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*Improving smallholder farmer incomes through strategic market
development in mango supply chains in southern Vietnam*

Study: 2.2 PBZ Alternative and Temperature Impact study

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Summary

The use of the soil applied plant growth inhibitor paclobutrazol (PBZ) improves mango yield, quality and assists in synchronising harvest maturity. Due to the critical importance of PBZ to commercial production systems of major international mango traders alternative gibberellic acid (GA) synthesis inhibitors have been investigated to replace PBZ should its use be restricted. The mango production system in southern Vietnam differs from other production regions in that the time between the application of the PBZ is up to 40 days less than the 100-120 days used internationally. Previous researchers, in trialling chemical substitutions for PBZ, have used the same duration between application of the inhibitor and floral induction treatments.

The aim of this study was to compare the growth responses of Cat Hoa Loc mango trees treated with the standard Vietnamese soil-applied PBZ and time of induction with trees treated with foliar applications of GA synthesis inhibitors; either uniconazole-P (UCZ) or prohexadione calcium (ProCal). The formulation of the sprays applied are based on published trials on other mango cultivars that have demonstrated comparable yield responses to conventional soil-applied PBZ treatments in other production systems.

The study was undertaken on the SOFRI research orchard using 5-year-old Cat Hoa Loc trees (planted at a spacing of 3 metres x 3 metres). Trees were grown on their own roots. A standard management practice was applied to the trees prior to the commencement of the trial. Trees were estimated to have a canopy diameter of 3 metres for the purpose of calculating chemical application. All trees were sprayed with 3 litres of an aqueous 3% solution of mono-potassium phosphate in August 2020. The gibberellin synthesis treatments were applied from 3 October to 16 October 2020. Prior to treatment, tree dimensions were measured to enable tree size and canopy volume to be compared. Trees were sprayed with potassium nitrate to induce flowers and the first floral initials were recorded on 17 November 2020. Fruit were harvested on 8 March 2021 and the number of inflorescences.tree⁻¹, number of fruit.tree⁻¹, and individual tree yields were recorded, from which the mean fruit size was calculated. Records were kept of the ambient orchard temperature using a data logger suspended within the tree canopy.

Flowering and fruit production was observed in all treatments. However, there was little or no yield in almost half the trees of each treatment. The treatments were applied to the trees as a block and not randomised and no statistical differences were observed. There were some high yielding trees in the ProCal treatment. The ProCal treated trees appeared larger than the trees in the other treatments that could be partly explain their higher yields. The trees in the UCZ and ProCal treatments appeared to have a premature vegetative flush that could have reduced their flowering and yield as immature vegetative flush is known to inhibit flower induction. Use of potassium sulphate sprays in the period between the application of the gibberellin synthesis inhibitor treatments, and treatment for floral induction may assist in preventing premature bud growth. The minimum temperatures recorded throughout the trial were above those thought to induce flowering in other mango cultivars. This suggests that the chemical treatments applied caused the responses observed. This would be more conclusive if temperature records were maintained for the entire trial. The proposed next steps should include:

- repeat trial using a fully randomised split plot design to allow more robust statistical analysis
- standardised duration between the application of the gibberellin synthesis inhibitors and stimulation of floral induction using potassium nitrate to 75 days to enable comparison with previous Vietnamese trials that suggested this was optimum for other mango cultivars
- the monitoring of maximum and minimum temperatures throughout the trials
- development of a screen to prevent radiant heating of the loggers
- the inclusion of 3% potassium sulphate sprays at days 25 and 50 after the application of gibberellin synthesis inhibitors to prevent the premature vegetative flushing of the UCZ and ProCal treatments.

