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Original Article

Physiological changes in pre-harvest dropped fruits in the pummelo cultivars '*Thong Dee*' and '*Khao Nam Phueng*'

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Abstract

This investigation of physiological changes in pummelo pre-harvest dropped fruit, termed "yellow fruit calyx symptoms" in Thailand, aimed to examine in two particular cultivars *Thong Dee* and *Khao Nam Phueng* grown in the central region of Thailand. The results show that the normal pummelo fruits of either variety had statistically more total non-structural carbohydrate (TNC) in their peel and pulp than did those of the dropped fruits. On the other hand, the leaves of normal fruit trees of both cultivars show less TNC than those found in the leaves of pre-harvest dropped fruit trees. There were significant differences in some plant nutrients in the leaves, peel and pulp of the dropped and normal pummelo cultivars. IAA concentration in fruit was determined with the result that normal fruits had a statistically higher IAA concentration than did those in pre-harvest dropped fruits. The PCR technique used for the greening disease test identified infections in leaves taken from the pre-harvest dropped fruit trees but none in leaves from the normal fruit trees. There were no differences in soil chemical properties between soil samples taken from the normal and pre-harvest dropped fruit trees. It seems likely that greening disease is the cause of 'yellow fruit calyx symptom' in Thailand and is the resulting from low TNC concentrations, low plant nutrients and low IAA concentrations in the pre-harvest dropped fruits in the pummelo cultivars.

Keywords: pummelo, pre-harvest fruit drop, physiological change, *Thong Dee*, *Khao Nam Phueng*

1. Introduction

Pummelo growers in Thailand have met pre-harvest fruit drop problems. The symptoms occur about three months after fruit set resulting in small fruit, yellow peel, slow rate of fruit growth, and the fact that the fruit calyx turns yellow. Finally, these fruits start to drop around 2-3 months before harvesting. The pummelo growers in the central region of Thailand have named these symptoms "yellow fruit calyx symptom". Over the last 2-3 years this problem has become quite serious with growers in Thailand losing both production and income. A shortage of carbohydrate, imbalances in plant nutrition and plant hormone as well as insect and pathogen have all been reported as causes of the fruit drop in pummelo and other citrus fruit (Davies and Albrigo, 1998). Nonetheless, the causes of pre-harvest fruit drop in pummelo are still unknown. This study has investigated the possible causes and physiological changes in pre-harvest "yellow fruit calyx symptom" in Thailand.

2. Material and Methods

The research was conducted in the pummelo growing areas of Nakhon Pathom. Similar size and age of *Thong Dee* and *Khao Nam Phueng* pummelo trees from five orchards were selected to set up the experiment. Fruit was collected from 10 pre-harvest dropped fruits and 10 normal fruits as a control group *per* cultivar *per* orchard at the fruit drop stage. The fruits were taken to determine the total non-structural carbohydrate (TNC), plant nutrient concentration, IAA concentration, and disease infection, as well as soil samples were taken to determine the soil chemical properties.

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2.1 Total non-structural carbohydrates

Peel, pulp and 10 leaves from each of the dropped and normal fruit trees were separated and taken for total non-structural carbohydrates analysis using the Nelson's procedure. All the samples were analyzed for total TNC by washing the samples in tap water then rinsing in distilled water and finally drying in a hot air oven at 65°C for 72 hrs. After drying, the material was ground in a Wiley Mill and stored in a desiccator. Total non-structural carbohydrates were extracted with 0.2 NH_2SO_4 (Smith, 1969) and determined by the Nelson reducing sugar procedure method described by Hodge and Hofreiter (1962) at the Central Laboratory, Kasetsart University, Kamphaeng Saen Campus.

