Hort Innovation

Final Report

Manipulating Mango Flowering to Extend Harvest Window

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Northern Territory Department of Primary Industries and Resources

Project code:

MG12012

Project:

Manipulating Mango Flowering to Extend Harvest Window – MG12012

Content

Summary

This project has investigated the climatic conditions associated with flowering in mango in the Northern Territory and evaluated chemical treatments that can be used to promote this process. It has shown that flowering is associated with the occurrence of anticyclonic winds related to south westerly winds, delivering low night temperatures leading to flower induction. For the first time, both the lower and upper temperature limits for flower induction have been described.

Aeroponics was used to evaluate the effects of the most widely used growth regulator on plant growth. This showed that uptake of the growth regulator occurs within 24 hours and that it affects both shoot and root growth. In the orchard it is applied months before flowering, supporting the conclusion that it is not directly implicated in floral induction. The ability of ethephon to selectively remove unwanted vegetative flush and allow regrowth at a time when floral inductive conditions are more likely has been demonstrated. Ethephon also appears to have the ability at an appropriate level of application level to slow flush development. The capacity of chemicals to promote bud break in mango under non-inductive and inductive conditions has been investigated. The most widely applied chemical used for this purpose in the Northern Territory has only a limited capacity to induce flowers without cool inductive conditions. It was shown that the uptake of potassium nitrate by the mango leaf occurs most efficiently at night. The ability of other gibberellin synthesis inhibitors like paclobutrazol to delay vegetative flushing and promote the development of mature leaves that are responsible for producing the floral inductive signal in mango were also investigated. These products were selected as they breakdown relatively quickly in the plant and soil. Further work is required to optimise the timing and amount of these chemicals applied as the response of trees to these gibberellin inhibitors was different that described for the current widely used gibberellin inhibitor paclobutrazol. The project outputs have been extensively promoted to the local and Australian mango industry.

Keywords

Flower manipulation; mango; Northern Territory; ethephon; paclobutrazol; climate; flower induction

Introduction

The Australian mango production period commences in late August in Darwin and ends around March with fruit from Mildura in Victoria or Gin Gin in WA. However, within each individual region or property, the harvest window may only extend for five to seven weeks. As enterprises and production develop within a region 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. As newer varieties are introduced and younger trees mature, this situation will become exacerbated. Reliable techniques to advance or delay fruit maturity on a property, to spread the harvest period, would improve harvest and packaging efficiencies, profitability and ensure the development of an export industry. Additionally, if the industry is to move to mechanisation in the harvesting process, such technologies will be critical.

These studies have focused on how plant growth regulators especially gibberellic acid inhibitors, can retard vegetative growth and alternative products can stimulate floral induction. The impact on root systems as well as the vegetative and floral components of the plant were studied in glasshouse and field experiments. Climatic factors, including heat units and agronomic practices were assessed. Results from all sectors of the project have been integrated into commercial trials to evaluate the combined effect of management options and flowering time. These options, in combination with crop forecasting and past production trends will allow producers to target harvest windows around peaks in production to meet specific market opportunities and result in a

more efficient use of resources.

Flowering in mango is dependent of mature leaves responding to inductive temperatures and generating a signal that is transmitted to the shoot apex. This leaf based signal causes floral differentiation in growing buds leading to flower production and eventually fruit development. In mangoes, gibberellic acid inhibitors are typically applied to soil to reduce tree vegetative vigor. These treatments aim to reduce gibberellic acid content in the apical buds which may inhibit flower development. Chemicals are also applied to the canopy that are thought to promote flower initiation, though it is not currently known whether they act on the leaf or shoot apex.

Methodology

This project was targeted at Northern Territory mango growers and investigated the climatic and physical constraints for flower manipulation. Despite the wealth of research and considerable grower evaluation, very few of the preceding options, either used alone or in combination have demonstrated consistent and reliable results. In discussions with the project leaders of the past and current HIA projects (MG 06005, MG10008 and MG06022), which have proposed strategies to manipulate the harvest window, limited progress has been achieved. The effectiveness of these treatments requires scientific evaluation, both alone and in-combination, to demonstrate they are effective, repeatable, sustainable, registered and do not have deleterious effects on fruit quantity and market access.

Use of climate data

In collaboration with the Bureau of Meteorology, investigations were performed to correlate flowering with climate impacts due to events such as the Madden-Julian Oscillation (MJO), which is a major fluctuation in tropical weather on weekly to monthly timescales. Additional data on the impacts, such as low night temperatures on vegetative and reproductive stages of the phenological cycle, were explored to determine the potential for a predictive model. Results of this work are presented in Appendix 1.

Ethephon

Ethephon was originally thought to be involved in the production of the signal for floral induction and potentially for accelerating the maturing process in leaves to make them responsive to inductive conditions. A simpler explanation is that, at an appropriate concentration, it selectively causes abscission of immature flush which inhibits floral induction. Ethephon has been shown to be effective in promoting flowering in NT mango orchards, but was not registered for this use in Australia. The response to ethephon is affected by concentration, pH of the spray formulation, ambient temperature, relative humidity and water quality. Based on the label description it recommended to use ethephon with hard water, which is found universally in the mango production regions in the Northern Territory. The development of appropriate spray formulations for removal of immature vegetative flush that would inhibit flower induction is presented in Appendix 1.The capacity of ethephon treated trees to respond to floral promoting chemicals after treatment is demonstrated.

The rapid action of ethephon on vegetative growth of mango trees means that it may have the capacity to be used as a quick acting inhibitor when applied at levels below which shoot abscission occurs. By comparing the effects of multiple sprays at low concentrations with that of a single high dose, it is possible to see if effects are cumulative or independent of previous treatments (Appendix

2).

Paclobutrazol and other foliar alternatives

Northern Territory mango growers almost universally use paclobutrazol to increase yield and access higher priced earlier maturing fruit. Circumstantial evidence suggests that in certain regions paclobutrazol has been applied at higher (non-registered) rates to improve production through an increase in early season fruit. The soil application of paclobutrazol is being scrutinised in a number of countries for its negative impact on the environment. For example, the compound is not registered for use on mangoes in the North America. There are a range of alternative products within the same grouping of plant growth regulators. Unfortunately, there have been limited studies on mango production with the focus on deciduous fruit crops. Some of these are recommended for foliar application, and have a half-life measured in days. In comparison, paclobutrazol can remain active in the soil for several months. These alternative products could allow for more reliable targeted application and reduce the risk of environmental impacts. The effects of paclobutrazol and other gibberellin synthesis inhibitors are explored in Appendix 3 and 4. The effects of paclobutrazol on root development and speed of uptake are examined using aeroponics in Appendix 5.

Thiourea and Potassium Nitrate

Although previous scientific reports on the use of potassium nitrate applied to Kensington Pride mangoes in Australia have shown little success in stimulating flowering. Reports from overseas on other varieties have demonstrated that potassium nitrate is effective. Northern Territory growers suggest that the utilisation of higher concentrations of potassium nitrate (4 %) compared to those reported previously in the Australian literature (1 %), with 2-4 applications in one season, are effective at promoting early fruit production. Applications of potassium nitrate in March/ April (dependent upon the phenological stage of the tree), with applications halted once the flowers appear, have been reported to be successful. Factors shown to be critical to the effectiveness of potassium nitrate and need to be taken into account including climate, age, and nutritional status of the tree and the physiological and temporal timing of application.

Other compounds shown to offer potential to induce early mango fruit production include thiourea. Agricultural consultants in the Northern Territory suggest that although thiourea is effective, its response is not consistent. Applications at too high a concentration can cause leaf burning. Thiourea has been used to increase early fruit production in pears and grapes. The ability of potassium nitrate, thiourea and thidiazuron to promote flower induction in mango under different inductive conditions are compared in Appendix 6, 7 and 8. The effects of adjuvants and timing of application of potassium nitrate are investigate in Appendix 9.

Tip pruning

In addition to the use of chemical treatments to remove immature flush and reset flushing patterns, mechanical pruning was investigated. By careful monitoring of regrowth and concurrent temperatures, precise temperature lower and upper limits for floral induction were defined for two commercial mango cultivars, Appendix 10.

Outputs
- Mango flower manipulation AMIA national mango conference June 2013
- NT R&D mango workshop February 2014

- On farm demonstration of the ethephon/KNO$_3$ trial June 18th 2014
- Mango flower manipulation. AMIA national conference 27th May 2015
- Master class on mango flower manipulation as part of the 11th International mango conference Darwin September 2015
- Mango flower manipulation mango grower meeting July 29th 2016
- 12th International mango symposium in Baise City, Guangxi China, from 10-16 July 2017 Present outcomes of flower manipulation trials
- Outcomes of the flower manipulation trial AMIA preharvest meetings Darwin and Katherine August 15th and 16th 2017
- Managing mango flowering and implications for growers AMIA national mango conference May 5-6th 2017

Outcomes

This project has furthered our understanding of the role of climate and growth regulators on mango flowering and subsequent fruit production in the Northern Territory's key production regions. These findings can be used to improve mango best management practices, while maintaining sustainable and profitable production systems in northern Australia.

This project has very precisely described the temperature requirements for flowering of mango cultivars in the Northern Territory. This is the first time that temperature thresholds for flowering of mango have been defined globally. This work has revealed the critical importance of cool nighttime temperatures and associated weather systems. It has demonstrated that differences exist between cultivars in both low temperature requirements and high temperature limitations for floral induction. The methods used to quantify these temperature requirements has the capacity to screen cultivars for their genetic capacity for climate change adaptation while also quantifying the ability of chemical treatments to modify these responses. The short periods of shoot development and inductive conditions to produce flowers highlights the sensitivity and vulnerability of this process.

Further studies are needed that integrate predicted climate change for major production regions under different emission scenarios using a range of forecasting models. A greater range of existing mango cultivars need to be screened to define their adaptive potential to enable climatic risks to the industry. This should be extended to cultivars that produce in hotter climates to determine the genetic potential of the available germplasm. This project has identified industrial chemicals that have the capacity to promote flower initiation under non-inductive conditions. Other acceptable options of decoupling climatic triggers from floral induction may be required if existing genetic and chemical treatments are insufficient to maintain a profitable mango industry.

There were difficulties in identifying the optimum concentrations for the application of gibberellin synthesis inhibitors, such as prohexidione calcium. However, this chemical has been shown to be as effective as paclobutrazol in promoting flowering in mango by previous work. They also have the advantage of being rapidly metabolised in the plant (~14 days) and biodegraded within 48 hours in the environment. It also has the advantage of being foliar applied so it is unlikely to impact root development and nutrient uptake. In comparison, when paclobutrazol was applied to the soil it reduced root development and consequently affected the uptake of nutrients. Foliar application of paclobutrazol was shown to have short-term benefits for promoting floral initiation but it is not currently registered for this purpose. In reviewing the use of gibberellin synthesis inhibitors on mango there is evidence that excessive use can delay ripening resulting in fruit with lower Brix levels.

Potassium nitrate was shown to have a weak effect on floral induction when weather conditions are unfavourable. However, it is effective in assisting the synchronisation of flowering when

temperatures are inductive. The project has also shown that the uptake of potassium nitrate is more effective at night. Applying potassium nitrate during none inductive conditions when trees are at the wrong development stage can promote vegetative growth that will inhibit future flower induction when suitable inductive conditions exist. Farm managers need to consider weather patterns likely to lead to cool night temperatures when initiation of tree growth using potassium nitrate will be most effective.

The difficulty in getting repeatable responses to ethephon appear in part due to the application during the day in northern Australia. It could also have been impacted by growers not irrigating trees in the Darwin region early in the season in an attempt to prevent vegetative flushing. In addition, ambient temperatures in orchards in the NT are routinely above label recommendations and the Delta T exceed the recommended bounds for efficacy. The results from night applications of ethephon have shown predictable responses at higher applications rates than previously suggested. A pH-modifying wetting agent was included in the formulation to manage the alkaline ground water conditions which may cause premature breakdown of the compound. These results have confirmed that ethephon can effectively control vegetative flushing to more effectively target growth during cooler inductive conditions. It has also shown that ethephon can act as a quick acting means to delay flushing without the residual effects of gibberellin synthesis inhibitors.

Recommendations

1. The methods developed for understanding temperature requirements for flower induction in mango need to be extended to other existing cultivars and potential commercial mango cultivars to understand the genetic and climatic limits for future production in northern Australia.

2. The capacity of chemical treatments to modify the inductive responses of mango inductive temperatures needs to be quantified.

3. The temperature requirements for flower induction for the different cultivars needs to be integrated with current climate models and emission scenarios to determine impacts on frequency of inductive conditions in current mango production regions.

4. The National Mango Breeding Program has selected cultivars that perform across all northern Australian mango growing regions under a generalised management system. The early flowering and cropping cultivars were not selected despite having high quality fruit. Crosses from this program should be re-evaluated to determine whether they have lower inductive temperature thresholds and consideration of early harvest windows.

5. Engineering of a foliar applied product that replicates the effects of the chemical promoters to initiate flowering should be explored. This could decouple flowering from environmental signals needed for flower and fruit production while meeting regulatory requirements.

6. Options for including thermography to characterise actual leaf temperatures and physiology to understand the differential responses between western and eastern sides to floral induction should be explored.

7. The use of protective shade structures under a range of growing systems to modify leaf temperatures within mango canopies to improve productivity of the western tree aspects should be considered.

8. The fate of soil applied paclobutrazol under a range of soil, irrigation and seasonal condition is needed to understand how leaching may impact groundwater quality.

9. The potential effects of high soil levels of paclobutrazol on mango ripening should be investigated to better understand its effects on the formation of sugars and flavours.

10. The capacity of ethephon, short-lived foliar applied gibberellic acid inhibitors such pro- hexidione calcium, and foliar applied paclobutrazol to the control post wet season vegetative growth to make quick changes to the flushing cycle.

11. Use of nitrogen and other fertilisers to promote early flush development after pruning to maximise the regeneration of the canopy and development of responsive foliage should be investigated.

Scientific publications

Journal article

Asis CA, Alexander T, Sarkhosh A, Umar M, and McConchie C. Optimising foliar nitrogen uptake of mango: Effect of adjuvant, leaf position and time of potassium nitrate spray. Acta Horticulturae 51: Accepted

Paper in conference proceedings

Asis CA, Alexander T, Sarkhosh A, Umar M, and McConchie C. 2018. Optimising foliar nitrogen uptake of mango: Effect of adjuvant, leaf position and time of potassium nitrate spray. Proceedings of the 30th International Horticultural Congress held in Istanbul, Turkey on 12-16 August 2018.

Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report.

Acknowledgements

Appendices

1. Inductive weather conditions and use of ethephon

1.1 Introduction

Climatic drivers for mango flowering in the Northern Territory

The Madden Julian Oscillation (MJO) is an atmospheric disturbance that travels eastward around the tropics and is associated with anomalous rainfall events. In the Northern Territory, the major meteorological predictive use for the MJO appears to anticipate rainfall events between October and March and has little or no influence on the occurrence of low night temperatures.

The cool night 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 (Figure 1).These winds bring the cool temperatures from central Australia to the northern areas of the Territory. The importance of this effect has previously been described in Batten and McConchie (1995).
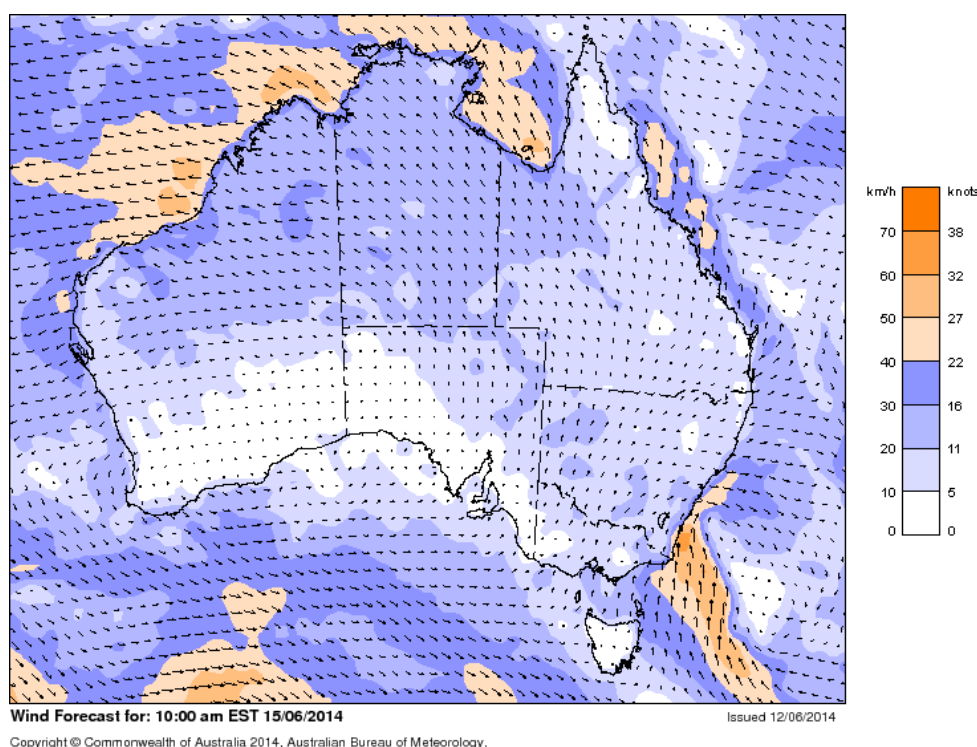
Figure 1. Wind map of Australia for 15th May 2014 showing the southeasterly winds. Wind strength is proportional to the size of the arrow and indicates the direction of air flow. The high-pressure system is in white and with low wind speeds. Wind spirals outward counter clockwise (http://www.bom.gov.au/marine/wind.shtml).

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). Further consolation is required to predict the amount of cooling and duration of chilling (hours below 20 °C) 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 cooler temperatures in Darwin (>20 °C) 5 to 7 days later.
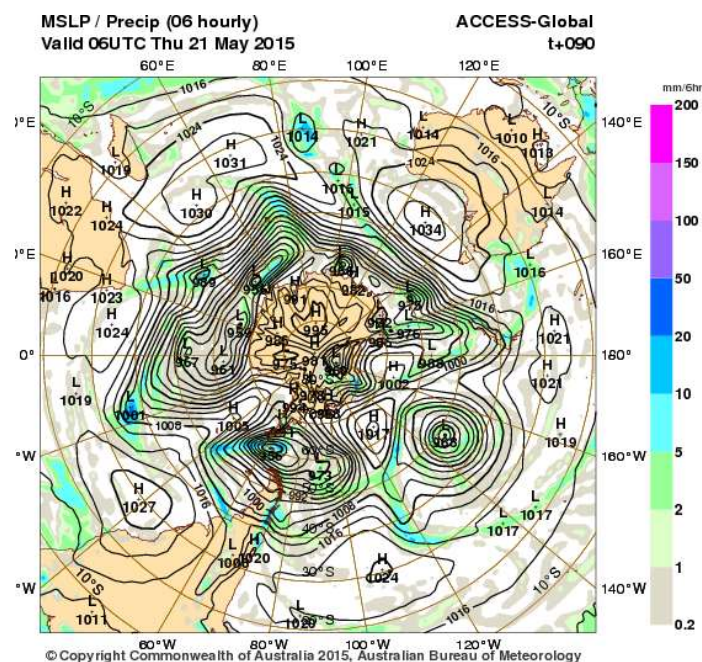
Figure 2. Map of barometric pressure showing areas of high pressure developing in the Indian Ocean and travelling eastwards. The high-pressure system shown over the Australian Bight at this time (21st May 2015) produced southeasterly wind in Darwin and night temperatures that went below 20 °C in Darwin regional production areas such as Noonamah and Berry Springs.

Use of ethephon to manage mango flowering

Ethephon is acidic and is neutralised when absorbed into the plant. At neutral pH, ethephon is unstable and dissociates into ethylene gas, phosphoric acid and chloride ions (REF). Once in the plant ethylene inhibits auxin transport across leaf abscission layer causing the abscission layer to form and the affected shoot to die or wither prematurely. Ethylene is thought to dissipate within days of application and the phosphate component is thought to be metabolised by the plant. Therefore, it is unlikely to result in residues in the fruit as it is being applied prior to flowering.