Context

Introduction

Manipulation of mango flowering using the soil-applied plant growth regulator, PBZ, and potassium nitrate sprays to induce flowering is widely used in tropical mango production in Mexico, South America and Asia (Davenport, 2009). PBZ functions to inhibit GA synthesis reducing the length of vegetative flush length and promotes the accumulation of reserves. Under Australian conditions this causes more rapid growth in response to stimulatory growth conditions (Blaikie *et al.*, 2004). Comparing the management systems used in Asia with those published for Mexico and Brazil indicates that there are marked differences in the scheduling of treatments and mango cultivars used. In southern Vietnam, detailed management systems have been developed for Cat Chu (Hau & Dien, 2009) and Cat Hoa Loc (Hau *et al.*, 2014) mangoes. In Vietnam, PBZ is applied 2–3 weeks after the commencement of vegetative growth that is stimulated by pruning following harvest. In Brazil, for Tommy Atkins, Haden, Keitt and other Florida mango varieties, flower initiation is stimulated by application of potassium nitrate (KNO₃) 100–120 days after application of PBZ (Albuquerque *et al.*, 2002; Oliveira *et al.*, 2020), while in Vietnam stimulation occurs 60–80 days after PBZ application. The use of PBZ improves mango yield, quality, and assists in synchronising harvest maturity (Davenport, 2009). Due to the critical importance of PBZ to commercial production systems of major international mango traders, alternative gibberellin synthesis inhibitors have been investigated to replace PBZ should its use be restricted (Hau *et al.*, 2018).

The biochemical stage of gibberellin formation that the different synthesis inhibitors act on differs. PBZ and UCZ are both triazoles that act at a similar early stage of gibberellin synthesis. They block the oxidation of ent-Kaurene, preventing the formation of the precursors for the formation of gibberellins. In comparison ProCal effects the final stages of gibberellin synthesis by inhibiting the formation of highly active gibberellins from inactive precursors (Verma *et al.*, 2010). ProCal is favoured as an option to replace PBZ because it breaks down relatively rapidly (half-life approximately 7–10 days) and is effective only in young shoots. Furthermore, the compound is translocated almost exclusively in the xylem and is unlikely to be accumulated in the fruit (Rademacher, 2000). It is also thought that ProCal breaks down in the soil within 24 hours of application.

In the past, the chemical thiourea was widely used in Vietnam to promote flower induction but recent recommendations promote the use of potassium nitrate as producing comparable responses.

The aim of this investigation was to compare the growth responses of Cat Hoa Loc mango trees treated with the standard soil-applied PBZ with alternate foliar applications of GA synthesis (either UCZ or ProCal). The formulation of the sprays applied are based on published trials on other mango cultivars that demonstrated comparable yield responses to conventional soil-applied paclobutrazol (PBZ) treatments. UCZ when applied to the soil had previously been shown to successfully replace PBZ in promoting flowering in the Dai Loan mango grown in the Cho Moi district (Hau *et al.*, 2018). In comparison, ProCal had not previously been investigated on Vietnamese mango cultivars.

The research team expected that growers would not consider a treatment requiring more than one application, and that treatments would need to be successful before trialling on mango properties.

Activities

Aim

Evaluate alternative products to PBZ that could be used to maintain high yields in Cat Hoa Loc should PBZ no longer be available.

Determine whether chemical inputs could be reduced for the off-season production of Cat Hoa Loc mangoes by using foliar applied gibberellin synthesis inhibitors including chemicals that breakdown more rapidly or require fewer chemical inputs.

Material and methods

Trials were performed on the SOFRI research orchard in Long Dinh commune, Chau Thanh district, Tien Giang province (10°24'13.0" N 106°16'33.9"E), using 5-year-old Cat Hoa Loc mango trees planted at a spacing of 3m x3m. The Cat Hoa Loc trees were grafted onto seedling Cat Hoa Loc rootstocks. A standard management practice was applied to the trees prior to the commencement of the trial. Trees were estimated to have a canopy diameter of 3 metres for the purpose of calculating chemical applications.

