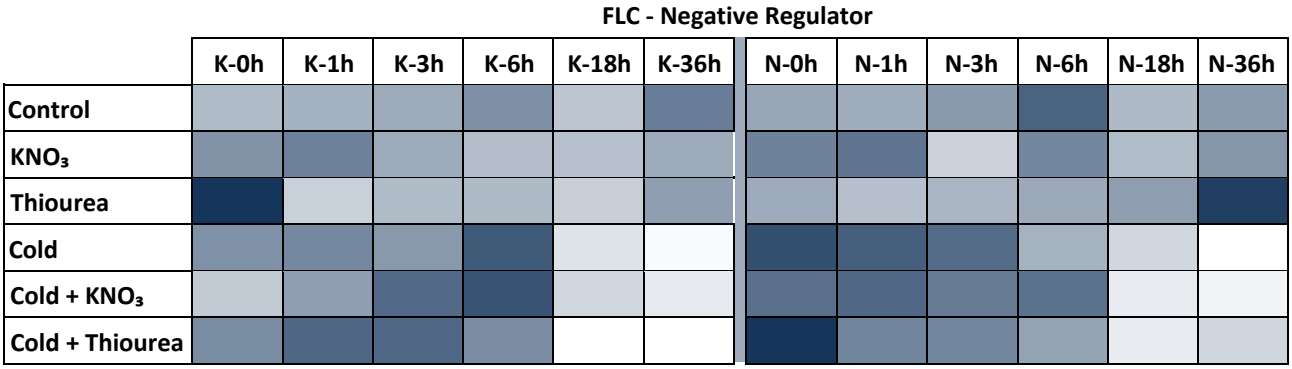

Figure 5-1. Heat map showing expression of the FLC gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

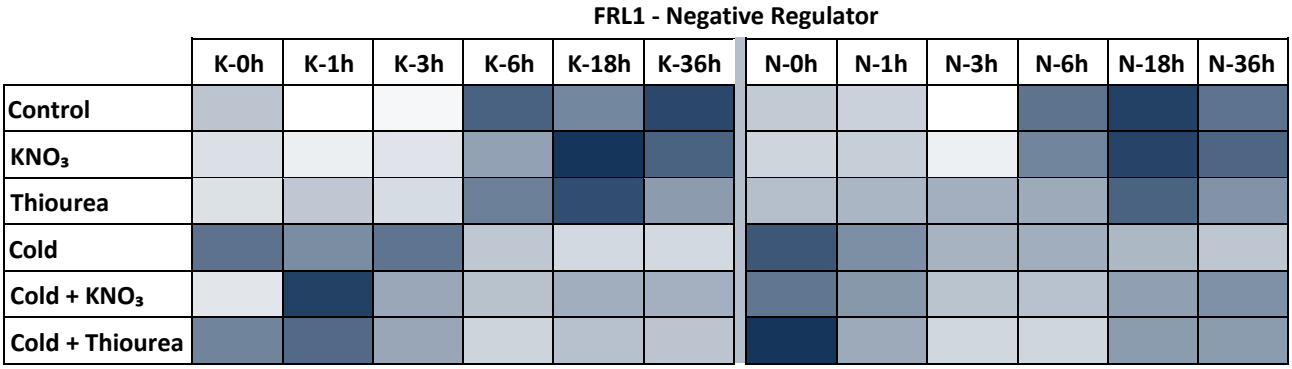

Figure 5-2. Heat map showing expression of the FRL1 gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