2.2 Plant nutrient concentration

Peel, pulp and leaves from the dropped and normal fruit were separated and taken for plant nutrient concentration analysis. The plant material was dried and ground before plant nutrient analysis. From this dried material the nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) concentration was determined. Levels of N were determined using the Microkjeldahl Method. P, K, Ca, Mg, Fe, Mn, Zn and Cu were extracted by HNO_3-$HClO_4$ (5:1) then the solution was left to cool down. Phosphorus in solution was determined calorimetrically by the Molybdate-vanadate yellow color method. K, Ca, Mg, Fe, Mn, Zn and Cu were determined using an atomic absorption spectrophotometer. All plant nutrient analysis were done at the Soil Plant and Agricultural Material Testing and Research Unit, Central Laboratory, Kasetsart University, Kamphaeng Saen Campus.

2.3 IAA concentration

Twenty-five of each of the normal and pre-harvest dropped fruits (five fruits *per* cultivar and *per* orchard) were collected. Peel from the fruit was separated and taken for IAA concentration analysis using high performance liquid chromatography (HPLC) detection (Crozer *et al.,* 1980) at the Laboratory Center for Food and Agricultural Products in Bangkok.

2.3.1 Extraction and partitioning

Peel samples were extracted twice with methanol, the methanolic extracts reduced to the aqueous phase in vacuum, and equal volumes of 1.0 M pH 8.0 phosphate buffer added. The sap was similarly buffered and all three samples were adjusted to pH 8.0 prior to partitioning five times against half-volumes of toluene. The aqueous phase was then slurried with insoluble polyvinylpyrolidone, filtered, adjusted to pH 3.0, and partitioned five times against two-fifth volumes dichloromethane. The three acidic, dichloromethane-soluble extracts were dried and reduced to dryness in vacuo.

2.3.2 High performance liquid chromatography

The solvent delivery system was a microprocessor-controlled Varian Model 5010 HPLC. Samples were introduced via a Perkin-Elmer Model 7105 sample valve and column cluates were monitored with either a Perkin-Elmer Model 650-10LC spectrofluorimeter or a Waters Model 440 UV absorbance monitor. Steric exclusion chromatography (SEC) was carried out on a 8-300 m-Spherogel (5 nm) column (Altex Scientific Inc., Berkley, U.S.A) using 0.5% acetic acid in tetrahydrofuran as a solvent at a flow rate of 1 ml min^{-1} (Crozier *et al.,* 1980). A 5-250 mm ODS-Hypersil column (Shanden Southern Instruments Inc., Sewickley, U.S.A) used for reverse phase HPLC was eluted with an increasing gradient of ethanol in ammonium acetate buffer (20 mM, pH 3.5) delivered at a flow rate of 1 ml min^{-1}.

2.3.3 Combined gas chromatography-mass spectrometry

Electron impact mass spectra of methylated samples were obtained at 70 eV with a Finnigan Model 4023 combined gas chromatograph-mass spectrometer (GC-MS) fitted with a 6'-2 mm OV-101, 100-120 mesh HP Chromosorb W column. The carrier gas was helium at 30 ml min^{-1}, the injector, jet separator, and source were operated at 225°C and the column oven at 180°C.

2.3.4 Solvents

All solvents were distilled in glass. Those used for reverse phase HPLC required special attention in order to minimize changes in the detector baseline during solvent-programmed runs. To this end ammonium acetate buffer was purified by running it through a 4-80 mm Bondapak C_{18} column, and technical grade ethanol was stored over granular charcoal for at least three days before being filtered through a fluorocarbon membrane filter (0.45 mm) and distilled. These treatments consistently produced solvents of higher quality than HPLC grade reagents purchased from commercial sources. Tetrahydrofuran for SEC was refluxed over Cu_2Cl_2 for 30 min prior to distillation after which it was stored in darkness under N_2 at 3°C for no longer than three days before use.