Ethephon is commercially sold in a range of concentrations ranging from 48 to 90 %. In Australia, it is registered for use on a range of crops for defoliating, selective fruit thinning, promoting evenness of maturity and inducing fruiting in pineapple. It is recommended not to apply it to weak or stressed plants and to use it in the daily temperature range from 18 to 32 °C. In addition, it should not be mixed with hard (alkaline water). These efficacy conditions are problematic for mango trees in the NT as many days during the dry season exceed 32 °C. In our trials, all applications were done at night to comply with the described temperature limits and based on delta T; the difference between the wet and dry bulb temperature, indicative of the evaporative potential. Practically this affects the rate that spray droplets decrease in size over time. Spray conditions for droplet deposition on the plant during Northern Territory dry season match delta T and wind conditions during the night .

It had previously be reported that 0.1 % Ethrel® (active ingredient (a.i.) 48 % ethephon) was 100 % effective at burning off flush less than 5 cm when sprayed on Kensington Pride mangoes (Lu,. 2005) and that flowering was improved. The side effects were that <5 % of recently matured flush was lost while 20-30 % of shaded leaves were abscised. This was dismissed as being insignificant as the shaded leaves were thought not to contribute greatly to the overall productivity of the tree.

In the second year, nearly 25 % of recently matured leaves were abscised while 36 % of old leaves were removed by the 0.1 % treatment but it was still effective at removing 100 % new flush. At 0.05

% ethephon removed 78 % of new flush, 1 % of newly matured hard green leaves and 17 % of old shade leaves. While the difference in responses between the years were noted, it was concluded it was quite safe for growers to experiment by themselves with sprays containing 0.05-0.1 % ethephon. However, ethephon was not registered in Australia for use on mango by the Australian Pesticides and Veterinary Medicines Authority (APVMA) and the NT chemical controller intervened and stopped the use of ethephon on mangoes.

Griffin (2011) also investigated the application of ethephon to mangoes in northern Australia and concluded that an application of 135 ml to 270 ml/ha resulted in a 14 % to 17 % increase in marketable fruit when applied to synchronise flowering. However, in Griffin (2011) much of the other spray rates and levels of ethephon are seemingly contradictory. A similar piece of research was completed by Sandry (2010) who concluded that the most suitable application rate was 192 ml/ha.

It is difficult to know precisely the amount of ethephon applied by Lu (2005). The date of treatment, night when inductive temperatures started and flowering commenced are summarised in Table 1. Based on a PowerPoint presentation retained by the Northern Territory Department of Primary Industry and Resources, it is estimated the treatment as described was sprayed to run-off with 0.1 % Ethrel® during day between 10 am and 3 pm. Sprayed with 0.1 % Ethrel® (48 % a.i. ethephon) @ estimated 4 L/tree and 200 tree/ha = 0.048 x 4 x 200= 38.4g ai/ha. This is considerably less than the dose proposed by either Griffin (2011) or Sandry (2010).

Table 1. Key dates of flowering events and commencement of inductive temperatures (adapted from Liu, 2005).

Year	Treatment date	Start of nights <18 °C	Flowering
2004	22nd April	11th June	7th July
2005	20th April	14th May	1st June

Commercially ethephon is sold in Australia for agricultural purposes at a range of concentrations from 72 % to 90 % active ingredient. So for the purposes of the report active ingredient (a.i.), is ai/ha or ai /L is presented.

1.2 Material and Methods

In April 2014, after receiving a permit to investigate the use of ethephon on mangoes by the APVMA, a field trial was established at Lambells Lagoon, outside of Darwin, on 20-year-old Kensington Pride trees. The trees were planted at a spacing of 8 X 10 meters (125 trees/ha) and were approximately 6 to 7 m tall. A Greentech 6000 L mist sprayer was used that was calibrated to apply 4 L per tree. Initial spray formulations were difficult because previous published trials had used backpack 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 were applied with 485 g ai/ha, using 9 L per 100 m^2 of canopy. This amount of spray was almost double the volume used by another collaborating grower. It was thought that using this quantity of spray would have resulted in a considerable amount running off. In addition, applying this volume of spray would have doubled 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 efficacy. Ground water used in the Northern Territory is generally alkaline, which may have led to hydrolysis of the ethephon releasing the ethylene prior to application. The adjuvant LI700® (Nufarm) was chosen as it acidifies the spray while increasing uptake by it corrosive action on the leaf cuticle. Ethephon spray alone has a pH of

3.5 while when combined with LI700® was pH 4.0.

The following five treatments were developed to investigate two 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 five treatments were applied to separate blocks (25 trees) within a single row. Two buffer trees separated each treatment. 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 spatial variation 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 °C and 32°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 °C and weighing the leaf drop for the two monitored trees per treatment (total 100 trees). The layout of the trial is shown in Figure 3.

Figure 3. *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 +0.25 ml/L LI700®, Blue = 290 g ai/ha ethephon, Red = 290 g ai/ha + 0.25 ml/L LI 700.*

1.3 Results & Discussion

The ethephon treatments had little or no effect on the development of flushes. Few if any immature leaves were abscised 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 0.25ml/L LI700® added (Table 2).

Table 2. 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.

Treatment	Mean weight of leaf fall tree^{-1} (g) Week 1^a	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 + 0.25 ml/l LI 700	256	1599	1855

^{a}Note all results have not been corrected for tree size with more leaves being shed from larger trees.

These results were disappointing as the ethephon treatments were intended to reset the flushing processes by chemically removing existing new leaf growth.

Based on these results, a new spray program was developed with increased concentrations of ethephon. To provide linkage between the two experiments, the highest ethephon spray formulation from the April trial was retained but the content of LI700® was increased from 0.25 ml/L to 1ml/L. A treatment including potassium nitrate (KNO_3) was added as cool inductive night temperatures were expected. Sprays were applied at 4 L/tree. The layout of the trial was similar to the earlier experiment but a new blocks of trees were selected. As previously described, the five spray treatments were applied to groups of 25 trees within a row. Treatments were separated by two 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 4).

 The revised spray treatments were as follows:

   1. Water plus LI700® 1ml/l

   2. 290 g ai/ha ethephon plus 1ml/l LI700® (0.67ml/L)

   3. 580 g ai/ha ethephon plus 1ml/l LI700® (1.34 ml/L)

   4. 870 g ai/ha ethephon plus 1ml/l LI700® (2.01 ml/L)

   5. 15 kg KNO_3 /ha 1ml/l LI700®
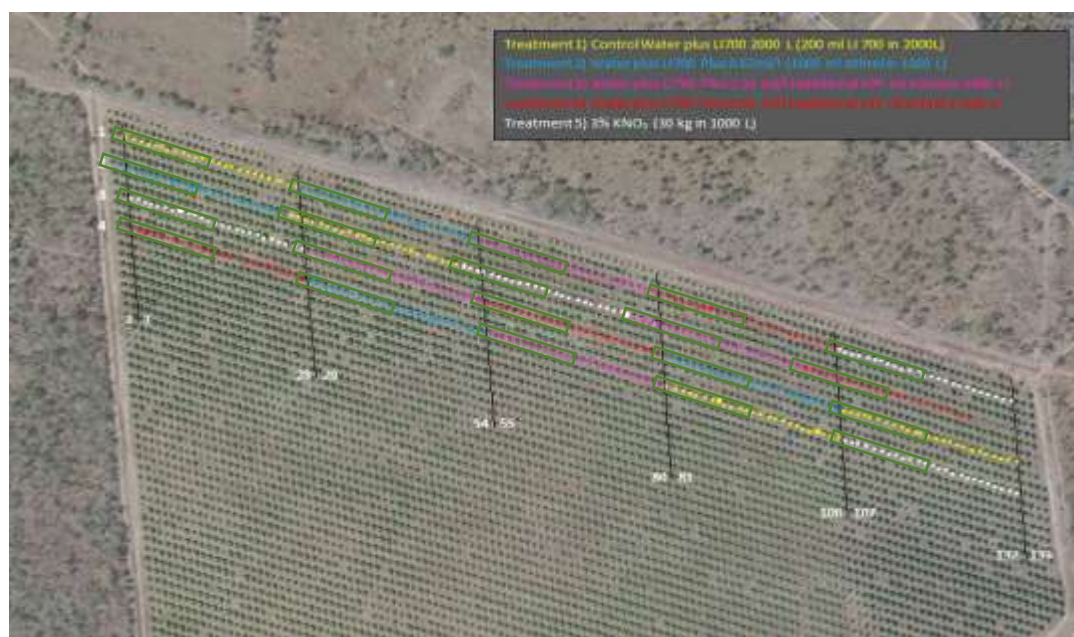
Figure 4. *Layout of the May 2014 trial at Lambells Lagoon investigating the effects of ethephon application levels and the response to KNO₃. Orange rectangles indicate trees treated with additional KNO₃ sprays. Colours as per key*

Twenty branches at different stages of flush development were tagged per spray treatment using the stages of flush development are shown in Figure 5. 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.

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 5. *Stages of buds tagged to identify stages affected by different ethephon and KNO₃ spray formulations.*

On the 10th June 2014 (20 days after treatment), 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 KNO₃ / ha (3 %) with 1 ml/l LI700® to stipulate flowering. Two days later, 12th June 2014, the same KNO₃ sprayed trees were sprayed again with 7.5 kg KNO₃/ha (1.5 %) with 1 ml/l LI700®. On the 10th August 2014, trees were assessed for percentage of terminals flowering.

Table 3. Mean percentage of terminals developing inflorescences on the east and west side of trees on 10th June 2014, following two 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	0.0	0.0
Ethephon 580 g ai/ha	0.0	0.0
Ethephon 870 g ai/ha	0.1	0.0

Only the KNO$_3$ treatments formed flowers (Table 3). The 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 the application on June 10th to have caused this flowering (Fig. 6) 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 cultivars.
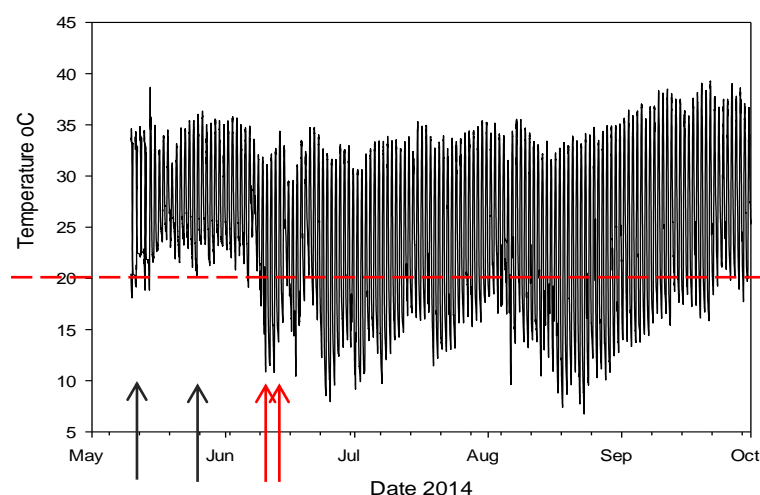
Figure 6. Maximum and minimum temperatures (°C) monitored at Lambells Lagoon during the period when ethephon and KNO$_3$ treatments were applied. The threshold temperature below which flower induction in believed to occur in mangoes indicated. The black arrows indicate the evenings that ethephon treatments 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 until September. New flowers were potentially being constantly recruited over this period. When assessed on 10th August 2014, the KNO$_3$ treated trees had consistently more inflorescences than the non-KNO$_3$ treated trees (Table 4). Similarly, the ethephon treated trees also performed well.

Table 4. 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.

Side of tree	Plus KNO$_3$		Minus KNO$_3$	
	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 5. 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 5. 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.

Treatment	Flush stage				
	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

1.4 Conclusion

These results indicate that in order to remove all stages of leaf flushing from a swollen bud to a soft green flush that 870g/ha of ethephon was the most effective treatment examined. This differs considerably from the recommendation of both Sandry (2010) and Griffin (2011) who recommended 192 ml or 270 ml of product (a.i. 48 % ethephon). This difference may be explained by our applications being applied at night and by a tractor-propelled sprayer. Subsequent to these trials, the collaborating grower used a spray formulation of 1000 g ai/L with 1 ml/1L LI700®, at 1200g ai/ha, to routinely control unwanted vegetative flush applied at night.

2. Use of Ethephon to delay flushing

2.1 Introduction

Another positive effect promoted by Liu 2005 from the treatment of Kensington pride mangoes with ethephon is the potential to 'hold up' growth. This was based on the lack of flush from trees treated with 0.1 % two or more weeks after treatment. It was suggested that Ethrel® concentrations of 0.05 to 0.7 % be used for this purpose and spraying be done after 5 pm.

2.2 Methods and materials

In a single row of more than sixty cultivar B74 mango trees were selected. From these, 36 trees of comparable size were identified and randomly numbered. All branches were tip pruned and 10 branches on each tree were tagged. These trees were allocated to three blocks of 12 trees each. Three trees of each block were allocated to the treatment. These treatments were:

      1. Untreated control.

      2. Single ethephon 0.5 g/L

      3. Single ethephon 0.25 g/L

      4. Four ethephon 0.25 g/L sprayed at fortnightly interval

Trees were tip pruned to force bud growth on April 10th and of these eight cut terminals were labelled to monitor their growth and evaluate the effect of the sprays. The bud growth was measured using the subjective scale described above. The responses to treatments were assessed on the 10th of July 2014, 14 days after the application of the last treatment. The mean of the subjective assessments are presented Table 7below. In addition, all the treatments were repeated a fortnight then a month later as shown in Table 6Table 7.

Table 6. Treatments applied to three replicates of individual tree of ethephon at the level and dates (Early Mid and Late) indicated. Trees were sprayed to runoff that was equivalent to 0.75 L.
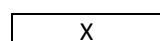
	Date					
	17/04/2014	31/04/2014	14/05/2014	30/05/2014	12/06/2014	26/06/2014
Early	X					
	X					
	X	X	X	X		
Mid		X				
		X				
		X	X	X	X	
Late			X			
			X			
			X	X	X	X

0.5 ai g/L

0.25 ai g/L once

0.25 ai g/L four times

Control: No treatment

X   Treatment date

2.3 Results

Table 7. Mean stage of flush following ethephon treatments evaluated on July 14th.

Treatment	Early	Mid	Late	Mean
Full X1	0.44	0.15	0.79	0.46
Half x 4	1.38	0.63	0.52	0.84
Half x 1	1.52	1.63	1.42	1.52
Control	0.50	2.21	2.46	1.72

The responses were variable as is evident in the control where the two of the untreated controls had no growth for the entire period (Table 7). Generally, the single spray of 0.5 ml/L was the most effective at reducing vegetative regrowth followed by the four-x spray of 0.25 ml/L ethephon. This treatment effect does not seem to be cumulative as these trees were exposed to twice amount of ethephon but seemed to have more or equivalent level of vegetative flush. The single 0.25 ml/L was the least effective at reducing flush development. These results appear to confirm that ethephon can be used as a means to delay vegetative flush and this is more effectively done using as single spray than multiple lower dose applications.

3. Investigation alternative of gibberellin synthesis inhibitors to the use of paclobutrazol for use on mango

3.1 Introduction

Investigation of range of gibberellin synthesis inhibitors have been trialed to determine whether any are suitable substitutes for paclobutrazol. The major advantage of these chemicals is that they are applied to the foliage reducing the risk that there will be residues in subsequent years that may result in a cumulative response to further applications. Paclobutrazol is known to affect root growth potentially affecting nutrient up take and nutrient partitioning in the plant (Kotur, 2006). In mango and other plant systems, leaf nutrient content varies both positively and negatively for different nutrients which may affect interpretation of nutritional standards such a leaf analyses. The three alternative anti-gibberellin synthesis compounds that have most widely been trialed in mango, are prohexadione calcium (calcium-oxide-3-propionyl-4-oxo-5 cyclohexene 3-carboxylate(ProCa), trinexapac-ethyl chloride (4-cyclopropyl methylene hydroxy acid 3, 5-dioxy cyclohexane carboxylic acid(TrixE), chlormequat chloride (2-chloroethyl trimethyl ammonium(CCC). Together they represent three of the four groups of anti-gibberellin synthesis groups (Verma et al., 2010). Both ProCa and TrixE operate at the final stages of gibberellin synthesis by inhibiting the formation of highly active gibberellins from inactive precursors. By comparison CCC functions to stop synthesis at early stages, preventing the formation of precursors and activity in the plant are characterized by preventing cell extension resulting in shortened inter nodes. Paclobutrazol also blocks early stages of gibberellin synthesis but downstream from where CCC is active. It blocks the oxidation of ent-Kaurene, which leads to the precursors for the formation of gibberellins.

Among the options Prohexadione calcium (ProCa) is the most favoured because it breaksdown relatively rapidly (half-life approximately 7 to 10 days) and is effective only in young shoots. Furthermore, the compound is translocated almost exclusively in the xylem and is, therefore, unlikely to be accumulated in the fruit (Rademacher, 2000). It is thought that ProCa breaksdown in the soil within 24 hours of application. TrixE is also an inhibitor that effects the end of the synthesis process of gibberellin. Applications of TrixE to mango was thought to be effective for 45 days after treatment (Mouco et al, 2010a). We have not included CCC in our studies as the previous work by Mouco et al., (2010a,b) using seedlings showed that it caused excessively compacted shoots.

The aim of this trial was to determine the capacity of two commercially available gibberellin synthesis inhibitors that are foliar applied to prevent vegetative flushing and respond to flower induction treatments.

3.2 Material and Methods

Two foliar applied anti-gibberellic acid treatments were investigated. The first of these examined the effects of prohexadione-calcium (Regalis® BASF) (referred to as ProCa) and trinexapac-ethyl (Primo MAXX® Syngenta). (TrixE) These chemicals had previously been investigated in Brazil to substitute for paclobutrazol to control vigorous regrowth after harvest and pruning (Mouco et al., 2010). In our investigations, we were aiming to control growth of Kensington Pride mangoes after the wet season (March-May). The trees we treated were sprayed with ethephon in April 16th and the first of three sprays applied on April 18th. The co-operating grower had previously treated these trees with paclobutrazol in December 2015. The formulation of the first spray is given in Table 8. The chemical content was increased in the second (applied 12th May) and third spray (applied 31st May) (Table 9). Note an additional treatment was included in the second and third spray of 50 g/L KH2PO4 (5 %W/V MKP) however were only three treated and control untreated trees. For the remaining treatments, there were four plots which were randomised in a single north-south

oriented row of 6-year-old Kensington Pride trees. The cumulative amount of chemical applied to the trees by the sprays is shown Table 10. There were single tree replicates within each plot. A single buffer tree separated each treated tree. Sprays were applied in the order the treatments are listed using a Stihl backpack mist sprayer and received 3 L of spray equivalent to run-off. The irrigation to the orchard commenced on May 14th. The trees were scored for vegetative growth at weekly intervals from May onwards. In all sprays contained 1 ml/L Pulse® Nufarm a modified polydimethylsiloxane wetter.

Table 8. Spray formulation of anti-gibberellic acid treatments applied April 18th 2016.

Treatment	Chemical
1	Control
2	0.03 g/L Prohexadione-Calcium
3	0.06 g/L Prohexadione-Calcium
4	0.1 g/L Prohexadione-Calcium
5	1.5 g/L Prohexadione-Calcium
6	0.12g Trinexapac-ethyl
7	0.30 g Trinexapac-ethyl

Table 9. In the second (12th May) and third spray (31st May) treatments the anti-gibberellic acid chemical concentrations were revised. An additional treatment of 50 g/L (5 %W/V) KH2PO4 and an additional set of controls was included.

Treatment	Chemical
1	Control
2	0.18 g/L Prohexadione-Calcium
3	0.37g/L Prohexadione-Calcium
4	0.5 g/L Prohexadione-Calcium
5	1.1 g/L Prohexadione-Calcium
6	0.45g Trinexapac-ethyl
7	0.9 g Trinexapac-ethyl
8	Control 2
9	50g/L KH_2PO_4

Table 10. Amount of active chemical applied to each tree in each spray treatment and the total for the spray series.