All trees were sprayed with a 3% mono-potassium phosphate (KH_2PO_4 , 0-52-34) on 24 September (day 268) and 1 October 2020 (day 275). The anti-gibberellin synthesis treatments were applied from 3 October (day 277) to 16 October (day 275) 2020. The application method, volume of spray and amount applied is shown in Table 1.

Table 1. Gibberellin synthesis inhibitor treatments applied to Cat Hoa Loc trees

Chemical	Application method	Amount	Date
paclobutrazol	Soil drench 1 g ai.L ⁻¹ (single application)	1g ai.m ⁻¹ canopy diameter	3 October 2020
prohexadione calcium	1500 mg.l ⁻¹ (single application)	3 L.tree ⁻¹ Total 4.5 g ai.tree ⁻¹	3 October 2020
uniconazole	Spray 1 : 0.33 g ai.L ⁻¹ Spray 2 : 0.33 g ai.L ⁻¹ Spray 3 : 0.66 g ai L ⁻¹ (three applications)	Spray 1: 3 L.tree ⁻¹ Spray 2: 3 L.tree ⁻¹ Spray 3: 3 L.tree ⁻¹ Total 4g ai.tree ⁻¹	3 October 2020 9 October 2020 16 October 2020

Source: Author analysis

All the gibberellin synthesis inhibitors were obtained in powder form. The PBZ contained 25% ai w/w. The chemicals used in this trial have been locally sourced and packaged, and we relied on this information for authenticity and content. The chemicals used were UCZ 5% w/w (see Figure 1), ProCal 10% w/w (see Figure 2) and PBZ 25% w/w (see Figure 3).



Figure 1. Locally sourced UCZ

Source: Dong Xhan, 2020

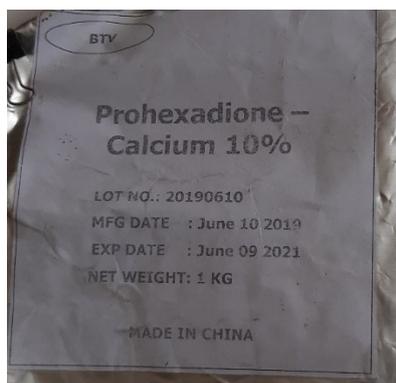


Figure 2. Locally sourced ProCal

Source: Author's image



Figure 3. Locally sourced PBZ

Source: Author's image

Prior to treatment, tree height (z) and canopy width perpendicular to the row (x) and parallel to the row (y) were measured. It was assumed that the canopy was an irregular ellipsoid (Charles-Edwards *et al.*, 1986), and the canopy volume (V) was calculated using the equation:

$$V = (\pi * (x-1) * y * z) / 6$$

One metre (1) was subtracted from x , the tree height to represent the skirted (pruned) height up to which branches were removed.

The trees were sprayed twice with a mixture of potassium nitrate and thiourea as described in Table 2. Observation of the responses to these treatments were made 17 November 2020 (day 322).

Table 2. Formulation of the first and second chemical induction sprays

Induction spray number	Date	Day of year (2020)	Inductive chemicals (g/18L)	Amount applied to each tree (L)
1	3.11.2020	308	120 potassium nitrate (0.67%) 40 thiourea (0.22%)	3
2	10.11.2020	315	60 potassium nitrate (0.33%) 20 thiourea (0.11%)	3

Source: Author's analysis

Hourly temperatures were monitored within a tree canopy by suspending a Temprecord RH humidity and temperature recorder (see Figure 4.). Temperatures were collected for the periods 15 October 2020 – 5 November 2020 and 27 November – 31 December 2020.



Figure 4. Temprecord RH humidity and temperature logger

Source: Author's image

Note: IC-953170ESCA, Temprecord International Ltd Auckland NZ

Following SOFRI standard practice, developing fruit were covered with white cloth bags on 1 January 2021 and were harvested on 8 March 2021 (day 67 of 2021). Data on inflorescence development, fruit number and yield was recorded.