2.4 Greening test

Seven of the normal and pre-harvest dropped fruit trees were used for a greening test: four for *Thong Dee* and three for *Khao Nam Phueng* cultivar. Leaf samples (6-7 leaves *per* tree) from each the normal and pre-harvest dropped fruit trees were collected and then taken to the laboratory at the Department of Plant Pathogens in the Faculty of Agriculture at Kasetsart University in Bangkhen, Bangkok for the greening test using the polymerase chain reaction (PCR) technique.

2.5 Soil properties

Composite soil samples were collected at 0-15 cm and 15-30 cm depth below the surface near normal and pre-harvest dropped fruit trees using auger at the edge of canopy. The samples were taken to the laboratory for soil chemical property testing: pH, EC, CEC, and soil organic matter (SOM), and at the same time P, K, Ca, Mg, Fe, Mn, Zn and Cu concentrations were ascertained. The methods used for soil chemical property and soil nutrient tests are identified in Table 1.

3. Results

3.1 Statistical analysis

All the parameters in Table 1 were statistically tested by the independent sample t-test method at the 0.05 significance level and yielded the following results

3.2 Total non-structural carbohydrate

Table 2 shows the TNC concentration (mg g^{-1}dw) in the leaves, peel, and pulp from the normal and pre-harvest dropped fruit of the *Thong Dee* and *Khao Nam Phueng* cultivars. On a dry weight basis, the normal pummelo fruits of both *Thong Dee* and *Khao Nam Phueng* cultivars had statistically more TNC concentration in their peel and pulp, 87.09 and 355.50 mg kg^{-1}dw, and 86.21 and 329.19 mg kg^{-1}dw, respectively, compared to the pre-harvest dropped fruit at 78.27 and 301.01 mg kg^{-1}dw and 80.24 and 273.09 mg kg^{-1}dw, respectively. The leaves of normal fruit trees in both cultivars showed statistically less TNC concentrations, 26.15 and 23.95 mg g^{-1}dw for *Thong Dee* and *Khao Nam Phueng*, respectively, than in the leaves of the pre-harvest dropped fruit trees at 35.86 and 30.28 mg g^{-1}dw for *Thong Dee* and *Khao Nam Phueng*, respectively.

3.3 Plant nutrient concentrations

Table 3 to 5 show the macro and micronutrient concentrations in the *Thong Dee* cultivar pulp, peel, and leaves of the normal and pre-harvest dropped fruits. This study revealed that the pulp of the normal fruits had statistically a lower N, Ca and Mg concentration than those in the pre-harvest dropped fruits; whereas, the pulp Cu concentration

Table 1. Methods for the Soil Chemical Property and Soil Nutrient Tests.

Parameter	Method
pH	1:1 soil:H_2O
Electrical conductivity	1:5 soil:H_2O
Cation Exchange Capacity	1 N NH_4Oac pH_7
Soil organic matter	Walkley and Black
Available P	Bray II
Extractable K, Ca and Mg	1 N NH_4Oac pH7
Extractable Fe, Cu, Mn and Zn	DTPA

Table 2. TNC Concentrations (mg kg^{-1}dw.) for leaf, peel and pulp of *Thong Dee* and *Khao Nam Phueng* Normal and Pre-harvest dropped fruits.

Treatment	*Thong Dee*			*Khao Nam Phueng*		
	Peel	Pulp	Leaf	Peel	Pulp	Leaf
Normal fruit	87.09	355.50	26.15	86.21	329.19	23.95
Pre-harvest dropped fruit	78.27	301.01	35.86	80.24	273.09	30.28
Sig*.	0.02	0.02	0.03	0.04	0.03	0.04

* significant difference at .05 level

Table 3. *Thong Dee* Macronutrient (%) and Micronutrient (mg kg^{-1}dw) Concentrations of the Normal and Pre-harvest Dropped Fruit Pulp.