Active ingredient	Treatment Number	Spray 1 Active ingredient applied per tree (g)	Spray 2 Active ingredient applied per tree (g)	Spray 3 Active ingredient applied per tree (g)	Total active ingredient applied per tree (g)
ProCa	2	0.09	0.70	0.70	1.49
	3	0.18	1.40	1.40	2.98
	4	0.30	1.88	1.88	4.05
	5	0.45	4.13	4.13	8.70
Trix E	6	0.36	1.62	1.62	3.60
	7	0.60	3.30	3.30	7.20
KH_2PO_4	8		150	150	300

3.3 Results

Prohexadione-Calcium (ProCa) appeared in increase vegetative flushing at the top of trees especially

evident for the highest concentration 1.1 g/L on June 6th (*Figure 7*). Growth was evident in the sides of the trees following ProCa treatment the trinexapac-ethyl (TrixE) reduced flush activity markedly and at the highest concentration tested stopped growth on the entire tree. The mono-potassium phosphate did not appear to have any effects on controlling flushing compared to the controls.

Figure 7. Response to ProCa (Prohexadione-Calcium), TrixE (Trinexapac-ethyl) or KH_2PO_4 *on production of vegetative flushes on Kensington Pride mangoes at the top, eastern and western sides of trees observed on the 6th June 2016.*

Eight days later, June 14th, the promotion of top growth due to Pro Ca treatment is evident with increasing levels with increasing amounts of ProCa application (Figure 8). The low level TrixE has also developed a vegetative flush at the top of the tree while at the highest TrixE level there is still not growth.
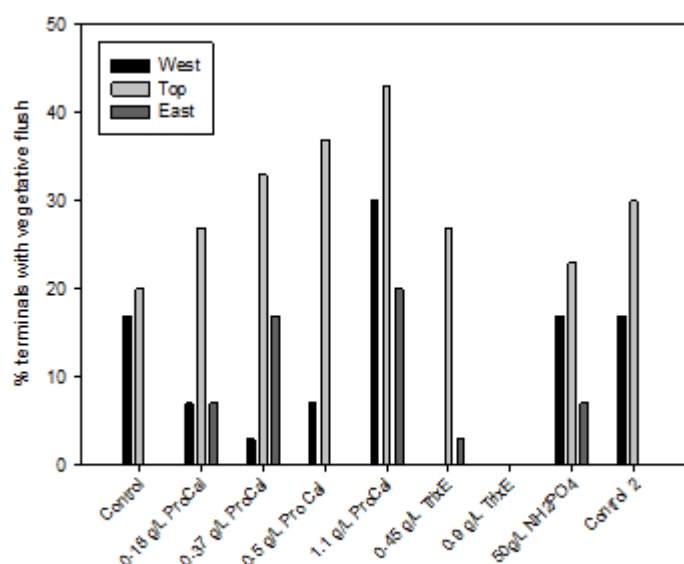
Figure 8. Response to ProCa (Prohexadione-Calcium), TrixE (Trinexapac-ethyl) or KH2PO4 on production of vegetative flushes on Kensington Pride mangoes at the top, eastern and western sides of trees observed on the 14th June 2016.

A fortnight later again the sides of the trees in the Pro Ca treatment have started a vegetative flush

but the TrixE treatment still appeared suppressed with still no flush activity evident at the highest TrixE application level (Figure 9). The Mono-potassium phosphate still resembles the control treatment.
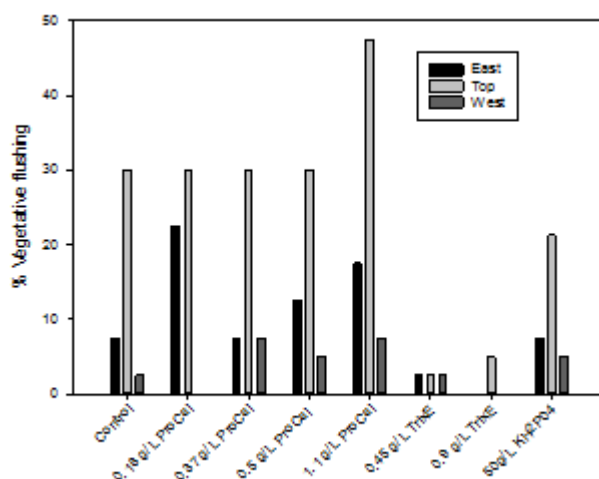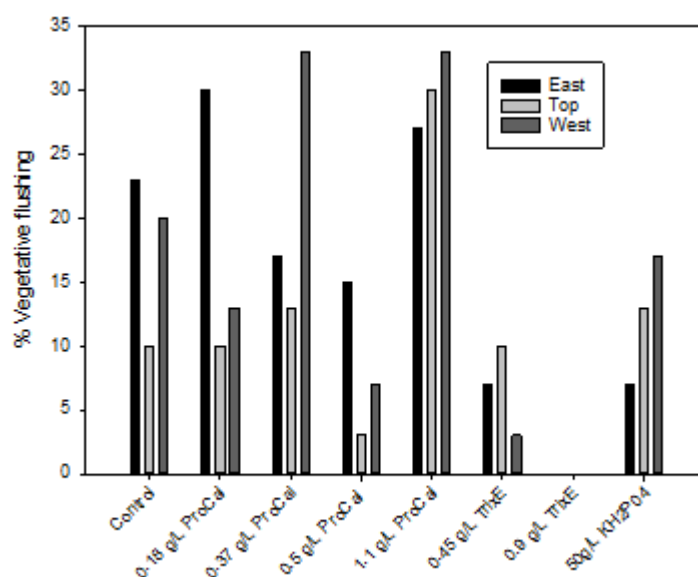

Figure 9. Response to ProCa (Prohexadione-Calcium), TrixE (Trinexapac-ethyl) or KH2PO4 on production of vegetative flushes on Kensington Pride mangoes at the top, eastern and western sides of trees observed on the 28th June 2016.

Flowering was evident on all treatments by July 28th (Figure 10). The higher levels of ProCa and TrixE appeared to lag behind the controls and lowest levels of ProCa application.
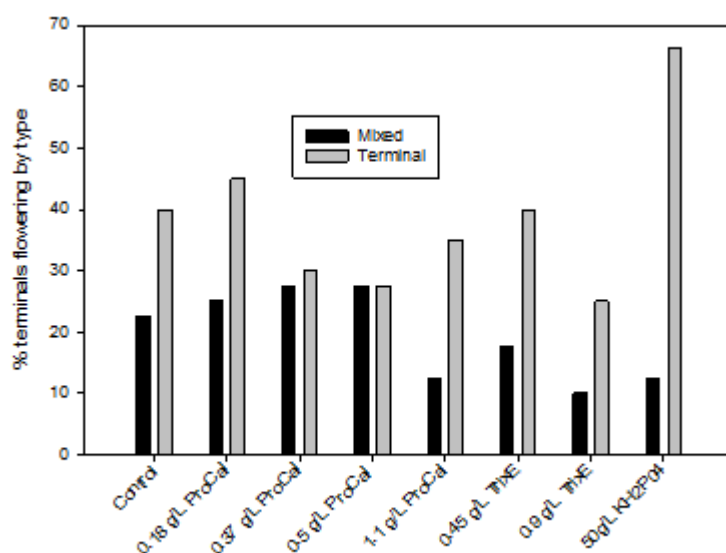
Figure 10. Response to ProCa (Prohexadione-Calcium), TrixE (Trinexapac-ethyl) or KH2PO4 on production of flower types either mixed (flowers and leaves) on Kensington Pride mangoes trees observed on the 28th July.

Extensive flowering had occurred on both eastern and western aspects of the tree. There appeared to be some depression in the level of flowering on the western side of the TrixE treated trees and the highest concentration of ProCa on 17th August (Fig 11).
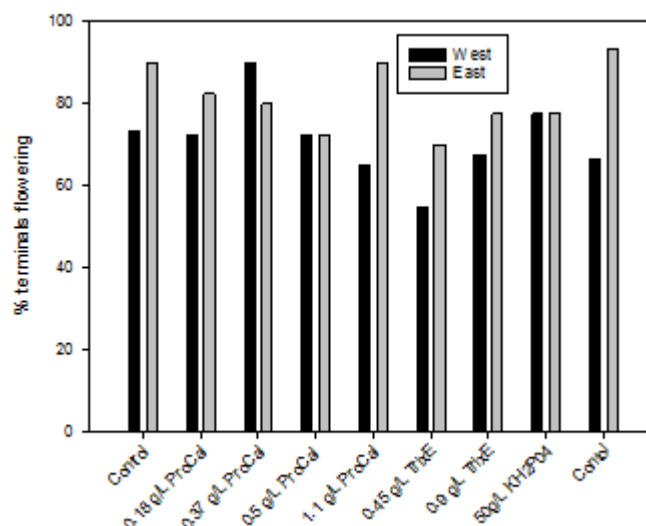
Figure 11. Percentage of terminals that developed inflorescences on the eastern and western side of Kensington Pride mangoes treated with ProCa (Prohexadione-Calcium), TrixE (Trinexapac-ethyl) or KH2PO4 on Kensington Pride mangoes observed on the 17th August 2016.

The most advanced flowering occurred on the mono-potassium phosphate treated trees, associated controls, and lowest level of ProCa treatment by the 25th of August. The increasing concentrations of ProCa appear to have prolonged or delayed anthesis, as did the Trix E treatments. There was no evidence to suggest flowering was advanced by these treatments. There were large fruit present on the controls, mono-potassium phosphate and lowest ProCa treatment at this stage. Small fruit were also present on other treatments. The trials had to be abandoned due to the sale of the property and were treated with ethephon to ensure treated fruit were not harvested if allowed to mature. No yield data was available due the shift crop development.
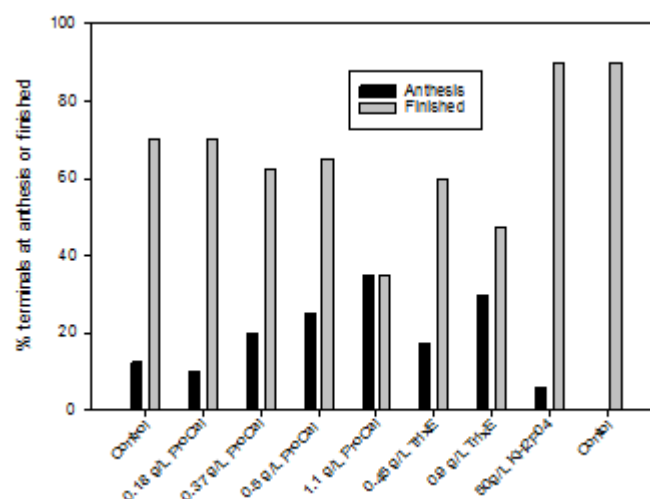
Figure 12. Percentage of terminals that had finished flowering or undergoing anthesis following treatment with either ProCa (Prohexadione-Calcium), TrixE (Trinexapac-ethyl) or KH_2PO_4 observed on 25 August 2016.

2.1.4 Discussion

Treatment with ProCa appeared to promote a leafy flush at the top of trees this is consistent with the work of Mouco et al., (2010) who also ProCa in potted plants promoted vegetative flush and increased the number of leaves produced by that flush at certain concentrations. Similarly, TrixE

was found to effectively prevent vegetative flushing and this effect was long lived. Our results for Prohexadione-Calcium differ considerably when compared to Perez Barraza et al. (2016), who were able to substitute this chemical for paclobutrazol to control tree vigour and induce flowering. In these experiments, ProCa was used at concentrations ranging from 0.15 to 0.50 g/L in four separate experiment in mature trees (Table 11). There was no evidence that flowering was advanced by ProCa as previously reported by Abdel Rahim et al., (2011) and Mouco et al., (2011). Unfortunately, the orchard was sold during the trial so we could not follow the crop through to maturity to determine if there were any yield benefits.

The response to ProCa has been compared with soil applications of paclobutrazol applied to the trunk of trees, or paclobutrazol and ProCa applied 30 days after pruning. In our trials, trees were pruned and treated with paclobutrazol in December and our treatments started in April. This delay may explain the differences in responses observed. This work needs to be repeated taking pruning date and application of paclobutrazol into account.

Based on the results Perez Barraza et al. (2016), we used a non-ionic wetter in our trials however, the Regalis ® label specified that it should be used with LI700®. In all our previous research with ethephon we have obtained more consistent results using LI700® than a non-ionic wetter on Kensington Pride. Based on the lack of response, future studies should consider using LI700® as the wetter penetrant. The label also specifies that ProCa should not be used within 3 days of using ethephon and we applied it 2 days after treatment in the first spray. The second and third spray treatments were not impacted by ethephon.

Table 11. Summary of the results of using Prohexidione Calcium or paclobutrazol on mango cv. Atoulfo in Mexico (Pérez Barraza et al., 2016).The control treatment is not described in this table but receive no chemical treatment. Off years were attributed to high temperatures (>30 $^\circ$C) and rain.

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Tree age (years)	10	8	15	15
Tree spacing (m)	7 x 5	5 x 5	9 x 9	9 x 9
Pro Cal mg L^{-1}	150, 250, 500	150, 250, 500	500	3 times 150, 500 1 time 1500
Applied days after pruning	4 times 0,10,20,30	4 times 30, 45, 60	0 0 + 10 0 + 10 + 20	3 times for 150 and 500 30, 45, 60 1 time for 1500
Wetter Inex	1ml L^{-1}	1ml L^{-1}	1ml L^{-1}	1ml L^{-1}
Standard Paclobutrazol treatment	500 mg L^{-1} applied 30 cm to base of trunk to saturation	500 mg L^{-1} applied 30 cm to base of trunk to saturation	500 mg L^{-1} applied 30 cm to base of trunk to saturation	500 mg L^{-1} applied 30 cm to base of trunk to saturation
On or Off year	On	-	On	Off
Flush growth/ Flowering	250 and 500 mg treatments reduced length of first vegetative flush but no differences after 2nd flush	500 shortest first flush but after 2nd flush all the same	All 1st flush shorter but the same after 2nd flush	Flowering % increase in 500, 1500 and PBZ treatments compared to control
Significant Yield difference	4 x 500 63 kg PBZ 75 kg Control 38 kg		1 x 500 74 kg 3 x 500 80 kg PBZ 80 kg Control 47 kg	1 x 1500 240 kg PBZ 237.5 kg Control 149.5kg

We had difficulty in determining the concentrations of sprays for use in these trials. The chemical rates for ProCa, TrixE and CCC treatments were developed from glasshouse based on potted plants treated with a single dose (Mouco et al., 2010). Both CCC and TrixE reduced plant grown by reducing the number of flushes and the internode length. The ProCa treated plants had increased leaf area with a single high dose, which is similar to that reported by (Mouco et al., 2010). ProCa and CCC bought the harvest forward by 12 days while PBZ as a soil drench or in combination a foliar spray ProCa advanced harvest 25 days but delayed ripening (Monco et al 2011). Based on the results these same chemicals were applied to 6 year old mango trees cv Kent planted 8x5 (Mouco et al 2011). The dose per plant was increased for ProCa to 1.5 or 3 g/tree from four sprays 20 days apart, for TrixE to one or 2g/tree from two sprays 45 days apart, and for CCC to 1.5 or 3.0 g/tree spread over three sprays 30 days apart. As a control Soil applied paclobutrazol at 4 g/tree was used or a combination of paclobutrazol 4 g ai/tree plus a single spray of ProCa 1.5g ai/tree both treatment containing paclobutrazol were more effective at controlling vegetative growth. However, after 230 days there were no significant differences in panicle development.

A different spray approach was taken by Abdel Rahim et al., (2011) who applied two sprays contain 0.5 g ai/l ProCa two weeks apart finishing July 31st, 2 months before the first flowers were recorded on October 4. Flowering was recorded over the next 2 months, 100 % flowering was observed on the treated trees, and no flowering occurred on the untreated controls over this period. No information on the amount of chemical applied to each tree was recorded.

The use of ProCa has also been investigated on mango cultivar Ataulfo in Mexico to mango vegetative growth and promote flowering (Pérez Barraza et al. 2016). In these trials mature orchard trees were used and the amount of ProCa was substantially higher. As previously investigated by Mouco et al. (2010). Multiple sprays were used presumably due to the short half-life of ProCa. There was considerable variability in the results of the experiments, which in part was explained by on, and off years for production. While the volume of spray used on each tree was not recorded levels as high as 1.5 g/L of ProCa were found to be effective.

Regalis® (Nufram, 10% ProCa) label rate for treating apples is to use 0.5 g/L to 0.75 g/L product on two to three occasions at 3 to 5 week intervals when new growth is <5 cm. If required, an additional spray can be used on very vigorous trees. For cherries, the label permits 1.0 g/L and two sprays at 3 to 4 week interval. It is recommended that this occur under slow drying conditions.

The mono-potassium phosphate (MPK) treatment was included as Vietnamese mango growers use two sprays of 0.5% MPK to obtain similar floral induction responses as potassium nitrate (KNO_3) and thiourea. In Brazil, MPK or monopotassium sulphate has been recommended to mature vegetative flush making it more responsive to floral inductive treatments. In our trials, there were no obvious differences in flowering of the MPK treatments.

In conclusion, the response to prohexadione calcium visibly differs to treatment with paclobutrazol in that it promoted a flush while paclobutrazol inhibits vegetative vigour. The concentration of ProCa which provides optimal benefit is between 0.5 to 1.5 g/L as either a single or multiple spray application. These treatments need to be combined with potassium nitrate to determine whether flowering can be advanced by these treatments.

4. Comparison of foliar applied paclobutrazol and trinexpac-ethyl

4.1 Introduction

Based on the responses to thiourea it was proposed to treat mangoes with growth inhibitors in June and attempt to induce flowering under non-inductive conditions in September and October. Currently the label use of paclobutrazol allows 20 ml of 25 % active ingredient to be applied to the largest mango trees as late as mid-February. The trees used in the current trials were treated in December so that by June it was approximately 160 days after treatment, well beyond the 90 days recommended in Brazil. Internationally, there are suggestions that in tropical regions considerably higher doses are applied to trees, approaching 9 to 10 fold. In Brazil, there have been a number of studies suggesting that with repeated use the soil microflora develops to rapidly breakdown paclobutrazol potential reducing its efficacy (Vas et al., 2015). There are no comparable studies in Australia and no long-term measures of efficacy in the range of environments in which mangoes are grown. It was considered that the trees would have low residual level of anti-gibberellin compounds in the leaves so attempts to increase these using foliar sprays was investigated.

While the literature suggested leaves do not readily absorb paclobutrazol, this has not been tested using a wetting agent (e.g. LI700®). Similarly, trinexapac-ethyl had proven the most effective of the foliar applied anti-gibberellic acid treatments.

4.2 Materials and methods

On June 9th a separate set of trees were sprayed using a backpack sprayerwith the chemicals listed in Table 12. As previously described there were four plots within a single row with randomised single tree plots with single tree buffers were used.

Table 12. Chemical composition of foliar applied growth regulators. All sprays contained 1 ml/L LI700®® (Nufarm).