Results and discussion

Results

Tree dimensions of the 18 trees used in the trial at the time of application of the plant growth regulators are shown in Table 3. The trees used for the ProCal treatment appeared to be taller than the trees allocated the PBZ and UCZ treatments so that the canopy volume also seemed larger. Immature foliage on trees treated with either of UCZ and ProCal reported marginal leaf burn. This was not observed on trees treated with PBZ.

Table 3. Tree dimensions, gibberellin synthesis inhibitor trial

Tree number	Diameter (m)			Height (m) (x)	Canopy volume (m ³)	No. of shoots per tree	No. of shoots infected by pests
	W-E (y)	S-N (z)	Average				
Tree 1	2.7	3	2.85	2.5	6.36	171	9
Tree 2	2.6	2.7	2.65	2.2	4.41	141	7
Tree 3	2.5	2.6	2.55	2.3	4.42	139	6
Tree 4	2.6	2.95	2.775	2.3	5.22	152	10
Tree 5	2.2	2.63	2.415	2.3	3.94	132	9
Tree 6	2.3	2.9	2.6	2.6	5.59	144	17
	2.48	2.80	2.64	2.37	4.99	146.50	9.67
Tree 7	3.2	2.9	3.05	2.6	7.78	213	5
Tree 8	2.7	2.4	2.55	2.2	4.07	124	8
Tree 9	2.9	2.5	2.7	2.6	6.07	144	10
Tree 10	2.6	2.4	2.5	1.9	2.94	130	7
Tree 11	2.7	3.3	3	2.3	6.07	109	6
Tree 12	2.3	1.9	2.1	2.1	2.52	123	17
	2.73	2.57	2.65	2.28	4.91	140.50	8.83
Tree 13	2.5	3.1	2.8	2.5	6.09	163	7
Tree 14	2.5	3.3	2.9	2.4	6.05	146	7
Tree 15	3.7	3	3.35	2.4	8.14	215	6
Tree 16	2.1	2.6	2.35	2.2	3.43	115	3
Tree 17	2.2	2.4	2.3	2.3	3.59	91	4
Tree 18	3.3	2.9	3.1	2.6	8.02	168	7
	2.72	2.88	2.80	2.40	5.89	149.67	5.67

Source: Author's analysis

Trees were observed after treatment with potassium nitrate and thiourea. Inflorescence development was observed on all treatments. The appearance of the leafy shoots and axillary inflorescence initials on trees are shown after treatment with PBZ (see Figure 5) and UCZ (see Figure 6) and ProCal (see Figure 7).



Figure 5. Shoot apices following treatment with PBZ

Source: Author's image

Note: Observed, 17 November 2020



Figure 6. Shoot apices following treatment with UCZ

Source: Author's image

Note: Observed, 20 November 2020 (left) & 7 December 2020 (right)



Figure 7. Shoot apices following treatment with ProCal

Source: Author's image

Note: Observed, 17 November 2020



Figure 8. Developing bagged fruit to protect from fruit fly damage

Source: Author's image

Per standard SOFRI practice, the bagging of the fruit with cloth bags prevented fruit fly damage (see Figure 8).

At harvest, the number of inflorescences and fruit per tree were counted and yield of fruit per tree weighed. The average fruit weight and mean anti-gibberellin synthesis treatment effects calculated (see Table 4). There was no significant difference in yield between treatments. This could possibly be improved in future trials by using a split plot design that may assist in accounting for any spatial variation and provide greater degrees of freedom for statistical analysis. There would appear to be greater levels of flowering in the UCZ and ProCal treatments, but it should be noted that the ProCal treated tree tended to be larger.