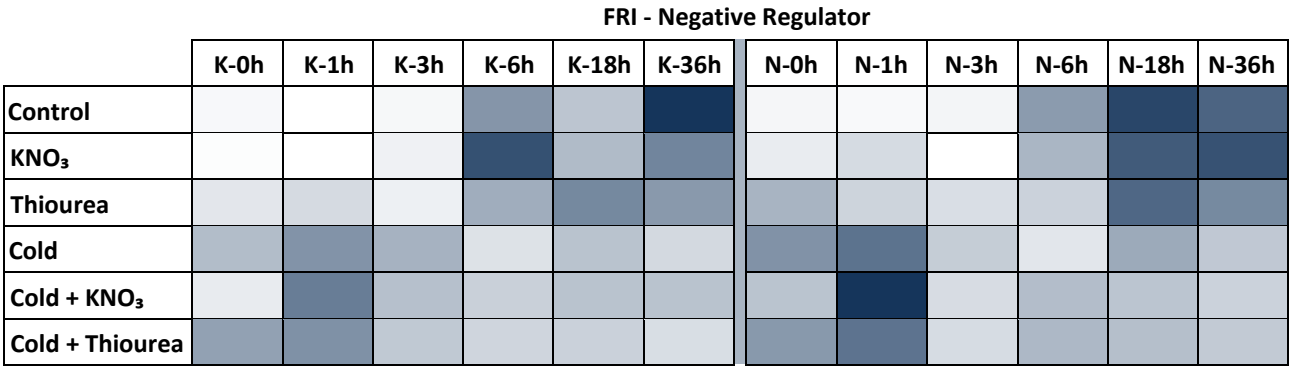

Figure 5-3. Heat map showing expression of the FRI gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the

figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

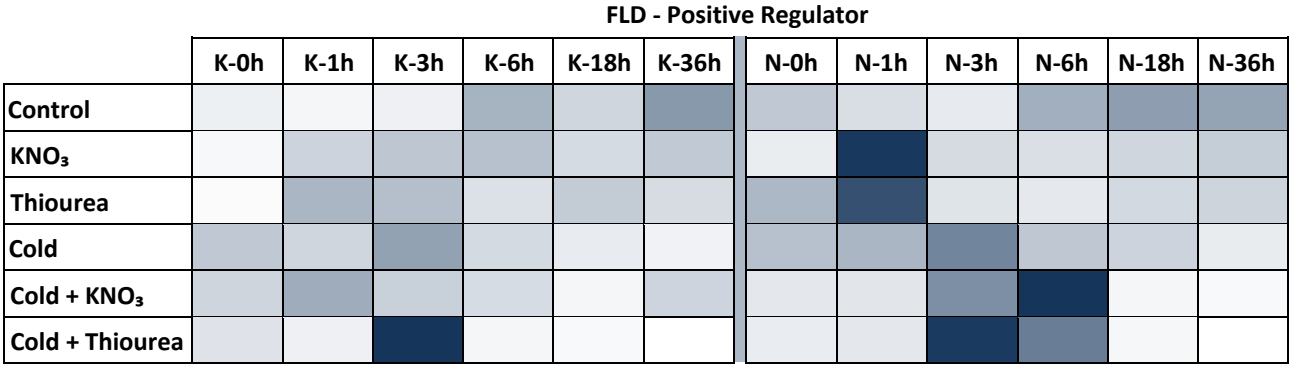

Figure 5-4. Heat map showing expression of the FLD gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest.

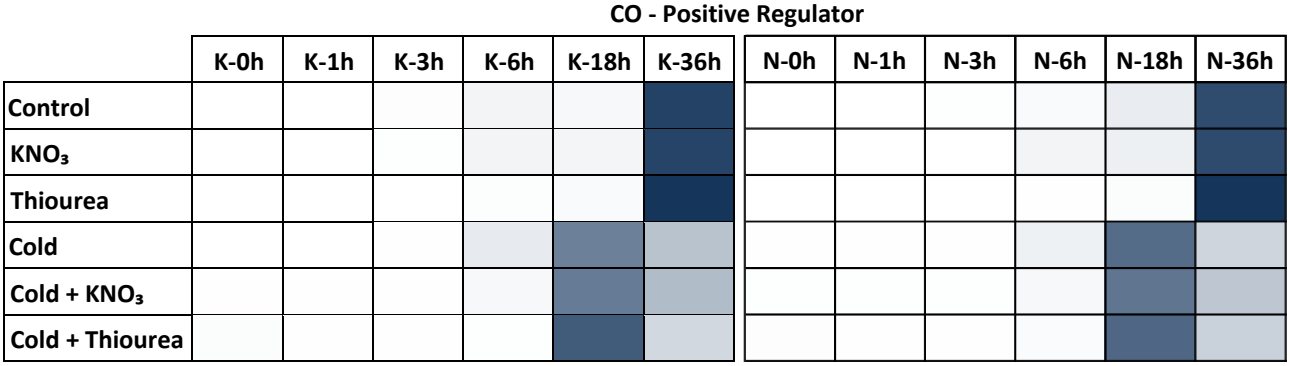

Figure 5-5. Heat map showing expression of the CO gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