Treatment	Chemical
1	control
2	1 g/L paclobutrazol
3	2 g/L paclobutrazol
4	3 g/L paclobutrazol
5	0.12 g/L Trinexapac-ethyl
6	0.24 g/L Trinexapac-ethyl
7	0.48 g/L Trinexapac-ethyl

4.3 Results & Discussion

The results were unexpected as all treated trees flowered about 1 month later. The most intense flowering was observed on the trees treated with 1 g/L active paclobutrazol. (Figure 13). There is evidence that the paclobutrazol was taken up by the tree as panicle length decreased with the increasing level of paclobutrazol applied (Figure 14). The trinexapac-ethyl appeared to reduce flowering but did seem to reduce panicle length at the highest concentration applied.
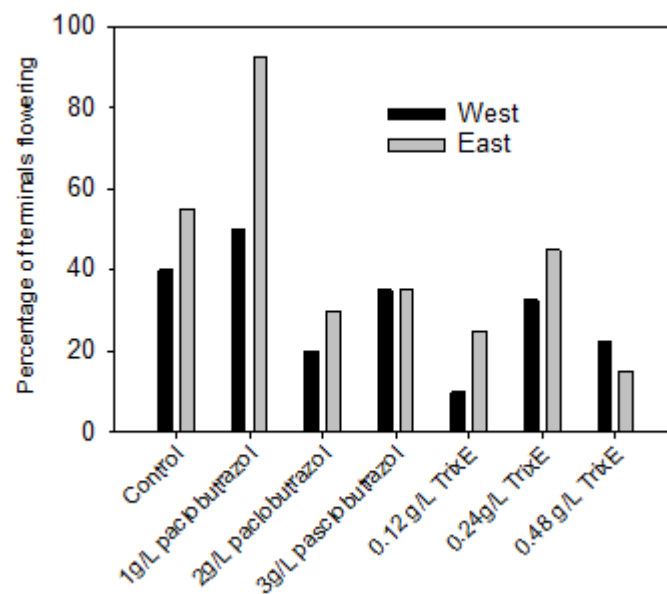
Figure 13. Mean percentage of terminals flowering on the west and east side of trees treated with different concentrations of foliar applied paclobutrazol or trinexapac-ethyl.
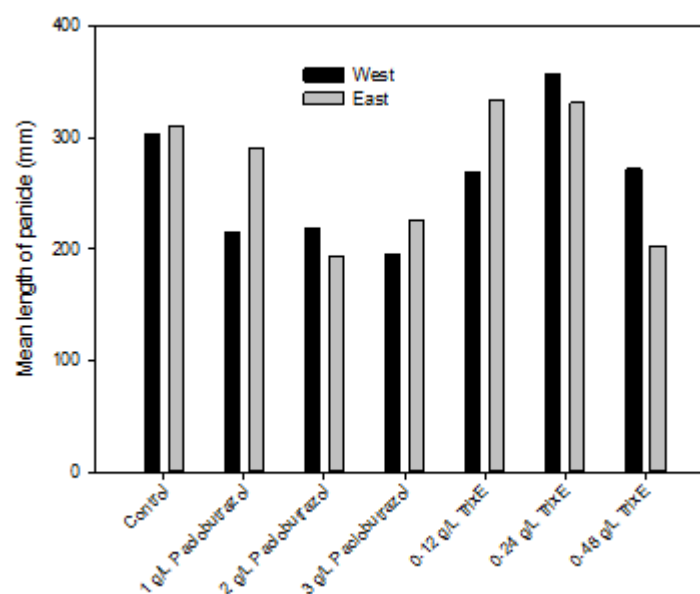
Figure 14. Mean length of twenty randomly selected panicles on trees treated with different concentrations of foliar applied paclobutrazol or trinexapac-ethyl.

There were some additional interesting observations. The flowers on the controls underwent anthesis over several weeks resulting in fruitlets with a range of ages that would make uniform maturity at harvest more complex. The flowers on the trees sprayed with two highest concentrations of paclobutrazol were sterile and appeared as dried husks. Therefore treatments should not exceed 1 g/L The flowers a lowest concentration of paclobutrazol were had synchronous anthesis, were fertile and developed fruitlets.

In the initial investigations on the efficacy of paclobutrazol in Kensington Pride mangoes comparisons were made between soil collar drenches and foliar sprays (Winston, 1992). Collar

drenches rates in this work were between 29 to 58 ml of active ingredient which were compared with 0.75 L /tree sprays of 1 ml or 2 ml per L active ingredient. The foliar sprays were applying 0.75 g or 1.5 g of paclobutrazol per tree using Agral 60 as the surfactant and were found no to affect tree growth. In our trial, we applied 3, 6 or 9 g of ai to the foliage of the trees using LI700®®. This was two to six times more active ingredient than used by Winston (1992) with potentially a more effective penetration. Our trees were sprayed at night to maximise the time taken for the sprays to dry and allow penetration even though paclobutrazol is a suspension. These sprays were applied 10 days prior to the commencement of floral inductive temperatures. Surprisingly the highest level of floral initiation was observed on the eastern side of the 1 ml/L paclobutrazol treated trees with all other treatments suppressing growth. However, the flowers that developed on the 2 and 3 ml/L paclobutrazol treatments were sterile. This was unexpected as paclobutrazol is meant to be primarily transported in the xylem but for a product absorbed by the leaf to reach the floral apex it would need to travel via the phloem. This suggests that the paclobutrazol has affected a secondary product that has been transported to the apices or the paclobutrazol has affected the apices directly.

The TrixE treatments had no obvious effects on the length of panicles. The levels of TrixE used were more than half the amount that stopped growth in earlier trials. Illustrating that there are temporal challenges to this efficacy of this compound.

5. Monitoring the uptake of paclobutrazol under aeroponics

5.1 Introduction

Paclobutrazol is widely used to reduce vigour in mango trees and to promote flowering leading to increased yields (Davenport, 2009). In Australia, paclobutrazol (PBZ) is registered on mango to be used as a collar drench at up to 20 ml product/tree applied 4 weeks after harvest and no later than mid-February. On sandy soils, it is recommended the tree commence vegetative regrowth after harvest prior to treatment. In practice, the chemical is diluted in several litres of water to assist in application and spread around the root zone. Paclobutrazol is a gibberellic acid inhibitor (Rademacher, 2000). It reduces tree vigour and decreases the time for vegetative flushes to become responsive to flower promoting chemicals such as potassium nitrate (Davenport 2009). It is applied 90 to 120 days prior to when flower induction is required. Palobutrazol (PBZ) has been found to reduce levels of gibberellins in subapical tissues (Guevara et al., 2012). In studies based in the warm tropics PBZ neither induced earlier flowering nor produced more abundant flowering but actually delayed flowering. This Indicates that PBZ on its own is not sufficient to induce flowering. Similar observations were made in other tropical areas and sub-tropical areas in the absence of cool temperatures. PBZ was not sufficient to promote flowering (Blaikie et al 2004, Yeshitela et al 2004). In Darwin, PBZ is applied from November until February to promote mango flowering from May to July.

The aim of the current investigation was to:

- Investigate the duration of exposure required for paclobutrazol uptake.
- Describe the impact of paclobutrazol on above and below ground plant parts.

5.2 Methods

Fruits of mango cultivar Kensington Pride were harvested from Jabiru Tropical Orchards, Berry Springs. Seeds were extracted and grown in potting mix of 3:1 peat moss and sand. Two-month-old plants were transferred to 1 L pots of perlite and pots suspended in cement sheets above 200 L plastic bins. There three plants per bin which were irrigated with culture media using a timer based irrigation system. The root systems were irrigated with a mist sprayer (35 l/h) (Sage Horticulture, Vic) that was regulated by Digisprink 2000 (Sage Horticulture Vic) controller to open the regulating solenoid every 8 min for 2 sec during daylight hours and every 40 min for 2 sec in darkness.

The components of the culture solution is shown in Table 13. During each preparation, the pH of the media was adjusted to 6.5 and electrical conductivity to 2.0 – 2.1 mS/cm using tap water. Plants were grown for three months before the application of treatments.

Prior to commencing the trial, roots were cut to a standard length of 300 mm below the base of the pots and shoot height measured from the top of the pot to the tip of the plant.

To expose plants to paclobutrazol, 1 mg/L of the active ingredient was added to the culture solution used to irrigate the plants. Plants were exposed to this media for 0, 12, 24, 48 and 96 h prior to washing roots with tap water and transferring to new bins irrigated with culture solution without any PBZ. Each treatment was replicated three times. A complete randomised design was used to allocate plants to bins and exposure times.

The following growth parameters were measured at fortnightly intervals:

- Shoot, length was measured from the base of the plant to the tip of the bud
- Plant diameter was be measured 1cm from the bottom of the plant
- Number of leaves were counted and flush status recorded

- Root length was be taken from the bottom part of the vase to the longest root tip.
- Stem diameters were measures at the base and tip of new stem (growth unit)

Table 13. The nutrient composition of aeroponics culture solution.

Element	Total Concentration (mg/L in solution)
Nitrogen	225
Phosphorous	51.5
Potassium	280.1
Calcium	190
Magnesium	28.4
Sulphur	46.2
Iron	2.05
Manganese	0.48
Boron	0.27
Zinc	0.12
Copper	0.05
Molybdenum	0.01

5.3 Statistical analyses

Data were 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.

5.4 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 12 hrs or more was seen to have a significant effect on inhibiting shoot growth/plant height ($P< 0.01$) (Figure 15 & Figure 16). Post-hoc Tukey's HSD tests showed that exposure times greater than 24 hours had less growth than the control and 12 hour treatment groups at the 0.05 level of significance. Exposure to 1 mg/active ingredient of PBZ for greater than 2 hours did not exhibit any further significant reduction in plant height.
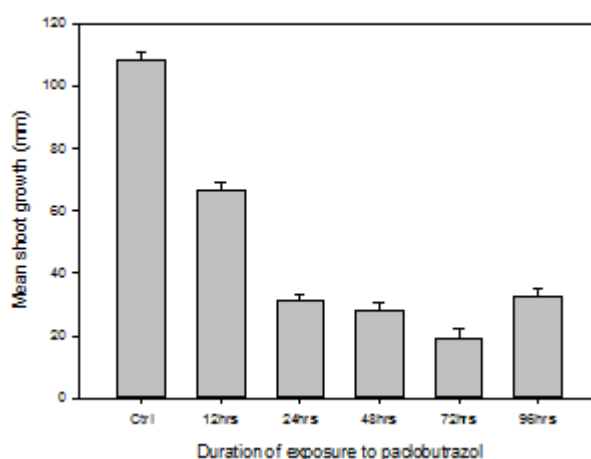
Figure 15. Mean height of mango plants exposed to 1 mg/L Paclobutrazol in culture media for periods up to 96 hours then monitored for two months.±SE.

Root length

Root growth was 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.
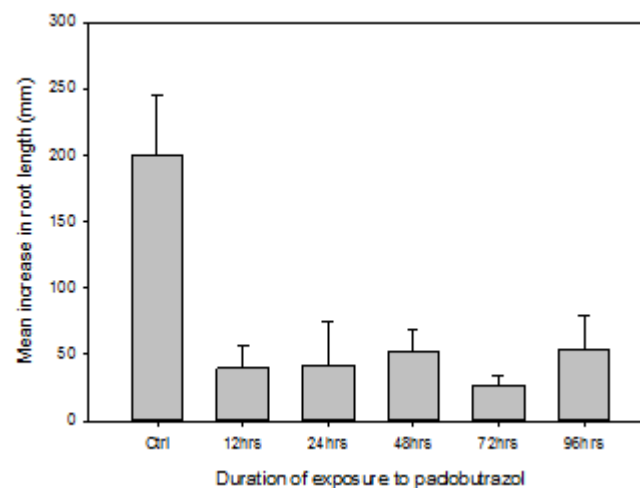
Figure 16. Mean Root growth of Kensington Pride mangoes two months after being exposed to a 1 mg/L aeroponic solution for durations up to 96 h ±SE.

New leaf production

After two months it was observed that paclobutrazol exposed trees developed fewer leaves than control group however, this was not statistically significant.
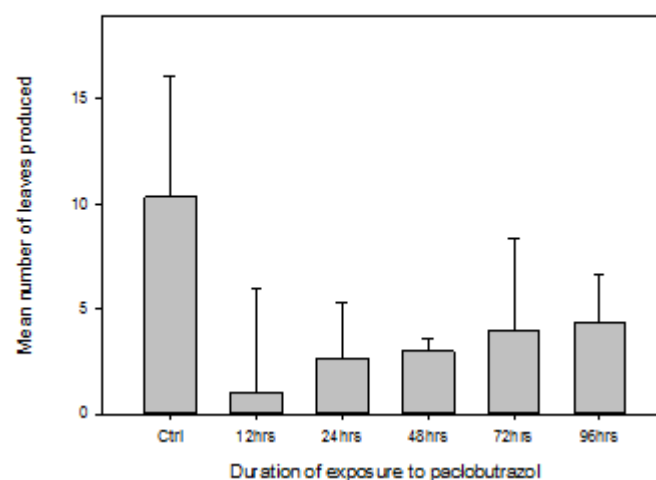
Figure 17. Mean number of new leaves developed on Kensington Pride plants expos to culture media containing 1 mg/L of paclobutrazol for the durations indicated then grown on culture media for 30 days before leaf counts.

5.5 Discussion

These results confirm the ability of paclobutrazol to inhibit vegetative growth in mango. This was evident by the reduction in above and below ground plant growth. While not statistically significant, it would seem that if plants were maintained for a longer duration the periodic flushing cycle would have slowed, resulting in less leaf development. This experiment showed that upon uptake of paclobutrazol, full inhibition was evident in roots after 12 hours while it took 24 hours exposure for the effect to be manifest in shoot growth. Since paclobutrazol is a suspension, it is possible the chemical particles could have bound to the root surface so that the washing of the roots was not sufficient to remove the product. This does not seem to have been the case as the inhibitory effects on shoot growth were progressive so that shoot growth after 12 hours was less inhibited than after 24 hours. If the particles had bound to the root after 12 hours, uptake could have continued after initial exposure for the 30 days that plants were allowed to grow after treatment. Developing a system in which the roots exposed to the paclobutrazol were physically removed would further support this conclusion. Singh (2001) has previously found that it took 30 days to affect gibberellin levels in mature mango trees. Abdel Rahim et al. (2011) also reported minimal changes in gibberellins in mature mangoes 4 weeks after soil application. In comparison, Naphrom (2004) cited in Bangerth (2009) found, in potted mango trees, a reduction in gibberellin levels for three weeks following soil application.

Assuming that the aeroponics system used reflects a similar response in orchard grown trees the uptake of paclobutrazol will be rapid while it is available in the root zone. This is supported by the reduction of gibberellins in treated trees and supports the conclusions of Guevara et al. (2012) that paclobutrazol itself does not induce floral initiation in mango since it is applied to mangoes months before floral induction. It is more likely that the increase in carbohydrate reserves and the slowing of the vegetative flushing cycle that allows the leaves of the most recent flush to age and become more responsive to inductive treatments and weather conditions such as low temperatures.

Previous studies have demonstrated modification of root distribution following paclobutazol treatment (Kotur, 2006) and changes in some mango leaf nutrient levels for Ca, Mg, Mn Fe, Zn and Cu. These changes have also been observed in other tree crops such peach following paclobutrazol treatment (Reiger, 1990). The time that paclobtrazol remains in the soil varies greatly depending on soil type and treatment history. In Brazil, it is believed that repeated use results in microbial selection and establishment of bacteria capable of biodegradation of paclobutrazol. This is highly influenced by soil type and moisture, with paclobutrazol (8 to 43 %) degradation occurring over a 40 day period (Vaz et al 2012, Vaz et al., 2015a; de Santo et al., 2013). Vaz et al. (2015b) showed that saturated soils with additional carbon resulted in almost complete breakdown of paclobutrazol after 70 days. Residues in the soil have been reported up to 150 and 300 days after application with residual effects lasting for up to 3 years (Reddy and Kurian, 2008).

The desired 120 days from application until the occurrence of cool weather will likely be exceeded if paclobutrazol is applied in November/December and may be exposed to extended periods of saturated soil over the wet season that can in extreme cases extend over three months. However if trees are not exposed to paclobutrazol prior to December, vegetative growth during the wet can become excessive. The current research shows that the uptake of paclobutrazol is very rapid so a strategy that adjusts the paclobutrazol treatment towards the end of the wet (February) could be investigated if there was a timely method to measure the residual quantity of paclobutrazol in the soil.

6. Effects of thiourea concentration and wetting agent on leaf abscission and axillary bud growth in mango.

6.1 Introduction

Thiourea is used for breaking dormancy in seeds, and stimulate shoot growth in deciduous crops such as pears and apples. It is widely used in Thailand, Vietnam and Cambodia for promoting out of season flowering in mango. It has previously been trialed in Darwin by NTDPIR staff as part of 'Deliverance Program' in Kensington Pride mangoes and to increase cropping in Honey Gold mangoes in Katherine (Winston, 2006). It is sold commercially as Dola (Dasco, Vietnam) in Cambodia and is used to promote off-season flowering in mango and durian and is recommended to be used at a concentration of 0.5 % with a spray volume of litres per tree. Tongumpai et al. (1997) reported that that concentrations of greater than 0.5 % thiourea resulted in excessive leaf drop that was exacerbated by use of wetting agents. To determine the concentration of thiourea in future trials the effect of varying thiourea concentrations and the inclusion different forms of wetters/penetrants.

6.2 Material and Methods

Five Kensington Pride mango trees were randomly selected within a single row of trees at Jabiru Tropical Orchards, Connelly Rd Lambells Lagoon on 24th February 2016 as shown in Figure 18.

Figure 18. Indicating the location of the experimental trees within a single row of trees at Jabiru Tropical Orchard. The eastern side of trees is to the right of the image

Twelve branches on each east and west side of the tree at waist height were tip pruned removing the ~10 cm apical portion of the branch, 24 branches per tree. On each branch, 10 leaves were counted back from the pruned apex and a piece of identifying flagging tape tied above the 10th leaf from apex. On 1st March 2016 the terminal leaves of one branch on each side of a tree was sprayed to run-off with one of the 12 spray formulations shown in Table 14. The number of leaves remaining on the treated branches were counted on the seventh, 14th, 21st and 30th March 2016 and any bud growth or production of leaves recorded. Daily maximum and minimum temperatures were recorded.

Table 14. Formulation of sprays used to investigate the response of Kensington Pride mango leaves to treatment with thiourea with Pulse® or LI700® compared no wetter.

Treatment Number	No Wetter	2ml/5L Pulse®	5ml/5L LI700®	Thiourea g/L
1	*			0
2	*			5
3	*			10
4	*			15
5		*		0
6		*		5
7		*		10
8		*		15
9			*	0
10			*	5
11			*	10
12			*	15
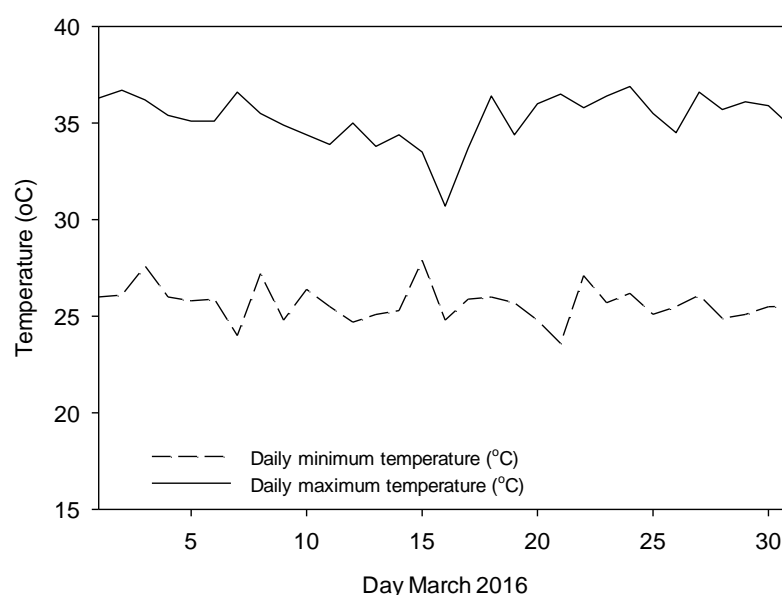
Figure 19. Maximum and minimum temperatures at Jabiru Tropical Orchards, in Lambells Lagoon NT, for March 2016.

6.3 Results and Discussion

This trial confirmed the observations of Tongumpai *et al.* (1997) application of thiourea at concentrations of 1.0 % and 1.5 % accentuated leaf drop when applied in water alone (Table 15). These effects were increased with the inclusion of LI700® or Pulse®. These effects were evident after 2 weeks at 1.5 % and increased up to 4 weeks. At 0.5 % thiourea there was leaf drop after 30 days post treatment, while using Pulse® caused leaf drop within a week of application in all the thiourea containing treatments. None of the water or water plus wetter treatments that did not contain thiourea appeared to cause pronounced leaf drop.

The effect of the treatments on different sides of the trees was very pronounced with two of the trees having very little growth on any of the treatments on the western side. In general, the treatments containing the wetters had greater development than the water no wetter controls.