Table 4. Tree responses to synthesis inhibitors, assessed 8 March 2021

Replicate number (1-6)	Inflorescences.tree ⁻¹	Fruit.tree ⁻¹	Average fruit weight g.fruit ⁻¹	Yield kg.tree ⁻¹
PBZ 1.1	21.00	10.0	478.50	4.79
PBZ 1.2	1.00	0.0	0.00	0.00
PBZ 1.3	12.00	0.0	0.00	0.00
PBZ 1.4	11.00	23.0	354.30	8.15
PBZ 1.5	7.00	1.0	387.00	0.39
PBZ 1.6	4.00	0.0	0.00	0.00
Mean PBZ	9.33	5.7	203	2.22
UCZ 2.1	30.00	3.0	402.67	1.21
UCZ 2.2	12.00	19.0	383.68	7.29
UCZ 2.3	9.00	8.0	334.25	2.67
UCZ 2.4	11.00	12.0	351.00	4.21
UCZ 2.5	21.00	0.0	0.00	0.00
UCZ 2.6	2.00	0.0	0.00	0.00
Mean UCZ	14.17	7.0	245	2.56
ProCal 3.1	2.00	0.0	0.00	0.00
ProCal 3.2	18.00	8.0	403.88	3.23
ProCal 3.3	8.00	0.0	0.00	0.00
ProCal 3.4	68.00	63.0	264.03	16.63
ProCal 3.5	26.00	0.0	0.00	0.00
ProCal 3.6	25.00	19.0	342.11	6.50
Mean ProCal	24.50	15.0	168	4.39

Source: Author's analysis

Information collected using the temperature loggers is presented in Figure 9. The temperature data can be used to interpret potential effects of temperature on flower initiation and fertilisation based on trees that failed to flower, or flowered and did not set and retain fruit. The period between 5 and 17 November 2020 would have assisted in understanding the conditions during flower induction and early flower development following the potassium nitrate and thiourea treatments. However, despite the night temperatures being markedly lower than the daily maximums by more than 10°C they appear to remain above 23°C suggesting that the resulting flowers were chemically induced and not the result of low temperatures. It is also notable that daily maximums regularly exceeded 35°C but this has not inhibited floral initiation in any of the treatments.

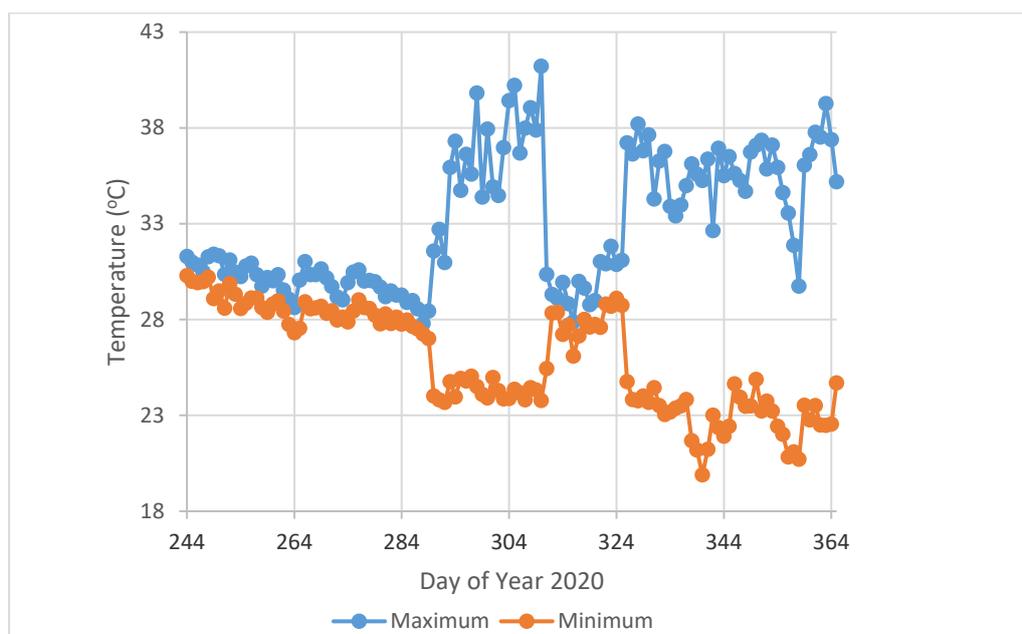


Figure 9. Minimum and maximum orchard temperatures, October – December 2020

Source: Author's analysis

Note: Temperature recording periods: 15 October – 5 November and 27 November – 31 December 2020
For the period between these dates, loggers were not used and records were not obtained.