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Normal fruit	1.81	.237	1.69	0.31	0.09	6.81	18.34	1.72	5.96
Pre-harvest dropped fruit	2.05	.248	1.88	0.43	0.11	4.45	15.10	1.86	5.50
Sig*.	0.004	0.454	0.108	0.002	0.010	0.019	0.551	0.602	0.627

* significant difference at .05 level

Table 4. *Thong Dee* Macronutrient (%) and Micronutrient (mg kg^{-1}dw) Concentrations in Normal and Pre-harvest Dropped Fruit Peel.

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Normal fruit	1.16	0.11	1.05	0.93	0.13	4.08	17.96	2.98	3.93
Pre-harvest dropped fruit	1.36	0.13	1.34	1.23	0.11	4.95	18.56	3.43	5.11
Sig*.	0.012	0.036	0.001	0.008	0.171	0.347	0.759	0.183	0.026

* significant difference at .05 level

Table 5. *Thong Dee* Macronutrient (%) and Micronutrient (mg kg^{-1}dw) Concentrations in Normal and Pre-harvest Dropped Fruit Tree Leaves.

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Normal fruit	2.81	0.16	2.08	6.10	0.40	106.33	272.27	24.18	48.33
Pre-harvest dropped fruit	2.68	0.17	2.18	4.90	0.33	68.83	264.05	20.33	43.09
Sig*.	0.38	0.58	0.46	0.04	0.02	0.40	0.79	0.27	0.65

* significant difference at .05 level

Table 6. *Khao Nam Phueng* Macronutrient (%) and Micronutrient (mg kg^{-1}dw) Concentrations in Normal and Pre-harvest Dropped Fruit Pulp.

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Normal fruit	1.85	0.25	1.73	0.33	0.10	6.97	17.35	2.28	8.76
Pre-harvest dropped fruit	2.09	0.27	2.01	0.39	0.11	6.26	15.35	2.14	7.35
Sig*.	0.012	0.44	0.11	0.20	0.24	0.58	0.70	0.71	0.33

* significant difference at .05 level

in the normal fruits was higher than those in the pre-harvest dropped fruits (Table 3).

The N, P, K, Ca and Zn concentration in the peel of the normal fruit were statistically lower than those in the pre-harvest dropped fruits but no significant differences were found between normal and pre-harvest dropped fruit for the other nutrients (Table 4). Concentrations of Ca and Mg in leaves from the normal fruit trees were seen as higher than those from the pre-harvest dropped fruit trees (Table 5).

Table 6 to 8 show the macro and micronutrient concentration in the *Khao Nam Phueng* cultivar pulp, peel, and leaves in normal and pre-harvest dropped fruit. From the study findings, the pulp of the normal fruit had statistically less nitrogen concentration than those in the pre-harvest dropped fruit. However, there were no significant differences in the other nutrients (Table 6).

The N, P, and Ca concentration in the peel of the normal fruit were statistically lower than those in the pre-harvest dropped fruit but no significant differences have been observed between the normal fruits and the pre-harvest dropped fruits for the other nutrients (Table 7). Concentrations of leaf Ca in the normal fruit trees were higher than those in the pre-harvest dropped fruit trees (Table 8).

3.4 IAA concentration

Normal fruits of both cultivars had statistically higher IAA concentrations, with 0.52 and 0.37 mg kg^{-1}dw for *Thong Dee* and *Khao Nam Phueng*, respectively, than those in pre-harvest dropped fruit, with 0.17 and 0.11 mg kg^{-1}dw for *Thong Dee* and *Khao Nam Phueng*, respectively ($t < 0.05$) (Figure 1).

The results from pathogen infection tests showed some pathogen infection in both the normal and pre-harvest dropped fruit of both cultivars. The infected pathogens found in the fruit calyx were *Lasiodiplodia theobromae*, *Fusarium moniliform*, and *Colletotrichum gloeosporioides* (data not shown). Pummelo leaves taken for the greening test

Table 7. *Khao Nam Phueng* Macronutrient (%) and Micronutrient (mg kg^{-1}dw) Concentrations in Normal and Pre-harvest Dropped Fruit Peel.