Table 15. Mean stage of flush development based on subjective assessment related to leaf development of ten tagged branches of five trees and all thiourea treatments combined for wetter treatments

Side of tree	Treatment Mean		Assessment week		
		Week 1	Week 2	Week 3	Week 4
East	Water	0.1	0.35	0.8	1.05
	LI700®	0.23	1.3	1.9	2.5
	Pulse®	0.1	0.88	1.48	1.73
	Mean	0.14	0.84	1.39	1.76
West	Water	0.1	0.6	0.85	1.05
	LI700®	0.2	0.38	0.63	0.83
	Pulse®	0.13	0.88	1.2	1.45
	Mean	0.14	0.62	0.89	1.11

These results are consistent with the observation by Tongumpai et al (1997) in other mango cultivars that treating mangoes with 1.0 % and 1.5 % causes increased premature leaf drop. Tongumpai et al (1997) commented that previous researchers had noted that these effects were even greater when surfactants are used. In our results the two forms of surfactant increased defoliation with Pulse® showing the highest level. These trees were treated between 10 am and 1 pm when trees were experiencing the highest daily temperatures. While 0.5 % thiourea in water did not cause leaf drop bud growth was lower on treatments that did include a wetting agent. Since bud, growth is essential for floral induction the lowest concentration of thiourea (0.5 %) was used in future investigation using Pulse® as the surfactant.

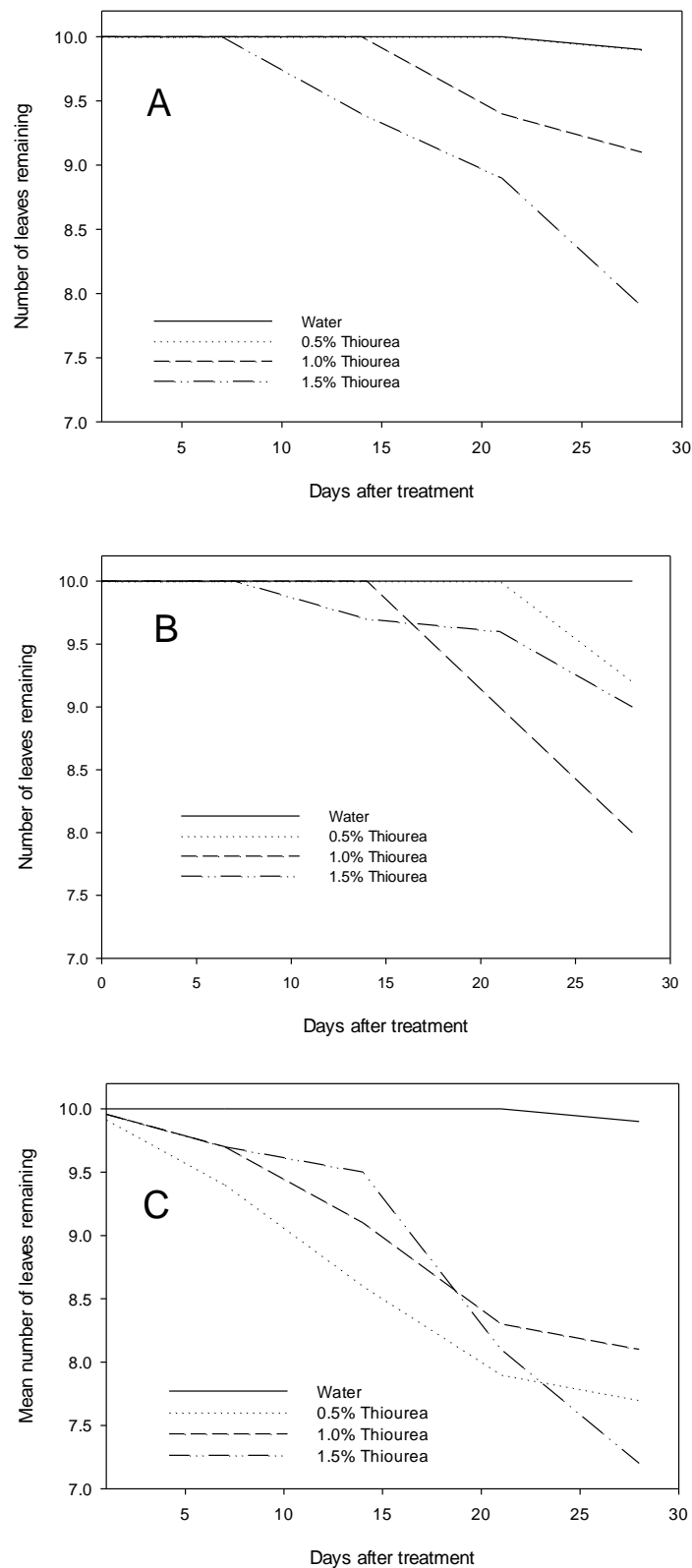*Figure 20.* A-C Response of pruned terminal branches of mango cv Kensington Pride bearing 10 leaves that were sprayed to runoff with Sprays containing A. No wetter B 1 ml/L LI700® (Nufarm) C 2).4 ml/L Pulse® (Nufarm). In the ± wetter treatment the sprays containing 0.5 g/L thiourea, 10 g/L thiourea or 15 g/L thiourea. Mean number of leaves remaining were counted at weekly intervals after treatment.

7. Comparison of the capacity of thiourea and potassium nitrate to promote flower induction in mango cv Kensington pride without inductive temperatures

7.1 Introduction

Winston et al. (2006) examined a similar formulation of thiourea at 0.5 % plus wetter to examine the impact on flowering, fruiting and quality in Katherine. The spray was applied on June 4th, 120 days after soil application of paclobutrazol on February 3rd. No record of the maturity of the flush treated was kept. The percentage of terminals flowering were recorded 3rd August and 8th September 2006 and crop harvested on 18th November. It was noted that there were no effects of thiourea on flowering, yield or fruit quality. The work on Honey Gold is confounded by temperature induction of flowering occurring at subsequent to the application of the thiourea treatments. This is similar to our results where thiourea caused earlier flowering in non-inductive conditions but as the trees entered the normal inductive conditions, the other treatments surpassed the effects of thiourea.

Previous investigation have examined the response of 14 cultivars by applying thiourea (0.5 %) 102 days after treatment with varying levels of paclobutrazol. About 40 days after paclobutrazol treatment trees flowered naturally but the crop failed due to anthracnose infection (Peris and Premachaudra, 2000). Trees were sprayed ~60 days later with thiourea and they found only four of the cultivars responded and flowered when observed three weeks after treatment. Trees treated with the highest level of paclobutrazol and thiourea flowering most intensely. Trees having had vegetative flush prior to thiourea treatment may explain the lack of response from other cultivars.

The effects of thiourea applied at concentrations from 0.5 % - 1.5 % on mango tree flushing and flowering when treated at increasing intervals after they had been treated with paclobutrazol (Tongumpai et al., 1997). They found thiourea triggered flushing on all the treated shoots they monitored but at 90 days post paclobutrazol treatments these flushes were vegetative, at 105 days, they were a mixture of vegetative and floral flushes and at 120 days, all flushes were floral. The control trees that were only treated with paclobutrazol only had ~70 % of shoots flushing, 50 % floral and 20 % vegetative. The bud break promoted by thiourea occurred 14-16 days after treatment but there was considerable phototoxicty. Tongumpai et al (1997) believed that paclobutrazol replaced the need for water stress to induce flowering and in comparing the response of paclobutrazol treated trees concluded that thiourea was more effective than potassium nitrate at 120 days at promoting floral induction.

Temperatures for the first 6 months of 2016 were exceptionally high with few night temperatures going below 20 °C until June 20th. There were a number of days prior to this when night temperatures went below 20°C this was only for a few hours and was insufficient to initiate flowering. The exceptional nature of regional night temperatures can be seen from the historical records of the numbers of days where daily minimums below 20 °C, 15 °C and 10 °C from January to June 30th (Table 16). The year 2016 represents the lowest number of days with minimums below 20 and 15 °C and the equal lowest for days, zero for days with minimums below 10 °C. Many of the days below 20 °C occurred after June 20th in 2016. This meant that floral induction due low temperature could not have occurred prior June 20th.

Table 16. Number a days with minimum temperatures below 20 °C, 15 °C and 10 °C for Middle Point (NT) from January 1st to June 30th for the years indicated. The current year, 2016, was the lowest or equal lowest in all cases. (BOM)

	Number of days		
Years	<20 ºC	<15 ºC	<10 ºC

2002	40	20	3
2003	39	13	1
2004	29	14	2
2005	48	17	2
2006	52	20	1
2007	39	9	6
2008	74	24	3
2009	35	12	3
2010	36	13	0
2011	64	34	12
2012	52	34	8
2013	39	4	0
2014	39	15	2
2015	60	25	2
2016	21	3	0

7.2 Aim

To prove that the use of potassium nitrate is as effective as the use of thiourea for the purpose of inducing flower panicle growth on mature Kensington Pride mango trees.

7.3 Material and Methods

Six treatments were replicated in four blocks in a single row of Kensington Pride trees at Jabiru Tropical orchard at Livingston Rd, Berry Springs. Within a block of trees, there was a single tree buffer between treated trees and treatments were randomised within the blocks. Single tree buffers were left between blocks. Prior to treatment 10 branches, that appeared to be about to flush (stage 1) were tagged on either side of the tree so that each tree had 20 tagged branches. Spray formulations are described below in Table 17.

Table 17. Formulation of the spray treatments applied to compare the effects of thiourea, potassium nitrate on bud break and flower induction in Kensington pride mangoes. All sprays including the water only control contain 1 ml/L Pulse® penetrant (Nufarm).

Treatment	Chemical 1	Chemical 2
1		
2	75g Thiourea	
3	75g Thiourea	450g KNO_3
4	450g KNO_3	
5	450g KNO_3	300g $KH2_2PO_4$
6	450g KNO_3	300g $NH_4H_2PO_4$

All tagged branches were checked on a weekly basis and growth was recorded. For counts and location of inflorescences the terminals on a tree were included not just the tagged branches. Trees were sprayed on the evening of May 18th and again on 26th May 2016 using the same spray formulations. Spraying occurred between 5 pm and 8 pm when the temperature ranged from 28 to 32 °C. The 12 L of spray were made up in 15 L containers using reverse osmosis treated water and transported to the orchard. The spray treatments were applied using a 15 L Stihl backpack mist sprayer. A single tank of spray treated the four single trees for that treatment in the four blocks. There was no residual spray after applying treatments so each tree received about 3.0 L. The

sprayer was rinsed 3 times between treatments and run to remove spray residue prior to use for the next treatment. Treatments were applied in the order listed Table 17 The randomisation within the row and replicates is shown in Table 18. The irrigation to the orchard commenced on the May 14th. Hourly temperature was logged using a Tinytag 2 (Hasting Loggers) location in a shrouded enclosure 1.2 m from the ground in an open area 10 m from the nearest tree (Figure 21).
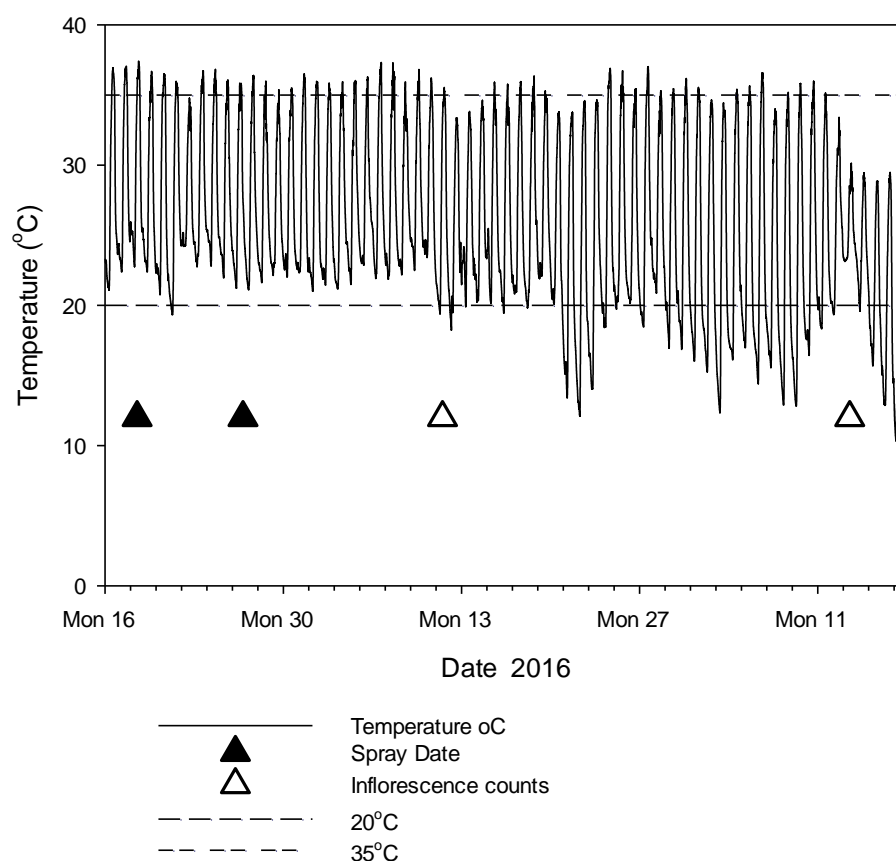
Figure 21. Log of the hourly temperatures during the period when treatments were applied and dates when inflorescence counts were made. Indicator lines for when the temperature exceeded 35 °C and went below 20 °C are indicated

7.4 Results

During the period leading up to and for three weeks after the last spray the minimum temperature went below 20 °C for 2-3 hours but were insufficient to cause low temperature induced floral induction. The maximum daily temperatures also consistently exceeded 35 °C. The effects of the thiourea and potassium nitrate trials on floral initiation 16 days after the last spray showed that sprays containing thiourea alone had the greatest number of inflorescences initiated and the inclusion of potassium nitrate appeared to reduce its efficacy (Figure 22). All the potassium nitrate sprays produced some inflorescences except that including monopotassium phosphate that produced no inflorescences on any of the treated four trees. No inflorescences were observed on the control trees that were treated only with water.
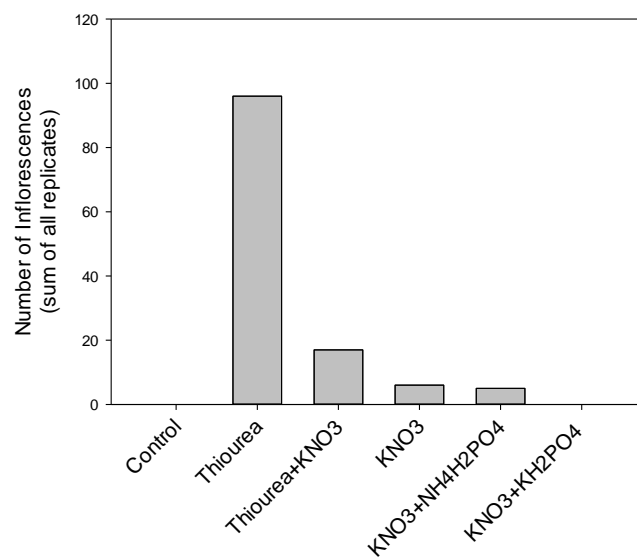
Figure 22. Sum of all inflorescences present on each treatment counted on 11th June.

The distribution of inflorescences in response to the thiourea only containing treatment were mainly on the shelter North Eastern and North Western side of the tree were also present on all eastern sectors of the tree (Figure 23). There were very few or no inflorescences produced on the north western and western sectors of all the trees and few in the southwestern and southern sectors.
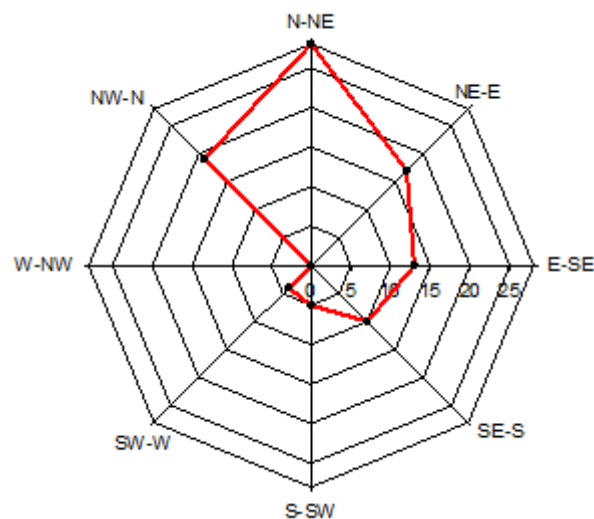
Figure 23. Distribution of inflorescences around the canopy of thiourea treated trees counted on 11th June. Numbers represent inflorescences to the right of the graphed point. E.g. at North the count represents inflorescences present in the sector of the trees North (N) to North East (NE).

Figure 24. Thiourea induced leafy inflorescence. Flowering branches developing in the leaf axils. This image was taken on 25th May 2016 and the day prior to the second spray on May 26th.

Inflorescences were evident as soon as one week after the thiourea spray application. The terminal leaves on the inflorescence shown abscised prior to anthesis. The second count of inflorescences was made on July 13th (Figure 25) well after cool night temperatures commenced on June 20th (Figure 21) and 32 days after the first count and 48 days after the last spray treatment. Inflorescences induction is now evident in the control trees and the marked difference between the thiourea treatment and those containing potassium nitrate has diminished (Figure 25). The potassium nitrate with MKP treatment has also produced inflorescences.
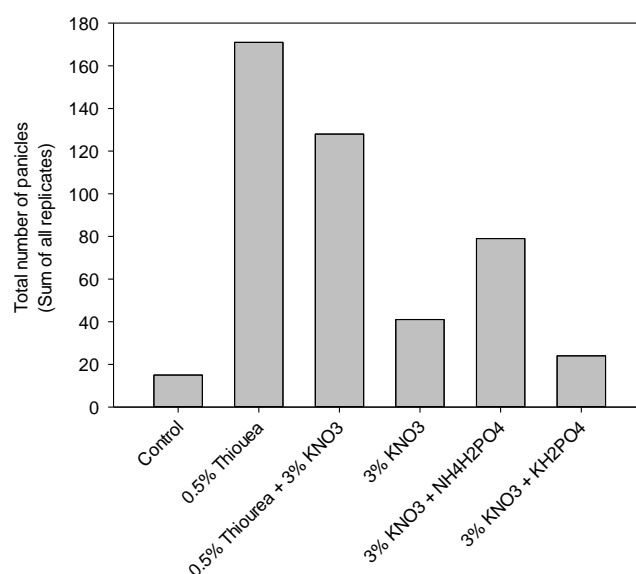

Figure 25. Sum of all inflorescences present on each treatment counted on 13th July 2016.

As the season progressed, the difference between the eastern and western side of trees diminished

and inflorescence development on the control and potassium nitrate treatments increased (Figure 26). Any advantage of the early flower induction appeared to diminish.
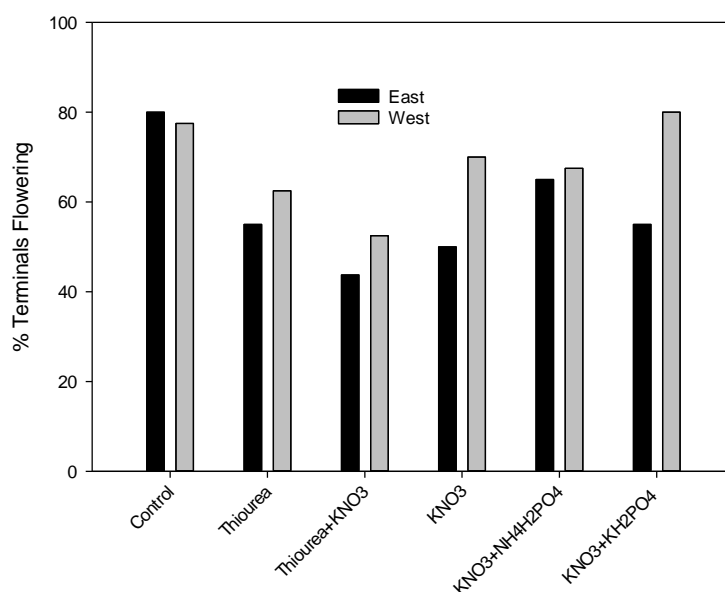
Figure 26. Assessment of flowering on the east and west side trees following treatment on August 17th 2016.