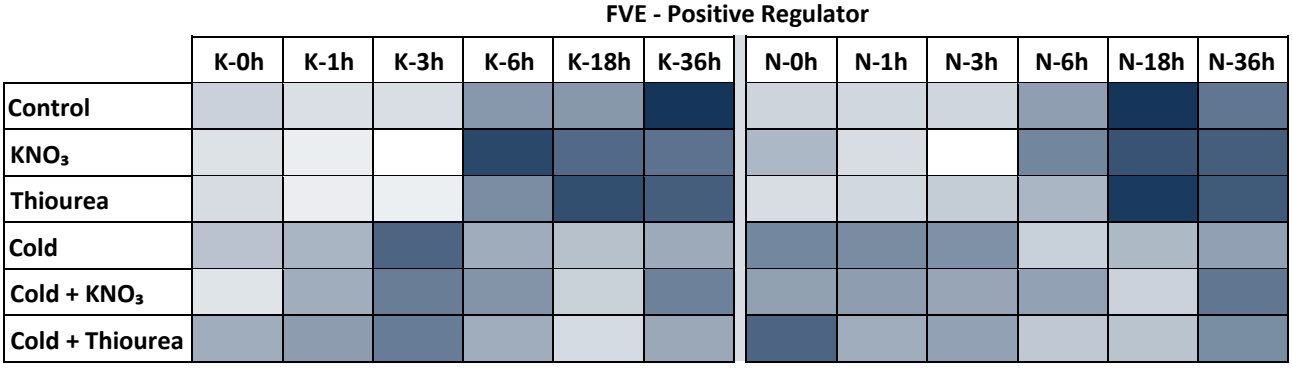

Figure 5-6. Heat map showing expression of the FVE gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

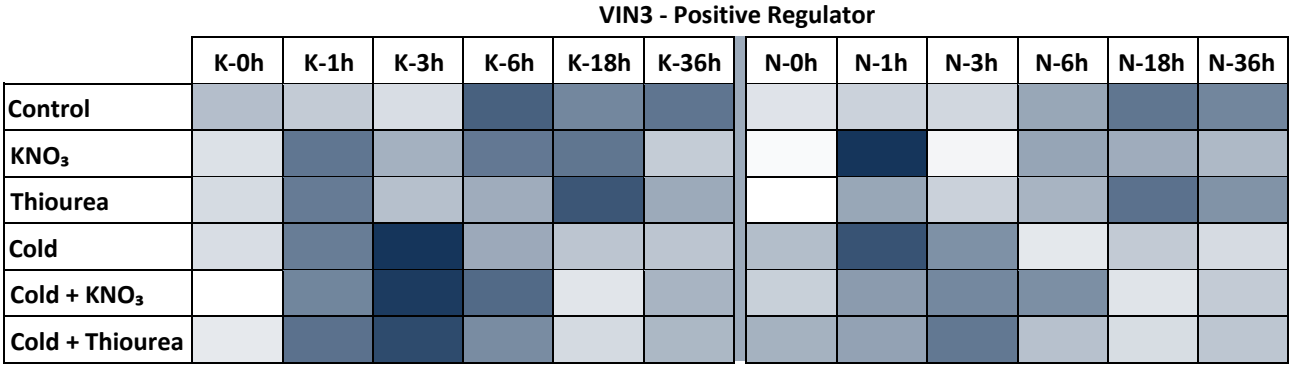

Figure 5-7. Heat map showing expression of the VIN3 gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