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Normal fruit	1.03	0.094	1.17	1.02	0.144	4.11	14.48	2.65	4.86
Pre-harvest dropped fruit	1.29	0.120	1.21	1.36	0.148	5.32	14.06	2.72	5.66
Sig.	0.003	0.029	0.610	0.034	0.675	0.292	0.892	0.805	0.423

* significant difference at .05 level

Table 8. *Khao Nam Phueng* Macronutrient (%) and Micronutrient (mg kg^{-1}dw) Concentrations in Normal and Pre-harvest Dropped Fruit Trees Leaves.

Treatment	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Normal fruit	2.62	0.12	1.88	6.75	0.49	124.29	322.07	26.41	46.53
Pre-harvest dropped fruit	2.49	0.12	2.06	5.70	0.42	85.83	298.26	21.67	38.70
Sig.	0.24	0.88	0.36	0.02	0.27	0.55	0.39	0.04	0.23

* significant difference at .05 level

by the PCR technique were shown in Figure 2. The PCR results showed greening disease infection in five (no. 6, 7, 8, 11 and 13) of the seven leaf samples taken from the pre-harvest dropped fruit trees (number 6, 7, 8, 9, 10, 11 and 13) but no infection in leaf samples from the normal fruit trees (no. 1-5, 12, and 14).

3.5 Soil properties

Soil samples from the normal and pre-harvest dropped pummelo fruit trees were taken to determine soil chemical properties. The results showed that there were no statistically significant differences in the chemical soil properties (Table 9 to 10).

4. Discussion

It is normal to find fruit drop in any crop production including citrus species. Many causes have been attributed to the shortage of carbohydrate, imbalances in plant nutritiones and plant hormones, poor fertilization, insects, disease, high temperatures, rainfall and defective irrational practices (Davies and Albrigo, 1998). Iglesias *et al.* (2006) reported that two successive abscission waves affecting flowers and developing fruitlets can be identified in citrus trees. The first wave induces massive flower and ovary abscission but the second reduces the number of growing fruitlets during the June drop. However, the pre-harvest fruit drop in citrus including pummelo especially at 2-3 months before harvesting is hardly ever reported. It seems, therefore, that the causes of pre-harvest fruit drop called "the yellow calyx fruit symptom" seen in Thai pummelo growing identified in this study

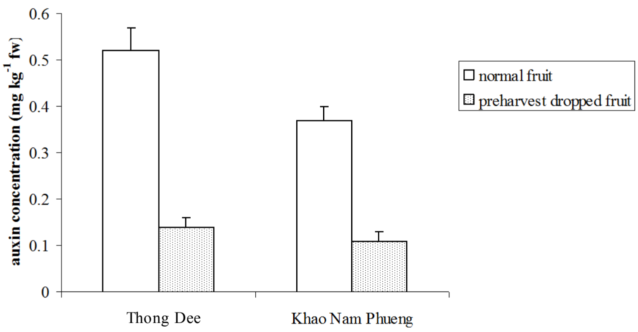

Figure1. IAA concentrations in normal and pre-harvest dropped fruits. Error bars show standard errors and data presented are the mean of 25 normal fruits and 25 pre-harvest dropped fruits *per* cultivar.

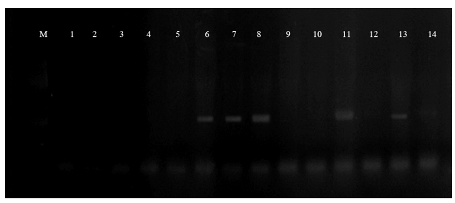

Figure 2. PCR detection of greening diseases. Number 1-5, 12 and 14 are samples from normal fruit trees. Number 6-10, 11 and 13 are samples from pre-harvest dropped fruit trees.

need to be clarified.