7.5 Discussion

Our results show that thiourea was able to induce flowering in Kensington pride mangoes under no inductive conditions more effectively than potassium nitrate. The lack of flowers on the western side of thiourea treated trees during none inductive conditions when the daily maximums were exceeding 35 °C suggests that the effects are temperature limited. Potassium nitrate was able to induce some flowers but there were less than 10 panicles in total formed on the four potassium nitrate treated trees. Including mono-potassium phosphate did not improve this response. It is notable that including potassium nitrate with the thiourea appeared to reduce the efficiency of thiourea in promoting floral induction.

It should be noted that thiourea is registered as an industrial chemical in Australia and is not permitted to be used in agriculture. The chemical regulator in the Northern Territory has issued notices to several growers and chemical distributors making them aware of these restrictions and prohibiting thiourea supply and sale.

8. Investigation of the effects of thidiazuron and thiourea on flowering in mango

8.1 Introduction

Thidazuron (Dropp® Bayer Cropscience) is a synthetic cytokinin like compound from a chemical group referred to as phenylureas. It is marketed by Bayer Crop sciences in Mexico as Revent® and in Chile as Splendor®. It used to break dormancy in a range of deciduous crops including apples, peach, pecans, blackberry and grapes. Erez et al. (2008) described it as a potent chemical for breaking dormancy in Rosacea and when used with mineral oil and KNO_3 or NH_4NO_3 as more potent than any chemical they had previously tested. It is known to increase fruit size in apples, pears, kiwifruit and melons. When used with gibberellic acid (GA_3) is described as increasing fruit size of parthenocarpic in mango by almost 50 % (Perez-Barraza et al. 2015). It is used in tissue culture to stimulate shoot proliferation in woody plant species. It was reported by Nunez-Elisea et al. (1990) as producing mixed or pure floral panicles when applied at 250 ppm (0.25 g/L) to axillary buds in decapitated and defoliated branches and stunted pure inflorescence when applied at 1000 ppm (1.0 g/L). These treatments were applied 2 months prior to normal floral induction. When applied after flower initiation sprays of 20 to 200 ppm caused thickening of the floral axis and enlarged flowers. In trials comparing the response of trees to paclobutrazol and prohexadione calcium while reducing vegetative growth and promote flowering it was found reductions in levels of gibberellins and increases in cytokinins occurred (Rahmin et al. 2011). To potentially mimic this a treatment was thidiazuron to elevate cyctokinins and trix ethy was that prevents conversion of precursor to highly active gibberellins were included.

8.2 Aim

The aim of these trials was to investigate the capacity of thidiazuron the induce flowering alone or in combination with other dormancy breaking compounds (thiourea or potassium nitrate) or a gibberellin synthesis inhibitors (trinexapac-ethyl).

8.3 Trial 1 - Material and methods

Eight treatments were replicated in four blocks in a single row of Kensington Pride trees at Jabiru Tropical Orchard, Darwin. Within a block of trees, there was a single tree buffer between treated trees and treatments were randomised within the blocks. Prior to treatment, 10 branches which that appeared to be about to flush were tagged on either side of the tree so that each tree had 20 tagged branches. All of these tagged branches were checked on a weekly basis and any growth was recorded. For counts and location of inflorescences on a tree were included not just the tagged branches. Trees were sprayed on the evening of June 15th and again on 4th July 2016 using the same spray formulations. Spraying occurred between 5 pm and 8 pm when the temperature ranged from 28 to 32 °C. The 12 L of spray were made up in 15 L containers using reverse osmosis treated water and transported to the orchard. The spray treatments were applied using a 15 L Stihl backpack mist sprayer. A single tank of spray treated the four single trees for that treatment in the four blocks. There was no residual spray after applying treatments so each tree received about 3 L. The sprayer was rinsed 3 times between treatments and run to remove spray residue prior to use for the next treatment. Treatments were applied in the order listed Table 18. The randomisation within the row and replicates is shown in Table 18. The irrigation to the orchard commenced on the May 14th 2016.

Table 18. Formulation of sprays to comparing the responses to thiourea and thidiazuron at different concentrations. The range thidiazouron sprays also included KNO3 or trinexapac-ethyl.

Treatment	DC-Tron (ml/L)	Non-ionic Wetter (ml/L)	Thiourea (g/l)	Thidiazuron (g/l)	KNO₃ (g/L)	Trinexapac-ethyl (g/l)
1		1				
2		1	5			
3	20		5	0.05		
4	20			0.05		
5	20			0.08		
6	20			0.17		
7	20			0.05	20	
8	20			0.05		0.08

8.4 Results & Discussion

Including oil with thiourea caused leaf necrosis (Figure 27) as was the case when oil was included with potassium nitrate but symptoms were less severe. The oil content was reduced to 10 ml/L in the second spray application. Thidiazron at the lowest concentrations, which was the theoretical limit of solubility in water, caused serious leaf and flower malformation rendering thidiazuron useless for the purpose of flower induction at the concentrations tested.

Figure 27. Leaves inside the tree canopy developed black necrotic spots following treatment with thidiazuron with oil contents of 20 ml/L in the first spray. Oil content was reduced to 10 ml/L in the second spray to reduce damage.

The effects of thiourea and thidiazuron are shown in flower assessments that were made on the 17th August (Figure 28). In general, only thiourea alone appeared to improve floral initiation compared to the control and the use of thidiazuron reduced floral development. In this case, thiourea was applied after cool inductive temperatures had started and there was comparable floral initiation on both the western and eastern sides of the tree. In sprays containing thiodiazuron

at increasing concentrations appeared to further reduced the level of floral initiation. There were no beneficial effects of including either potassium nitrate or trinexapac-ethyl in the spray formulations and the concentrations indicated. It is interesting to note that including trinexapac-ethyl in earlier trials had been effective and suppressing bud growth formed inflorescences. When combined with thidiazuron it appeared to reduce bud break compared to thidiazuron alone. This result does not assist in resolving whether reduced levels of gibberellins and elevated cytokinins is associated with flowering in mango.
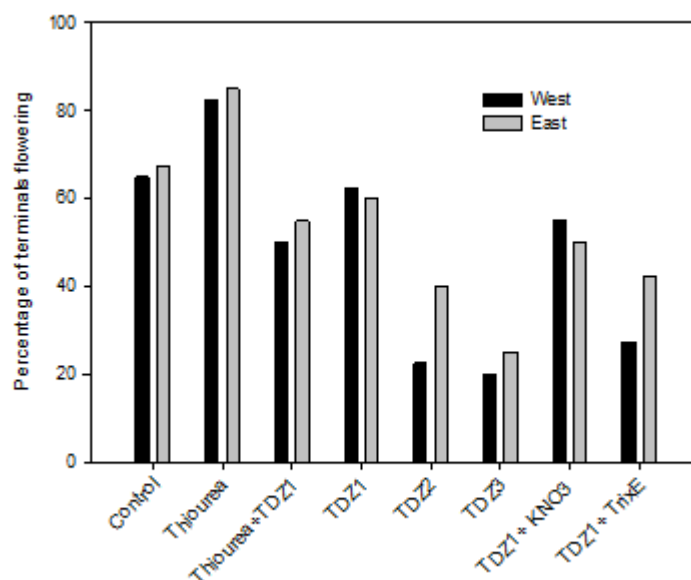
Figure 28. Flowering response to a range of thidiazuron formulations assessed on 17th August. thidiazuron 0.05g/l = TDZ1, thidiazuron 0.8 g/l =TDZ2, thidiazuron 0.17 g/l = TDZ3, TrixE= trinexapac-ethyl

Other effects of thidiazuron included reduction of leaf development, thickening of leaf lamina and crinkling of leaf margins (Figure 29).

Figure 29. Effect of thidiazuron 0.08g/L cause leaves to become crinkled with marginal burnt 29 Aug 2016

Treated trees also developed multiple cauliferous stunted inflorescences in leaf axils (Figure 30 and Figure 31). The cauliferous flowers extended on some braches more than a meter down the branch from the shoot apex represent 3 or 4 flushing cycles.

Figure 30. Multiple cauliferous flowers along terminal branches. New leaves subtending branch apex were thickened and crinkled

Figure 31. Multi-cauliferous clusters of flowers formed extending over 1m back from branch terminal

8.5 Trial 2 - Material and methods

A second trial using higher concentration of thidiazuron was establish. This used the same design as described above (Section 8.3, page 52). Trees were sprayed once one evening of June 21st using the spray formulations shown in Table 19.

The same basic design used for the seven treatments but only a single spray applied in the evening of 21st June. To determine if oil was the most effective penetrant a treatment using LI700® (Nufarm) was included for comparison. The spray formulations are shown in Table 19and included DC-Tron (Caltex Australia) and LI700® as adjuvants for the thidiazuron. The same basic randomisation, buffering and plot design was used as previously described in other trials.

Table 19. Formulation of sprays with high concentrations of thidiazuron (TDZ) with oil (DC-Tron) or a buffering penetrant (LI700®)

Treatment	DC-Tron (ml/L)	LI700® (ml/L)	Thidiazuron (g/L)
Control	0	0	0
1	10	0	0
2	10	0	0.25
3	10	0	0.5
4	10	0	1
5	10	0	2
6	10	0	4
7	0	2	2

8.6 Results & Discussion

As previously reported thidiazuron caused serious flower malformation and leaf, damage compared to the control (Figure 32). It was interesting to note that LI700® appeared to have the most severe effects on thidiazuron uptake and malformation. While at the concentration tested thidiazuron did not have any beneficial effects on floral initiation the responsiveness of mango to this chemical would suggest that its ability to produce seedless mangoes in combination with gibberellin sprays in cultivars prone to forming nubbins should be investigated.
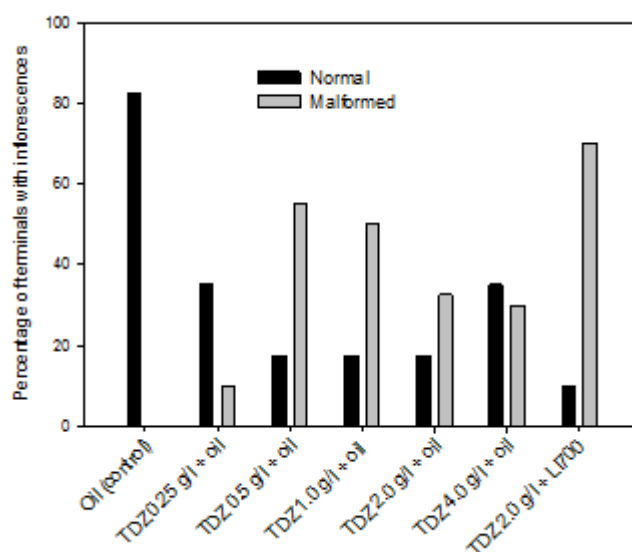
Figure 32. Effects of high levels of thidiazuron (TDZ) with oil (DC-Tron) or LI700® on flower development on Kensington Pride. Malformed inflorescences formed clusters of flowers with no obvious pedicle.

The thidiazuron continued to cause crinkled leaves and bud break along the branches and swelling of terminal buds (Figure 33). When buds did grow, they were fascinated and stunted (Figure 34 and Figure 35). Terminal panicles when they did develop were compressed and stunted (Figure 36).

Figure 33. Enlarge swollen terminal bud resulting from applications of thidiazuron, July 14th.

Figure 34. Fasciated and stunted terminal inflorescences caused by thidiazuron, July 14th.

In some treatments TDZ (0.25 g/L), there were high levels of initial fruit set (Figure 37). These fruits

may have not been pollinated as thidiazuron has been used in combination to promote retention and growth of nubbin fruit in mango cv atauflo (Pérez-Barraza et al 2015).

Figure 35. Stunted inflorescences caused by thidiazuron. July 17[th.]

Figure 36. Stunted and condensed inflorescences caused by thidiazuron July 17th

Figure 37. Multiple fruits set on a terminal inflorescence formed after treatment with thidiazuron. These may not have been pollinated and resulted from parthenocarpy.

9. Optimising foliar nitrogen uptake of mango: Effect of adjuvant, leaf position and time of potassium nitrate spray

CA. Asis, T. Alexander, A. Sarkhosh, and C. McConchie

9.2 Introduction

Foliar application of fertiliser is another method of supplying nutrients to the plants when high rates of loss may occur for soil applied fertiliser. It is also recommended when plant's growth internal demand and environmental conditions interact to restrict the transfer of nutrients to the critical plant organ. In these conditions, plants absorb the nutrients more rapidly from foliar spray than through the soil (Marschner. 1995; Fernandez et al. 2013). Through the foliar application, growers can provide nutrient to the crop at the right time to coincide with critical stress events such as growth flushes, flowering, and fruit set (Weinbaum, 1988; Stevens, 1994). This method also addresses the increasing concerns about contamination of groundwater by fertiliser (Alexander and Schroeder, 1987; Stevens, 1994). Thus, foliar fertiliser has become an important tool for the sustainable and productive management of crops especially those of commercial importance worldwide (Alexander and Hunsche, 2016).

In mango production, growers regularly apply foliar potassium nitrate (KNO_3) as a management practice to promote flowering especially during off season (Yeshitela et al., 2005). Flowering is the first of several events that set the stage for mango production each year. Moreover, the timing and intensity of flowering greatly determine when and how much fruits are produced. Control of the timing of flowering and its intensity allows growers to harvest their crops at the most profitable times. A reliable flowering is necessary to obtain consistent mango production (Davenport, 2007). However, the role of KNO_3 in flowering is not fully understood.

The process of foliar penetration of a leaf applied solution occurs through circular cracks and imperfections, stomata, leaf hairs and other specialized epidermal cells (Fernandez et al., 2013). Thus, the structure and composition of the cuticle, as well as the morphology, distribution, and size of the stomata, play a role regarding the penetration of foliar sprays (Fernandez and Eichert, 2009; Fernandez et al., 2013). Moreover, factors related to the physiological state of the plant such as root temperature, root osmotic potential, the age of leaf or the current nutrient status modulate the effectiveness of foliar fertilization (Weinbaum, 1996; Fernandez and Ebert, 2005). The prevailing environmental conditions such as relative humidity, light, and temperature also affect the physical-chemical properties of the applied solution (Schönherr and Luber 2001; Schönherr et al., 2005; Fernandez et al., 2013).

In mango production, growers currently apply a range of KNO_3 spray formulations. Thus, it is necessary to elucidate the factors that optimise nutrient uptake from KNO_3 application in mango. The objective of this study was to determine the effect of adjuvant, position of leaves and time of application on the leaf nitrogen content of mango cv. Kensington Pride.

9.3 Materials and methods

The experiment was conducted at the Kensington Pride orchard in Katherine Research Station, Australia. The site is located 14.47°S; 132.31°E with an elevation of 110 m. The soil type is haplic mesotrophic red Kandosols (Isbell, 2018) with 6 % clay, 19 % silt, 75 % sand and sandy loam soil texture. The soil had 1.35 % C, 0.08 % total N, pH of 6.0, electrical conductivity of 78.2 µS/cm, and cation exchange capacity of 12.0 c.mol+/kg soil.

The study was a 2 x 2 x 4 factorial experiment arranged in randomised complete block design with 5

replicates. The treatments were spraying to run off 3 % KNO_3 with 0, 100, 250 and 500 mL Li-700/100 L solution on tagged mango terminals from the east (sunny side) and west (shaded portion) of trees in the morning (8 am) or night (8 pm) time side of the east-west orientated rows of trees (Figure 40). Ten trees were selected with five trees were used for 8 am spraying and five trees for 8 pm spraying. In each side of the tree, 20 leaf terminals with growth stages of BCCH scale 319 (Rajan et al., 2011) were tagged and labelled according to the treatment. Spraying was done on both sides of the leaves and leaf terminals for other treatments were covered with a bag to avoid contamination from spray drift. Leaf samples were collected at 4, 12, 24, and 48 hours after spraying, rinsed thoroughly with running water and finally washed with distilled water. Samples were oven-dried at 60 °C until constant weight had been attained, ground and analysed for total nitrogen using the Kjeldahl method (Bremner and Mulvaney, 1982).

The data on total nitrogen content of the leaf samples were statistically tested based on the analysis of variance (ANOVA) with means separation at 5 % LSD using the Statistical Tools for Agricultural Research version 2.0.1 software (http://bbi.irri.org).

9.4 Results & discussion

Analysis of variance showed that total nitrogen contents were comparable in mango leaves across rates of adjuvant, time of application and location of the leaves at 4 and 12 hours after spraying but varied significantly at 24 and 48 hours after spraying. The total N content of leaves varied significantly with time of application, which was observed 24 h after spray application of KNO_3. Moreover, the total nitrogen content of leaves differed with the time of KNO_3 spray (Figure 40). When KNO_3 spraying was done in the evening (8 pm), leaf N content was higher than those sprayed in the morning were.

Figure 38. Changes in the total N content of mango leaves from the west and east part of the tree as influenced by KNO3 spray.
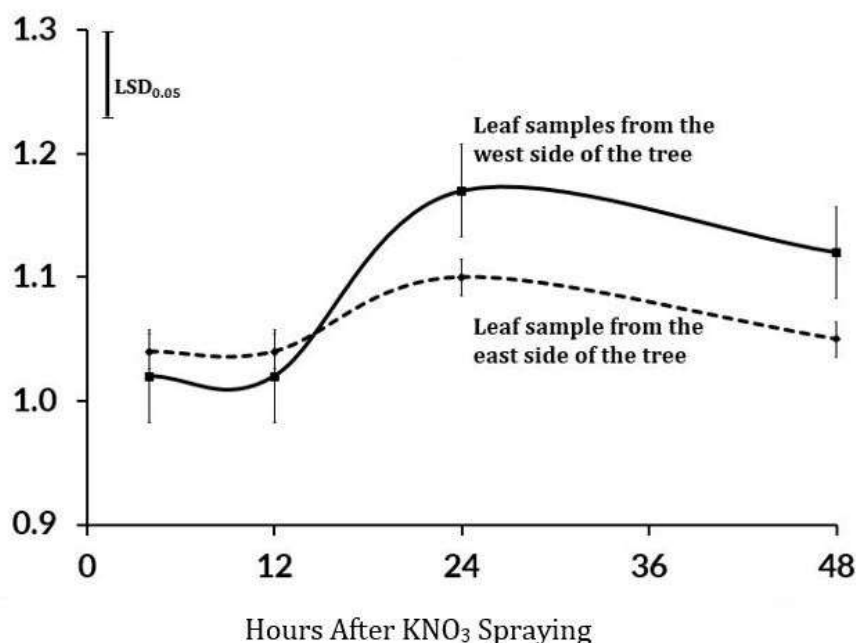
Figure 39. Changes in the total N content of mango leaves from the west and east part of the tree as influenced by KNO3 spray.

This difference in the total nitrogen content with the time of KNO_3 application could be attributed partly to the higher ion penetration during the night owing to cooler temperature and higher relative humidity. According to Schönherr et al. (2005), foliar penetrations of salts requires very high humidity, which is expected to be highest when the dew occurs during the night. Stagnant air layers over stomatous leaf surfaces at low wind velocities will favour rates of penetration when the humidity in bulk air is 100% or lower. Moreover, at high relative humidity, drying of the salt deposit is delayed, and cuticular permeability may increase through hydration (Schönherr and Luber, 2001).