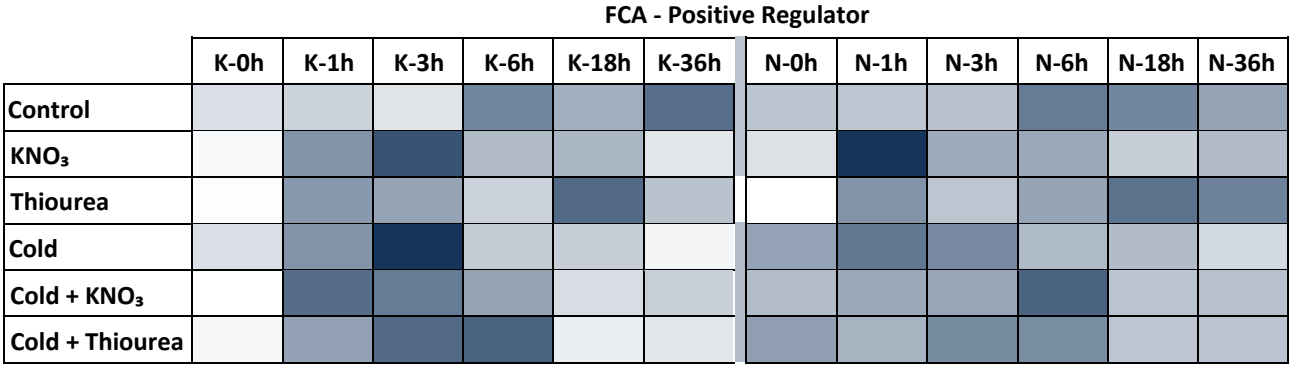

Figure 5-8. Heat map showing expression of the FCA gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

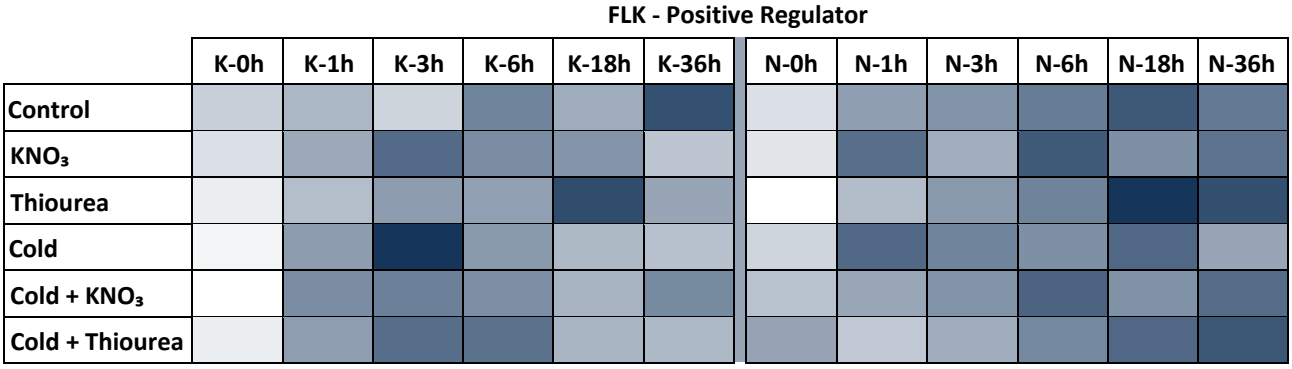

Figure 5-9. Heat map showing expression of the FLK gene at increasing time points after treatment. Treatment application is displayed as 0h. Treatments are listed on the left of the figure. Kensington Pride results are on the left, NMBP1243 results on the right. The darkest colour represents the highest level of expression, white represents the lowest

5.5 Discussion

The results of this experiment show that these treatments have an impact on the expression of the chosen flowering genes. There are also differences in expression

between the two cultivars.