Discussion

In the hormonal model of flower initiation proposed by Davenport (2009) the use of PBZ is thought to reduce the level of GA that is believed to inhibit floral initiation. Exogenous applications of gibberellin support this interpretation as they have been shown to inhibit flowering. In Vietnam, exogenous sprays of gibberellin can be used to inhibit flowering and ensure vegetative growth following pruning after harvest (Hau, 2008).

These trials have successfully shown that comparable yields can be achieved using either PBZ, UCZ or ProCal. However, tree yields were low and inconsistent in all treatment with around half the trees in all treatments failing to either flower or set and retain fruit. The time between application of treatments and inductive sprays was much shorter than best practice for southern Vietnam. This may have reduced the time for trees to accumulate reserves required to support a crop but could also be due to the treatments failing to prevent vegetative growth that is known to inhibit flower induction (Davenport, 2009). Options for statistical analysis were limited by the design of the trial future investigations should consider using fully randomised split plots enabling spatial variation with orchards to be considered.

The time between application of the gibberellin synthesis inhibitors was around 30 days, which is much shorter than previous investigations using PBZ and UCZ in Vietnam. Hau *et al.*, (2018) previously compared the effects of PBZ and UCZ in Dai Loan mango. In their trial they compared PBZ at 1.5 g a.i.m⁻¹ canopy diameter with UCZ at 1.0, 1.5 and 2.0 g.a.i m⁻¹canopy diameter then applied KNO₃ 45, 60 and 75 days after bud induction treatments (DABIT). They had the highest yields when PBZ or UCZ were applied at 1.5 ga.i. m⁻¹canopy diameter and KNO₃ was applied 75 DABIT. These results suggest that cropping responses could be improved by extending the time between application of the gibberellin synthesis inhibitor and the inductive chemical treatment to 75 days.

It should be noted that the gibberellin synthesis solubility differs markedly: saturated UCZ solution contains 8.4 mg/L at 25°C, saturated PBZ solution contains 26 mg/L at 20°C and saturated ProCal solution contains 174 mg/L at 20°C (National Library of Medicine, n.d.).

In tropical regions autonomous flower induction has been described to occur when temperatures do not regularly go below 18°C (Ramirez & Davenport, 2012). In southern

Vietnam, the occurrence of minimum temperatures below 18°C is rare but on-season production occurs after the annual period when lowest minimum night temperatures occur and do not require nitrate spray for this induction. This suggests that low night temperatures are associated with flower induction in some cultivars but do not necessarily require minimums below 18°C. If this is the case, the induction of flowering may not be due solely to the chemical treatments. Chemical flower induction in the tropics either by nitrate or cool temperatures occurs much more rapidly, in a matter of days (Hau *et al.*, 2014, Clonan *et al.*, 2021) compared the 2–3 months described for subtropical and temperate production regions (Davenport, 2009). The observation of inflorescence development in all gibberellin synthesis inhibitor treatments within two weeks after treatment with potassium nitrate and thiourea is consistent with these observations.

In these current trials, a combination of potassium nitrate and thiourea was used to initiate flowers. The concentration of both these chemicals was much lower than the published commercial concentrations for the use of these chemicals of 3% for potassium nitrate and 0.5% for thiourea.