The total nitrogen contents was higher in mango leaves in the west than those in the east side of the tree at 24 and 48 hours after spraying (Figure 41), indicating a higher nitrogen use efficiency in the leaves from the west than those in the east part of the tree with KNO_3 application. Reports have shown that nitrogen-use efficiency diminishes at higher irradiance and may be enhanced at low irradiance (Evans 1989a; Rosati et al., 1999). Seemann et al. (1987) also reported that greater N partitioning to RuBP carboxylase at low irradiances for *Phaseolus* and *Alocasia* plants. Leaf nitrogen content is closely correlated with leaf photosynthetic capacity and nitrogen use efficiency in several species (Field and Mooney, 1986; Hirose and Werger 1987). Moreover, when light is the source of variation in leaf N content, the photosynthesis and nitrogen relationship shows great scatter because of partitioning of nitrogen among the different nitrogen fractions (Evans 1989b; Rosati et al., 1999). The result also indicates that leaf position is significant factor during leaf sampling to evaluate the nutrient status after foliar application.
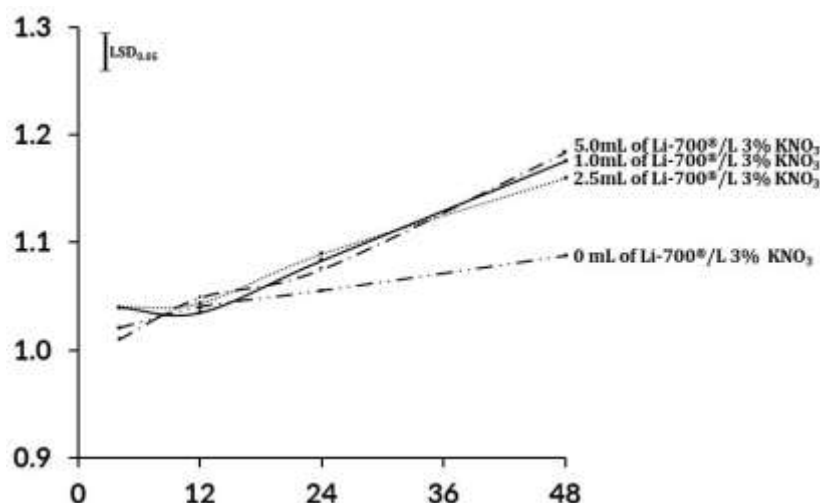
Figure 40. Changes in the total N content of mango leaves as influenced by KNO3 spray with varying level of adjuvant Li-700®.

Addition of adjuvant LI-700® also increased the total nitrogen content at 48 hours after spraying. However, total nitrogen content did not vary significantly with different levels of adjuvant such that total leaf N content in leaves applied with 1 mL LI-700®/L of KNO_3 spray solution was similar with those applied with 2.5 mL, and 5 mL/L of KNO_3 spray solution, respectively (Figure 41). This result indicates that for foliar application of 3 % KNO_3 the small amount of at least 1mL/L spray solution is enough to provide an adjuvant effect on N uptake from KNO_3. This amount is substantially lower than the recommended rate of 3mL/L spray by the manufacturer

9.5 Conclusion

The study showed that leaf nitrogen content was higher in leaves that were sprayed with adjuvant than those without adjuvant and when the application was applied in the evening. Total nitrogen content was also higher in leaves from the shaded part of the tree that those exposed to sunlight. These results indicate that addition of adjuvant and spray application in the evening assist nitrogen uptake of mango leaves following foliar application of KNO_3.

10. The impact of different tip-pruning times on flowering, yield, and maturity in two Australian mango cvs. Honey Gold and B74 in the Katherine region

Ali Sarkhosh, Mike Khal, Maddison Clonan, Trevor Olesen and Cameron McConchie

10.1 Abstract

This study aimed to take advantage of the progressively cooler weather in the Katherine region (northern Australia), occurring from April to July, to investigate the effect of climate on developing mango buds. The experiment was conducted using seven-year-old commercial mango cultivars, Honey Gold and B 74, 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 to approximately 10 cm 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 the experiment from April to November climate data including: temperature (mean, maximum, minimum), chill and heat sums, cumulative chill and heat (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. Effect of temperature during the development stage when floral induction occurs, defined, as 3 days either side of when lateral buds were 10 mm in length, was determined. By relating these conditions to the fate of the bud, it was possible to identify climatic conditions associated with floral induction and the production of a vegetative flush. Results indicate that temperatures below 18 °C and less than 32 °C for Honey Gold and 35 °C for B74 promoted floral induction. Dry matter measurements indicate that tip pruning at the right time may be an effective agro-technical tool to delay harvesting time for the studied mango cultivars. The result showed that combination of cool weather (<18 °C) and tip pruning when applied in appropriate climatic conditions, is a promising alternative strategy to the use of paclobutrazol for sustainable mango production in the region.

10.2 Introduction

Controlling growth to stimulate the formation of vegetative and reproductive buds is common practice in fruit tree management. Mangoes trees are pruned to remove diseased branches, define tree structure, induce early flowering and improve fruit quality (Yeshitela et al, 2003; Davenport, 2006). Flower manipulation via pruning can encourage out-of-season production, regulate production timing and improve productivity (Ramírez and Davenport, 2010). Flowering in mango is an essential physiological stage for fruit production.

Davenport et al. (2006) suggested that flowering responses in mangoes could be separated into tropical and subtropical climatic regions. Tropical conditions based on USDA climate classification temperatures rarely go <18 °C (Jordan, R. 2001). Subtropical conditions are described as having winter temperatures that are regularly <10 °C. It is now well established that mango leaves produce a floral promoter that is transmitted to the bud resulting in floral induction; for this to occur the bud needs to be actively growing (Batten and McConchie, 1995). Davenport (2000, 2003, 2006, and 2007) has subsequently termed this as (bud) initiation, reinterpreting earlier investigations. The coincident of initiation and induction is dependent on environmental conditions. However, the capacity of a growing bud to develop an inflorescence is not restricted to when the floral promoter is present at the commencement of bud growth but rather it is responsive in buds exceeding 1 cm in length (Batten and McConchie, 1995).

In tropical regions, floral induction occurs in buds that have attained a sufficient time at rest since the previous flush (Ramirez and Davenport, 2010). Supporting evidence for this research in Columbia where stimulation of growth of flowering was achieved by potassium nitrate applied as a foliar spray or tip pruning (Ramirez et al. 2010).

Cold temperatures, below 18 °C, are one of the main factors in mango flower induction. The consequence of temperature are more obvious where flower development happens after or during exposure to floral inductive cool temperatures (Davenport, 2009). Removing of the apical bud on terminal shoots in mango just prior or during the flowering period induces the development of normally suppressed axillary buds adjacent to the point of cutting (Reece et al., 1946).

Katherine is a major mango production region in Northern Territory with a subtropical climate, with the distinct wet season (December to March) and dry season (April to November). Annual rainfall is around 1000 mm and mainly received in December to March. Average temperatures range from 25 °C to 35 °C, with an occasional 40 °C maximum daytime temperature during November and December, usually accompanied by very high humidity. During April to July temperatures regularly dropping down below 20 °C overnight. Cold weather and synchronization of vegetative growth are two main key factors of mango flowering. Synchronization allows all the stems in the canopy to be at a similar physiological stage of maturity (Davenport, 2000). Synchronized growth is achieved by tip pruning of all stem terminals on a tree (Davenport, 2003, 2006). Tip pruning not only produces a uniform flush of vegetative growth through the canopy but also reduces flower-inhibiting factors produced by immature vegetative flushes (Davenport, 2000, 2009).

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 B74, applied at four weekly intervals. Utilising the cold weather from April to July during the dry season in Katherine region, this study could investigate tip pruning as an alternative strategy for managing flowering and mango production in Katherine.

10.3 Materials and methods

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 the Katherine region. Evaporation far exceeds rainfall from April to October and orchards were both irrigated throughout the trial. During this dry period night, temperatures can fall to below 10 °C but may also remain above 20 °C. Exclusively two large-scale growers in the region using specific cultural practices unique to each producer grow these cultivars. Twenty four trees of each cultivar on Kensington Pride rootstock were selected from 7-year-old trees for each cultivar. All trees were subjected to commercial pruning after harvest and treated with paclobutrazol by January 1st the levels applied to B74 were high by industry standards. A randomised complete block with six pruning treatments was applied to each cultivar, resulting in three trees pruned every four weeks, with 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 at approximately 10 cm above the last internode for each branch on each tree for each pruning time. Twenty branches were randomly selected for measurement and tagged around the canopy on each tree.

Measuring of variables

Two data loggers (Tinytag Plus 2, Hastings Data Loggers, NSW, Australia) were installed on each site to measure hourly temperature and relative humidity (RH %) hourly from week 13 to week 46

(harvesting time) of the year. During this period temperatures below 20 ˚C were calculated as chill sums as well as heat sums calculated as follows (Moore, 2013):

$$\left[\frac{(\max temp + \min temp)}{2}\right] - 12$$

Where:

Max temp = daily maximum temperature $^\circ$C

Min temp = daily minimum temperature $^\circ$C

Equation 1

A heat sums calculator is a tool for predicting fruit maturity in mango in Australia. Heat sums are calculated by summing the degree-days calculated above for a set period. Different mango varieties have slightly different heat sum requirements to achieve picking maturity. Generally growers count heat sums from flowering at stage 6 of panicle emergence (the first time the floral buds can be seen) to the time that dry matter (DM %) reaches to certain percentage for each variety. Recommended DM % for both cultivar Honey Gold and B74 is approximately 15 % percentage as harvesting index. DM percentage is used as an indicator of maturity by most mango producers in Australia.

After one week of pruning, length (mm) of the axillary flush below the point of cutting was recorded as weekly vegetative growth for each measurement branch. For all treatments, flowering time (week of the year), percent canopy flowering (percentage), the number of panicles emerging below of cutting point, and the number of fruit per tree were recorded. For each pruning time, 20 fruit per tree (60 per treatment) were harvested in week 46 of the year (second week of November). Fruit quality metrics were recorded including Brix $^\circ$, using digital reflectometer (two times per fruit from both side); fruit weight (g) and dry matter (DM %).

In this study, DM % was measured using traditional destructive techniques as well as the now industry-standard infrared spectroscopy (NIR) method. For the traditional DM percentage method a 1 cm deep sample from both sides (centre) of the fruit were taken using an apple corer. The samples were peeled, measured for fresh weight (g), dried at 65 $^\circ$C for 48 hrs and measured for dry weight (g). Destructive DM % was calculated using the formula below (Owens and Moore, 2013) and non-destructive prediction of DM % was measured by pre-collaborated near infrared spectroscopy F-750 NIR unit (Felix Instruments, WA, USA) (Subedi et al., 2007).

$$DM\ \% = \left(\frac{Dry\ weight\ (g)}{Wet\ weight\ (g)}\right) * 100$$

Equation 2

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). Shoots that were damaged throughout the measurement period were excluded from the analysis, as they could not be determined as floral or vegetative. Percentage of shoots that flowered was calculated for each tree and each prune event. Regression analyses were performed using SigmaPlot (Systat Software Inc, San Jose) for each tree individually. Equation 1 was then used to estimate the day on which the flowering or vegetative shoots (analysed separately), were 10 mm in length. To identify the days at which shoots were 10 mm for a flush event, the days identified for comparable vegetative or flowering shoots were combined using equation 2. The mean minimum, mean and mean maximum temperature was calculated for the 7 day period

surrounding the day at which 10 mm was reached, to determine 3 days before and 3 days after this development period was attained. A three-parameter sigmoidal curve was fitted to the relationship between percentage flowering terminals and each temperature measure. This was used to describe the sentinel temperatures for flower induction.

Equation 3.

$$y = \frac{a}{\left(1 + e^{\left(\frac{-(x-x0)}{b}\right)}\right)}$$

Where:

x =day of the year

y=length of shot

Equation 4.

$$Day\ of\ year = (no.of\ veg.shoots * veg.shoot\ etimate\ of\ day\ at\ 10\ mm) \\ + (no.of\ fl.shoots * fl.shoot\ estimate\ of\ day\ at\ 10\ mm)$$

10.4 Results & Discussion

Temperature collected during the study period was used to calculate day/night temperature fluctuation (difference between maximum and minimum daily tenmperature, ℃), cumulative chill (daily hours <20 ℃) and cumulative daily heat sums described above. This was performed for the entire flush cycle, from the started of flush/bud growth until flowering or ceasation of vegeative growth. These are presented for each site in figures 43 and 44 along with RH percentage.
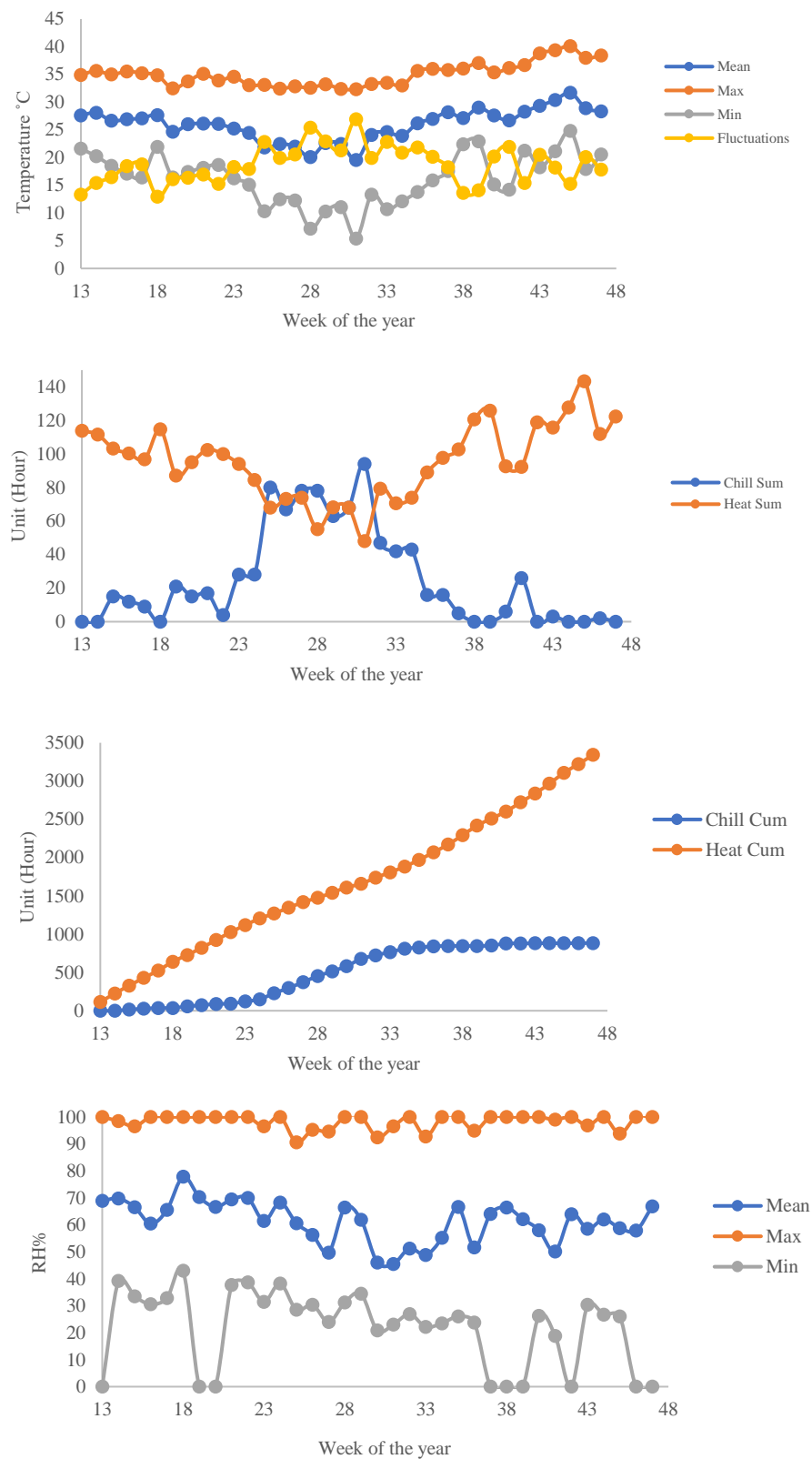
Figure 41.Weekly climate data including; temperature (mean, max. min), chill and heat sums, cumulative chill and heat units, and relative humidity (mean, max, min) for 'B74' plantation site at NT Land Development Farm in Katherine region (Florina Road, 14°32'44.2
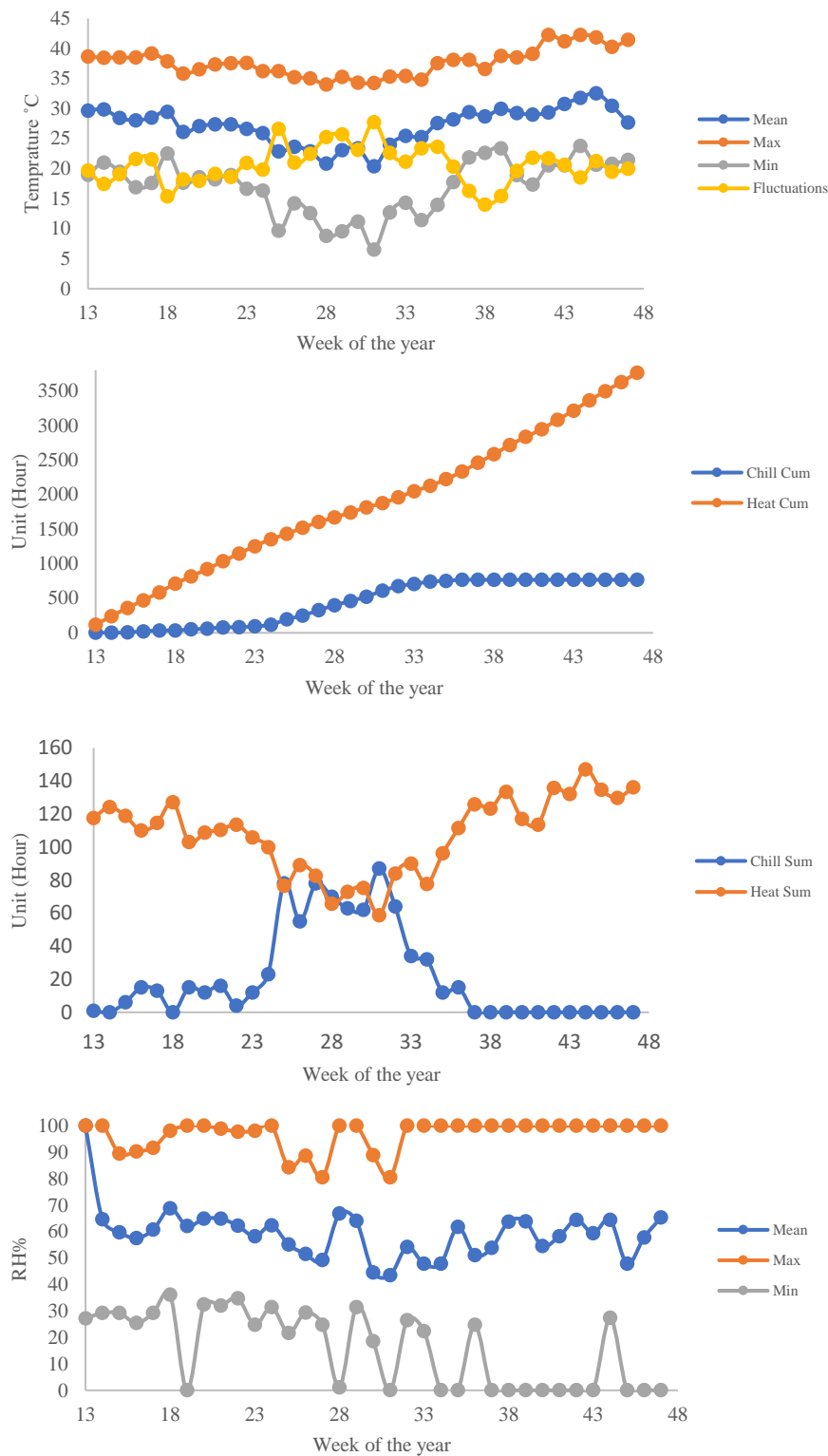
Figure 42. Pattern of vegetative growth (pruning time one) and inflorescence elongation (all treatments) for both cultivar mango cultivars during week 13 to week 46 of the year based on climate data provided in Fig. 43 and 44 for both plantations in Katherine.

The effects of different pruning times on terminal growth, flowering, fruiting and fruit quality are presented in *Table 20* and Table 21. The earliest regrowth following pruning was observed 2-3 weeks after tip pruning for the buds close to the cutting point, regardless of treatments. This is consistent with other mango studies (García De Niz et al. 2014). For tip pruning events 1 and 6, the vigor (length, mm) of the first and second growth cycle were not statistically different to the unpruned tree (control) in either cultivars. Flushing patterns for both cultivars based on data collected for tip-pruning event 1 is shown in Figure 43.