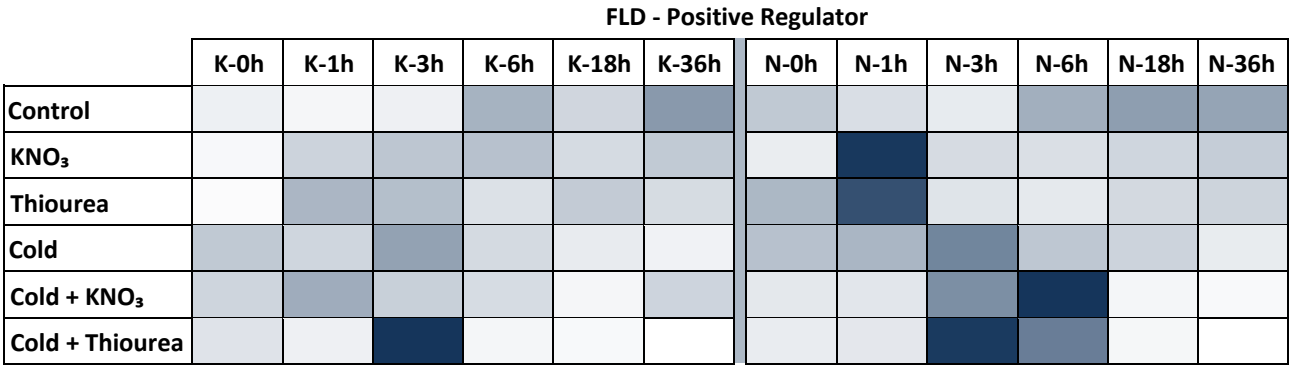

Figure 5-4 shows that the thiourea treatment is increasing expression of the positive flowering regulator FLD, this could support observations that thiourea is a floral inducer. Figure 5-1shows the expression of FLC decreasing over the time course in response to the cold, cold with KNO_3 and cold with thiourea treatments, similar results can be seen in another negative regulators FRI and FRL1 (See Figure 5-3 and Figure 5-2) .If flowering is being induced a decrease in the expression of negative regulators would be expected, which could suggest that these treatments are inducing flowering.

These results are supporting the hypothesis that the expression of flowering regulation genes is influenced by chilling and application of KNO3 and Thiourea. However, more work needs to be done for further confirmation.

6 Work to be done

6.1 Identification of flowering gene homologs in Mango transcriptome

- The sequences found in the mango transcriptome that align to *Arabidopsis* FT can be cloned into a vector and transformed into an *Arabidopsis* FT mutant, this will show if the gene can function in *Arabidopsis* to induce flowering and indicate that it could possibly be functional in mango.
- More work can be done on the transcriptome and genome data from mango. More genes can be identified and also introns and promoter regions can be identified in the genes already identified.

6.2 Investigation of regulation of flowering in Mango

- This work needs to be repeated over another season and on trees that are more likely to flower well.
- Samples collected in this work can be used to do transcriptomic analysis to gain a large amount of data showing genes up or down regulated in samples that flowered and samples that didn't.

- The data from the first year of sample collection needs to be paired to the bud pictures and further analysed.
- Samples need to be analysed using FT primers that have been optimised since the beginning of this work.

6.3 Effect of temperature, potassium nitrate and Thiourea on expression of flowering related genes in Mango

- This work has been repeated over a longer time period, the samples have been collected and are part-way through being processed and analysed.
- All samples need to be analysed using FT primers that have been optimised since the beginning of this work.

7 References

AFGAN E., BAKER D., VAN DEN BEEK M., BLANKENBERG D., BOUVIER D., et al., 2016 The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. Nucleic Acids Res. 44: W3–W10

AMASINO, R. 2010. Seasonal and developmental timing of flowering. The Plant Journal, 61, 1001-1013.

BANGERTH, K. 2009. Floral induction in mature, perennial angiosperm fruit trees: similarities and discrepancies with annual/biennial plants and the involvement of plant hormones. Scientia Horticulturae, 122, 153-163.

CAMACHO, C., COULOURIS, G., AVAGYAN, V., MA, N., PAPADOPOULOS, J., BEALER, K., & MADDEN, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics*, *10*, 421. http://doi.org/10.1186/1471-2105-10-421

CHAILAKHYAN, M. K. 1936. New facts in support of the hormonal theory of plant development. Comptes Rendus De L Academie Des Sciences De L URSS, 13, 79-83.

CHOUARD, P. 1960. Vernalization and its relations to dormancy. Annual Review of Plant Physiology, 11, 191-238.

DAVENPORT, T. 2009. 5 Reproductive Physiology. The mango: Botany, production and uses, 97.

DAVENPORT, T., ZHANG, T. & YING, Z. Isolation of genes potentially regulating mango flowering. Proceedings of the 33 rd Annual Meeting of the Plant Growth Regulation Society of America. Quebec City, 2006a. 109-110.