Many researchers have reported fruit drop in citrus due to low carbohydrate in fruits caused by the lack of carbohydrate translocation (Davies and Albrigo, 1998; Ruiz

Table 9. Soil pH, EC, CEC and Moisture Content (%) at 15 cm depth in Normal and Pre-harvest Dropped Fruit Tree Samples.

Treatment	pH	EC	CEC	Soil Moisture Content
Normal fruit trees	5.6	1.8	24.94	6.29
Pre-harvest dropped fruit trees	5.9	2.2	26.54	6.42
Sig*.	>0.05	>0.05	>0.05	>0.05

* significant difference at .05 level

Table 10. SOM (%), Macronutrient Concentrations (%) and Micronutrient Concentrations (mg kg^{-1}) at 15 cm Depth in Normal and Pre-harvest Dropped Fruit Tree Samples.

Treatment	% OM	P	K	Ca	Mg	Fe	Mn	Cu	Zn
Normal fruit trees	3.12	1617.26	550.96	2908.62	786.96	150.94	65.61	15.91	23.67
Pre-harvest dropped fruit trees	3.26	1666.46	590.26	2974.78	857.65	133.9	57.08	14.53	23.85
Sig*.	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

* significant difference at .05 level

et al., 2001). However, this often happens in the June drop at which stage the fruits are still small and not 2-3 months before harvesting when they are much larger. Based on the evidence in this study, the pulp and peel TNC concentrations in the normal fruits from both cultivars were higher than those in the dropped fruits (Table 2), which accords with other findings for mango, apple, and sweet cherry (Blanusa *et al.,* 2006). Ruiz *et al.* (2001) have reported that a shortage of carbohydrate causes a drop of fruit because the sucrose concentration in leaves decreases causing greater competition for the developing fruit. However, this study had found the reverse and leaf TNC concentration from the normal fruit trees were lower than those from the dropped fruit trees (Table 2). This suggests the shortage of carbohydrate in the dropped fruits is not directly caused by low photosynthesis in the leaves but may instead be caused by low photoassimilated translocation from the leaf to the pummelo fruits. Normally, the photoassimilates is tranlocated from source (leaf) to sink (fruit) in the fruit trees. Tuomi *et al.* (1989) and Oitate *et al.* (2011) suggested that photoassimilates produced by the leaf in a shoot are preferentially consumed in sink organs in the same shoot, and largely contribute to the vegetative and reproductive growth of this shoot. The photoassimilated translocation into fruits in orchard trees has been most extensively studied using the ^{13}C tracer. Volpe *et al.*(2008) examined effects of the sink-source balance on translocation of photoassimilates from adjacent non-reproductive shoots to peach fruits. Zhang and Tanabe (2008) examined translocation of photoassimilates from various types of shoot to pear fruits in adjacent shoots. Therefore, it could be that the dropped fruits received low photoassimilates from the leaves due to the poor carbohydrate translocation resulting in the low TNC in the dropped fruits but the high TNC in the leaves of dropped fruit trees.

An imbalance of plant nutrient may be the cause of the fruit drop in citrus including pummelo. Findings from the current study indicated that there were some significant differences in nutrient concentrations in pulp and peel in the dropped and normal pummelo fruits from both cultivars (Table 3-8). However, the leaf nutrient concentrations from the dropped and normal fruit trees were within the acceptable range for citrus and pummelo (Change *et al.,* 1992 and Maneepong, 2008). The implication here is that the pre-harvest drop of pummelo fruit in this study apparently was not caused by an imbalance of or unsuitable plant nutrient concentrations in pummelo trees. Although, the significant differences in some plant nutrients in peel and pulp of the normal and dropped fruits in both pommelo cultivars were found. Although, the IAA concentration in the pre-harvest dropped fruit was lower than in the normal fruit for both cultivars (Figure 1), it clearly showed that the dropped fruit had a hormone imbalance. This follows the evidence reported for other fruit crops such as the apple. Ward (2004) found that the fruit drop in apple depends on inhibited plant hormones and NAA. In citrus fruit, it has been reported that the natural fruit drop take two stages. The first stage is the fruitlets drop to reduce competition between fruit on the trees. The second is the pre-harvest fruit drop caused by a decrease in auxin concentration in the fruit reported by Spiegel-Roy and Goldschmidt (1996) and Ruiz *et al.* (2001). There are many reports showing that the decrease of IAA concentration is responsible for fruit drop. Jacksen and Osborne (1972) and Kostenyuk and Burns (2004) reported that IAA plays a major role in regulating the sensitivity of abscission zone to ethylene. It is suggested that endogenous ethylene production increases and abscission occurs only after the IAA content decreases in the abscission zone in citrus. Osborne (1989) found that endogenous plant hormones are involved in fruit abscission. The concentration of endogenous auxin in the abscission zone must decrease below a certain threshold to promote abscission. In addition, concentration of IAA in the pedicles and fruitlet of the falling mango is lower than that in the retention fruit (Bain *et al*., 1997).