Figure 43. Inflorescence and vegetative growth below pruning point in Honey Gold a) when tip pruning was performed in week 17, 21, 25, 29 of the year, b) when tip pruning was performed in week 13 and week 33 of the year in both produced 2 to 5 axillary vegetative flushes below cutting point.

As most of the buds below the cutting point in tip pruning events 2, 3, 4, and 5 produced inflorescences, there were not enough vegetative buds available for these pruning times to be analyzed for vegetative flush growth pattern.

Table 20. *Influence of six different tip pruning events on vegetative, floral and fruit characteristics (mean ± standard error (SE)) in mango cv. Honey Gold in Katherine.*

Pruning time	Week of the year	% Terminals 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°C
Control	-	51 ± 2.22	62 ± 1.45	17.33 ± 0.39	18.29 ±0.19	479±19.04	7.12 ± 0.19	28	1869	452
Times 1	13	51.23 ± 1.22	61 ± 1.45	17.23 ± 0.39	18.39±0.19	469±19.04	7.15 ± 0.19	28	1869	452
Times 2	17	95 ± 1.45	67.6 ± 1.2	17.34 ± 0.26	18.49±0.30	497±20.98	7.10 ± 0.13	28	1869	425
Times 3	21	86.66 ± 1.56	66.66 ± 0.88	17.09 ± 0.25	18.22 ±0.32	504.73±20.2	6.65 ± 0.11	29	1799	443
Times 4	25	93.33 ± 1.43	69.33 ± 1.33	17.13 ± 0.39	18.23±0.39	478.18±20.99	6.61 ± 0.14	30	1743	434
Times 5	29	93.33 ± 1.7	67 ± 1.24	17.07 ± 0.39	18.13±0.39	473.17±20.99	6.5 ± 0.14	32	1561	354
Times 6	33	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00			122

Table 21. Influence of six different tip pruning events on vegetative, floral, and fruit characteristics (mean ± standard error (SE)) in mango cv. B74 in Katherine.

Pruning time	Week of the year	% Terminals Flowering	Number of fruit/tree	NIR dry mater	Traditional Dry matter	Fruit weight (g)	Brix	Flowering time (week of the year)	heat Unit Cum	Chill cum < 20°C
Control	-	57.23±1.23	112±1.45	17.5±0.4	18.2±0.2	399.58±15.21	9.22±0.16	28	2028.8	398
Times 1	13	58.12±1.32	114±1.33	17.5±0.4	18.2±0.2	399.58±15.21	9.22±0.16	28	2028.8	398
Times 2	17	83.23±1.55	113±1.25	17.41±0.23	18.13±0.29	415.23±10.81	9.1±0.17	28	2028.8	376
Times 3	21	90.13±1.72	111±1.23	17.26±0.22	18.10±0.28	421.15±16.32	9.1±0.16	29	1958.8	399
Times 4	25	100±0.0	121±1.65	17.29±0.23	18.13±0.25	395.28±10.36	9.1±0.18	29	1958.8	344
Times 5	29	63.12±1.56	116±1.73	16.20±0.24	16.90±0.26	344.25±12.23	7.56±0.19	32	1752.0	310
Times 6	33	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0			93

 When the pruning occurred in weeks 13 (April) and 33 (August) of the year, the number of vegetative lateral shoots below cutting point varied between 2 to 4 (Figure 44). Of the buds produced from tip-pruning at time 1, about 50 % of shoots that were initially vegetative produced terminal inflorescences after the initial vegetative flush matured (length >100 mm, and hard and dark green leaves) and receiving around 400 hours chill sums over the duration of the two flushing cycles (Table 20 and Table 21). However, in both cultivars vegetative buds did not produce terminal inflorescences when tip pruning was performed in August (week 33). This was due to the lack of coincident growth with cool conditions even though chill sums for hours below 20 °C was recorded >125 hours for Honey Gold and >95 hours in B74.
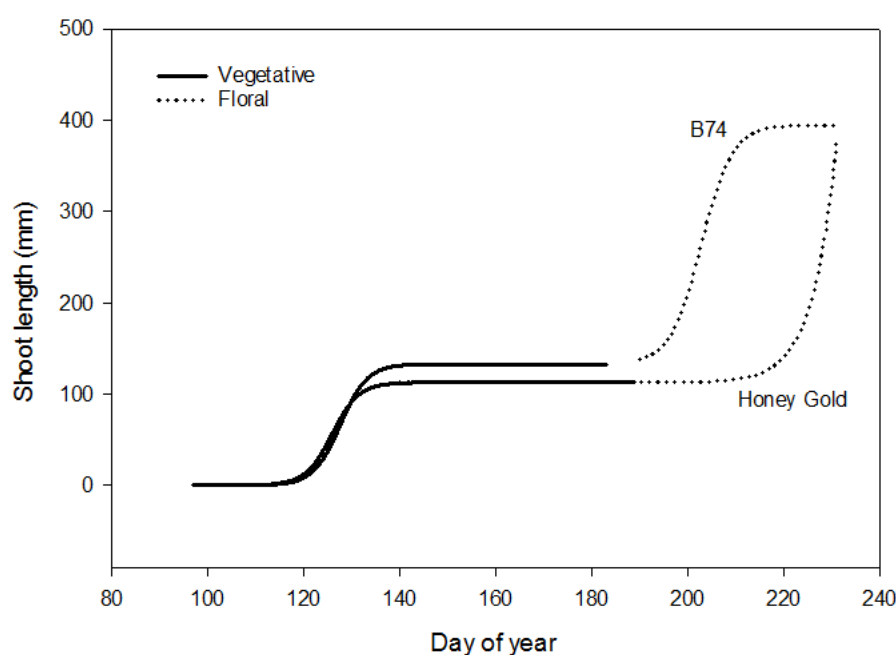

Figure 44. Mean shoot length of shoots that formed on terminal inflorescences from the second flush after pruning time 1 in Honey Gold and B74.

Superficial examination of the fate of flushes reveals similar responses in both cultivars at the different pruning times. The highest proportion of proportion of floral shoots were observed when pruning was performed in weeks 17, 21, 25, and 29 of the year, where the proportion of floral shoots was 86 % to 95 % for Honey Gold and 63 % to 83 % for B74 (Table 20 and Table 21). In B74 the proportion of floral shoots was lower (63 %) when trees were pruned later (week 29), and eliminated in both cultivars when tip pruning was performed very late (i.e. week 33). The proportion of floral shoots from pruning in week 13 for both cultivars was recorded from 51 % to 58 % for Honey Gold and B74 respectively.

Across the entire flush cycle monitored in Honey Gold more than 420 hours chill sums (<20 °C) was recorded for unpruned trees and pruning events 1, 2, 3, and 4, chill sums were 354 hours for pruning event 5 and 122 hours for pruning event 6. In B74 more than 340 hours chill sums (<20 °C) was recorded for unpruned trees and pruning event 1, 2, 3, and 4, chill sum for pruning event 5 was 310 hrs, 93hrs for pruning event 6. The highest fluctuation in day/night temperatures was recorded at 25 °C in week 25 and 27 °C in week 31 for B74. The greatest fluctuation in day/night temperatures was recorded at 27 °C in week 25 and 28 °C in week 31 for B74 (Figure 41 and Figure

42).

The failure of flowers to develop on following pruning event 6 in both cultivars (Table 20 and Table 21) confirmed that these mangoes need a combination of cold temperatures and hard dark green mature leaves flower induction (Shu and Sheen, 1987; Whiley et al., 1989; Chaikiattiyos et al., 1994; Nunez-Elisea and Davenport, 1994; Batten and McConchie, 1995; Wilkie et al. 2008).

As with other studies, this study found the number of panicles produced below the cutting point ranged from 2 to 5 in trees tip pruned at events 2, 3, 4, and 5 and resulted in a higher proportion of terminals flowering than control and pruning event 1 trees (Yeshitela et al., 2003, Oliveira et al., 2017).

In both studied cultivars, tip pruning increased the number of panicles/tree but not the number of fruit/tree (Table 20 and Table 21). The number of inflorescence panicles was increased on tip-pruned branches (event 2, 3, 4, and 5) but panicle size was reduced in comparison to control and pruning event 1 (Fig 45). Reduction in panicle size may reduce the number of flowers in a panicle due to within panicle competition for photo assimilates (García de Niz et al., 2014; Oliveira et al., 2017). Furthermore, the sexual expression of flowers in mango are influenced by environmental factors such as luminosity and temperature and the proportion of hermaphrodite flowers in the panicle may change from 2 to 75 % influenced by these by these factors as well as the location of the panicle on the tree (Lima-Filho et al., 2002).

There appeared to be a reduction in Brix° and dry matter (DM%) as flowering occurred later in the year, as has been found elsewhere (Oliveira et al., 2017). Delaying the DM percentage accumulation by tip pruning suggests tip pruning at the right time could be used as an agro-technical tool to advance or delay harvesting time for both cultivars. Chang and Leaon (1987) reported that tip pruning in mango could cause the development of inflorescences from auxiliary buds and delay harvesting for 20-30 days, which is in contrast with our results in this study where there was no statistically difference in fruit quality across the pruning treatments. The heating unit cumulative from anthesis to harvesting recorded in the current study, that varied from 1562 to 2028 in both cultivars (Table 20 and Table 21), this could be explained by heating unit experienced by developing fruit have a greater role in determining mango maturity than pruning date. 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.

The duration of the period between the cessation of growth of vegetative flush that result from pruning event 1 and the commencement of terminal growth on these shoots that produced flowers was approximately 40 days (Figure 44). This is considerably shorter than 4 or 5 months for terminal stems that have attained in rest since the previous flush under tropical condition to support floral induction (Nunez-Elisea and Davenport 1994, 1995 Davenport 2003 and Ramirez et al., 2010). This may reflect that the higher mean daily temperatures in Katherine of >30 °C are correlated with prematurely creating responsive mature hard green flush. The terminal buds on the second flush of pruning event 1 were 10 mm at approximately the same time as the flowering shoots from tip-pruning events 2-5.
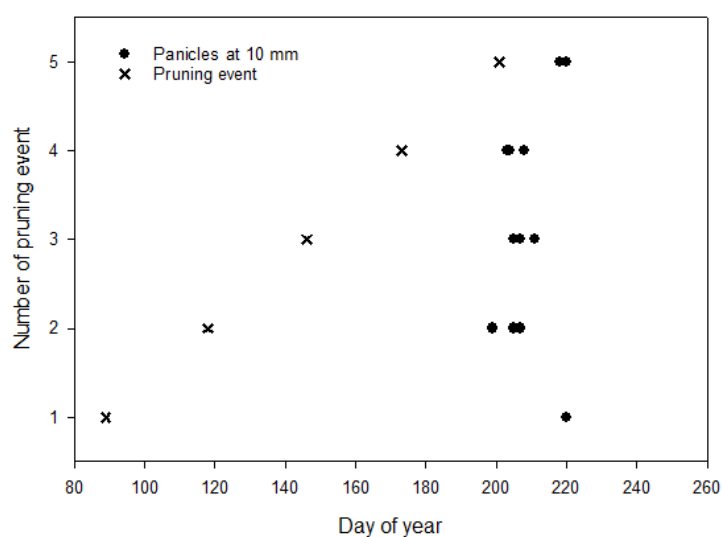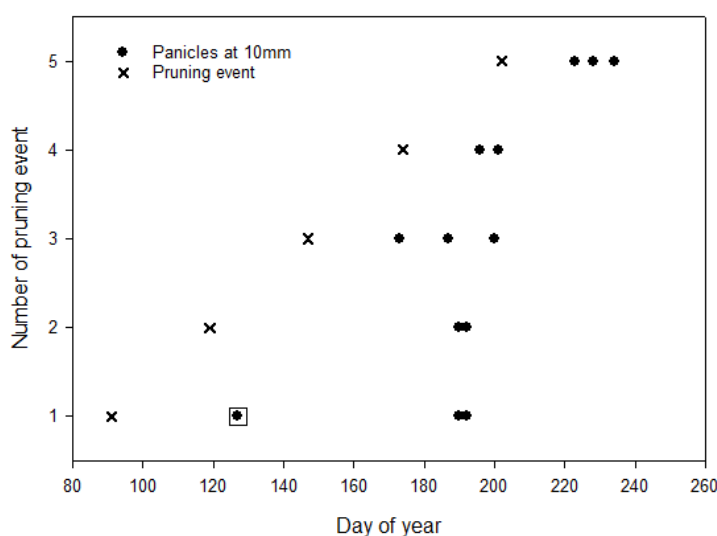
Figure 45. The estimated day of the year that "Honey Gold" shoots that formed inflorescences were 10 mm following tip-pruning event1-5. Pruning 6 is not included as all shoots were vegetative

In Honey Gold, the time that floral buds were 10 mm in length occurred around day 200-220 despite the period between pruning events 2, 3, 4, and 5 being a month apart (Figure 45). Even on the buds on the second flush that produced flowers from pruning event 1 were 10 mm at about the same time. In B74 the spread of dates that flowering buds were 10 mm in length occurs from day 170-240 (Figure 46). There is an outlier from pruning event 1 and flowered when the minimum temperature was 24 °C at day 125. The B74 orchards were treated with high levels of paclobutrazol early in the year, which may have affected this response. Paclobutrazol is an inhibitor of gibberellin synthesis that is widely used to control vegetative growth and assist in aging leaves to make them responsive to inductive conditions (Davenport, 2009). The fitted curves for the temperature that occurred on the days preceding and after the buds were 10 mm (Figure 47) indicate that temperatures above 32 °C in Honey Gold and 35 °C in B74 inhibit floral initiation while temperatures below~18 °C in Honey Gold and 18.5 °C are required for floral initiation.

Figure 46. The estimated day of the year that "B74" shoots that formed inflorescences were 10 mm following tip-pruning event 1-5. Pruning 6 is not included as all shoots were vegetative. Note the outlier with the square around the dot in pruning. This could possibly be due to a late application of paclobutrazol.

The physiological data to support the demarcation of mango flowering into tropical (rarely below 18 °C) and subtropical regularly below 10°C is limited. In a key case study of tropical mango production in Columbia (Ramirez and Davenport, 2010) the temperature was monitored at a site separate to the orchards being investigated and had a mean monthly minimum temperature of 18.5 °C. This is the threshold minimum temperature for floral induction in B74 but no daily data from within the study orchards in Columbia. Similar results were reported by Abdel Rahim et al. (2011) in a region considered tropical, where flowering occurring without trees being exposed to temperatures below 18 °C based on mean monthly minimums. Precise 6 hourly temperature data in Guevara et al. (2012) demonstrated flowering in mango cv Tommy Atkins occurred without temperatures below 20 °C but trees treated with potassium nitrate and paclobutrazol flowered before the untreated controls that in turn preceded the paclobutrazol only treated trees. It is notable that the daily maximum temperatures leading up to the potassium nitrate promoted flowering are below 31 °C compared to the preceding maximum temperatures of around 33 °C. In Honey Gold, we found that temperatures above 32 °C inhibited flowering so that reduction in temperature shown in Guevara et al. (2012) could have been critical for floral induction. It was concluded that the key factor governing flowering in the tropics is age of the last vegetative flush (Davenport 2003, 2009, 2010; Ramírez and Davenport 2010). In the Columbia, study discussed above the mean monthly temperature never exceeded 30°C so maximum temperatures could also have been important.
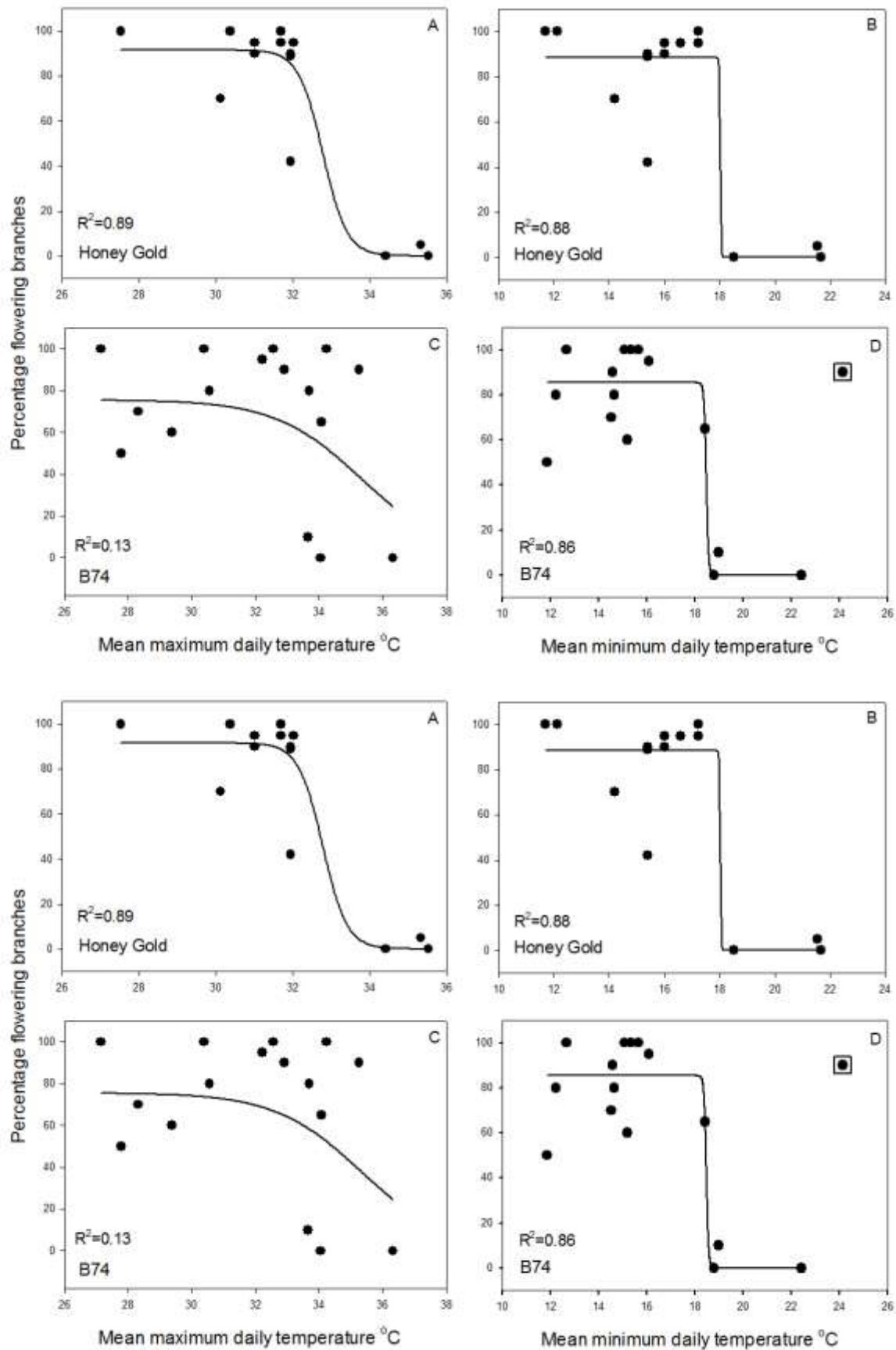
Figure 47. Fitted curves of the percentage of flowering branches for "Honey Gold" against the mean maximum temperature (A) and mean daily minimum temperature(B) for the 7 days including the day when shoots achieved 10 mm. Fitted curves of the percentage of flowering branches for "B74" against the mean maximum temperature (C) and mean daily minimum temperature (D) for the 7 days including the day when shoots achieved 10mm. Note the outlier in D in the square where flower induction occurred with a minimum temperature of 24 °C.

10.5 Conclusion

Cool ambient temperatures are detected in mature hard green mango leaves and transmitted to growing shoots where flower development occurs. We have shown that axillary bud development can be triggered by tip pruning of branches in both Honey Gold and B74 cultivars. This experiment illustrates that cool weather, with chill sums <20 °C over 300 hrs (unit) during axillary buds development, is needed for flower bud initiation and differentiation, especially in early weeks after tip pruning performed. However, further investigation through regression analysis noted that the length of time in which inductive conditions occur can in fact be narrowed to within a seven day period. Some commercials mango cultivars in Australia, such as Kensington Pride, are very low yielding. For these cultivars, we have shown that tip pruning has the potential to improve canopy flowering significantly when performed at the right time.

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