DE LOS SANTOS-VILLALOBOS, S., PARRA-COTA, F. I., DE-FOLTER, S. & PEÑA-CABRIALES, J. J. 2012. Primers to amplify flowering locus T (FT) transcript in mango (Mangifera indica) and their potential use in other angiosperms.

ENDO, T., SHIMADA, T., FUJII, H., KOBAYASHI, Y., ARAKI, T. & OMURA, M. 2005. Ectopic expression of an FT homolog from Citrus confers an early flowering phenotype on trifoliate orange (Poncirus trifoliata L. Raf.). Transgenic research, 14, 703-712.

GARNER, W. & ALLARD, H. 1920. Effect of the Relative Length of Day and Night and Other Factors of the Environment on Growth and Reproduction in PLANTS1. Monthly Weather Review, 48, 415.

HU, G., LIN, S., YE, Z., XU, C. & ZHANG, S. 2003. Isolation and sequence analysis of LEAFY homologous gene from mango. Subtropical Plant Science, 33, 1-4.

KARDAILSKY, I., SHUKLA, V. K., AHN, J. H., DAGENAIS, N., CHRISTENSEN, S. K., NGUYEN, J. T., CHORY, J., HARRISON, M. J. & WEIGEL, D. 1999. Activation tagging of the floral inducer FT. Science, 286, 1962-1965.

KEARSE, M., MOIR, R., WILSON, A., STONES-HAVAS, S., CHEUNG, M., STURROCK, S., BUXTON, S., COOPER, A., MARKOWITZ, S., DURAN, C., THIERER, T., ASHTON, B., MENTJIES, P., & DRUMMOND, A. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data.Bioinformatics, 28(12), 1647-1649.

KLEBS, G. 1918. Uber die Blutentbildung bei Sempervivum. Flora (Jena), 128, 111–112.

KNOTT, J. E. Effect of a localized photoperiod on spinach. Proc. Am. Soc. Hortic. Sci, 1934. 152-154.

KOBAYASHI, Y., KAYA, H., GOTO, K., IWABUCHI, M. & ARAKI, T. 1999. A pair of related genes with antagonistic roles in mediating flowering signals. Science, 286, 1960-1962.

KORESSAAR T & REMM M. Enhancements and modifications of primer design program Primer3. Bioinformatics 2007;23(10):1289-91.

LUO, C., HE, X., CHEN, H., JIANG, Y., GAO, M. & LI, Y. 2009. Cloning and bioinformatic analysis of the AP1 homolog gene from mango. Genomics and Applied Biology, 28, 851-858.

NAKAGAWA, M., HONSHO, C., KANZAKI, S., SHIMIZU, K. & UTSUNOMIYA, N. 2012. Isolation and expression analysis of FLOWERING LOCUS T-like and gibberellin metabolism genes in biennial-bearing mango trees. Scientia Horticulturae, 139, 108-117.

TOURNOIS, J. 1914. Etudes sur la sexualite du houblon. Annals des Sciences Naturelles, 19, 49–191.

TRÄNKNER, C., LEHMANN, S., HOENICKA, H., HANKE, M.-V., FLADUNG, M., LENHARDT, D., DUNEMANN, F., GAU, A., SCHLANGEN, K. & MALNOY, M. 2010. Over-expression of an FT-homologous gene of apple induces early flowering in annual and perennial plants. Planta, 232, 1309-1324.

UNTERGASSER A, CUTCUTACHE I, KORESSAAR T, YE J, FAIRCLOTH BC, REMM M & ROZEN SG. Primer3--new capabilities and interfaces. Nucleic Acids Res. 2012;40(15):e115.

WILKIE, J. D., SEDGLEY, M. & OLESEN, T. 2008. Regulation of floral initiation in horticultural trees. Journal of Experimental Botany, 59, 3215-3228.

ZHANG, H., HARRY, D. E., MA, C., YUCEER, C., HSU, C.-Y., VIKRAM, V., SHEVCHENKO, O., ETHERINGTON, E. & STRAUSS, S. H. 2010. Precocious flowering in trees: the FLOWERING LOCUS T gene as a research and breeding tool in Populus. Journal of Experimental Botany, erq092.

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