Insights

PBZ is sold as powder in Vietnam and made into an aqueous suspension prior to applying on the base of the tree in the rootzone. The white powdered commercial product contains 10-25% active ingredient. PBZ is believed not to be phloem mobile meaning that it is not translocated to developing mango fruit. The observation that brix levels reduced in Cat Hoa Loc with increasing levels of PBZ (Hau *et al.*, 2014) is consistent with the significant negative relationship reported in Palmer mangoes in Brazil where a reduction of total sugars (reducing sugar and non-reducing sugars) was also found in mango trees irrigated with PBZ at 0.7 – 1.9 g a.i. m⁻¹ canopy diameter prior to flowering (de Souza *et al.*, 2016). This suggests that either PBZ directly or indirectly affects the accumulation of starch or the conversion of fruit reserves to sugars. The foliar applications of gibberellin synthesis inhibitors have the potential to reduce chemical inputs and reduce exposure of roots to the inhibitory effects of growth regulators.

The chemical manufacturer of the locally sourced UCZ and ProCal are unknown beyond the labelling on the packaging. Both these chemicals are sold under license internationally as aqueous suspensions by Sumitomo/Valent Sumagic® 0.5g L⁻¹, Fine Agrochemicals Ltd as Concise® (UCZ) and in Australia by Sumitomo Chemical Australia Pty Ltd as Sunny 50® (50g L⁻¹). ProCal is sold internationally as granules by BASF (ProCal) as Apogee® (275g.kg⁻¹ w/w) and Regalis plus® (100g.kg⁻¹w/w). both these products on the Australian market are extremely expensive costing more than AUD500.00 per package 1 L Sunny® or 1.5kg Regalis Plus®. Alternative sources are not registered for agricultural use in Australia.

The previous published results for foliar application of UCZ in mango used UNI 50 SC (powdered form of UCZ) that contained 50g L⁻¹ of UCZ (de Souza *et al.*, 2016). The UCZ used in the current trials was locally sources where it is marketed as powder Stoplant 5WP and contained 50g.kg⁻¹ w/w UCZ and sold in 100g packs for USD25.00. In comparison ProCal was available in bulk for USD10 kg and contained 100g.kg⁻¹w/w. These prices could be reduced further by directly sourcing the chemicals from a Chinese supplier such as King Tech corporation. While the safety of alternate sources of these chemicals needs to be confirmed as suitable for food production, southern Vietnam potentially has access to price competitive alternatives to PBZ.

Due to the low solubility of the triazoles it is unclear whether there is sufficient time for the powdered UCZ to dissolve when product is supplied as a powder and prepared under orchard conditions. Since the chemicals such as UCZ are more soluble in organic solvents such as ethanol, investigation of stock solutions of these synthesis inhibitors in benign solvents that are then added to water to ensure saturation is achieved could be examined.

The previous investigations of soil application of UCZ were undertaken to provide options to maintain production should access to PBZ be restricted. It should be noted that both PBZ and UCZ are from the same family of chemicals. In the USA (greatest importer of mangoes worldwide), both UCZ and PBZ are only permitted for use on ornamental plants, while ProCal can be used on fruit trees such as apple.

Commercial formulations of ProCal (Regalis plus®) contain pH modifying buffers and recommendations for use of UCZ suggest use of buffers to reduce spray pH to 4.5-5.5. This was not considered as part of these investigations. There are also a range of adjuvants that could also be assessed to determine if they assist in the uptake of these growth regulators. Due to the number of potential variables and limited access to orchards, screening protocols need to be developed, such as potted plant trials, to rationalise potential considerations for further development.

There is also considerable development required in adapting any spray formulation to the high-volume pump-based spray equipment used almost universally in southern Vietnam mango orchards. Mature mango trees are routinely sprayed with 15 L of product for floral initiation and pest treatment (Van Son, personal communication). It is unclear whether there is a linear uptake of gibberellin synthesis inhibitor with increases in the spray concentration or if the total amount of chemical applied to a tree controls the subsequent growth responses.

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