Therefore this might be a promising avenue of thought that a low auxin concentration in the pummelo fruits may be the cause of this pre-harvest fruit drop (Figure 1). Interestingly, the result shows that the application of synthetic auxin to fruit does inhibit the fruit drop at this stage. Agusti *et al.* (2006) have reported that 2, 4-DP applied to mature fruit decreases fruit drop in the sweet orange. While Anthony and Coggins (1999) found that 2, 4-D inhibits the pre-harvest fruit drop in citrus fruits in general. Observations of the symptoms of the pummelo pre-harvest fruit drop identified as “yellow fruit calyx symptom” has found harder and smaller leaves compared to those of normal fruit trees. These symptoms have not normally been found in pre-harvest dropped fruit trees caused by a low auxin concentration in the fruit. Therefore, it is possible that beside auxin also other causes are possible affecting the pummelo pre-harvest fruit drop in this research, such as plant disease.

Disease infection has been one of several factors resulting in the drop of fruits. In this study, *Lasiodiplodia theobromae*, *Fusarium moniliform*, and *Colletotrichum gloeosporioides* were all found at the fruit calyx in the normal and pre-harvest dropped fruit of both cultivars (data not shown). Therefore, it is unlikely that these microorganisms are the cause of the pre-harvest fruit drop in this study, but it could be a secondary infection. Greening disease has been one of several diseases found in citrus production and may be the cause of fruit drop in citrus species. The disease is caused by phloem-limited bacteria in the genus *Candidatus Liberibacter* and attacks the vascular system of plants. Three species of bacteria have been described, which include *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus*, and *Candidatus Liberibacter americanus* (Texeira *et al.*, 2005). *Candidatus Liberibacter* spp. has been known to be the causes of greening disease, which is highly destructive to citrus production in Asia, Africa, and South America. Although, primarily affecting sweet orange and mandarin, greening disease has been observed for a long time in pummelo and *Candidatus Liberibacter asiaticus* was exclusively associated with the disease symptoms in pummelo (Deng *et al.,* 2008). This was supported by Garnier and Bove (2000) who found that in Cambodia, Laos and Myanmar, *Candidatus Liberibacter asiaticus* infected various citrus cultivars including pummelo.

Results of the PCR greening analysis on the leaves from the pre-harvest dropped fruit trees in this study indicated that it was infected by phloem-limited bacterium, but not in the normal trees. Hardened and small leaves, small fruit, poorly colored fruit and fruit drop, all symptoms of greening, in both the cultivars tested was similar to the symptoms reported for other citrus species (Davies and Albrigo, 1998). Some greening symptoms were not observed in the pre-harvest dropped fruit trees in this study such as yellow leaves, leaf mottling, yellow shoots, twig die-back, poor flowering and stunting. Greening symptoms do vary according to cultivar, tree maturity, time of infection, stage of disease, and other abiotic or biotic agents that affect the tree (Texeira *et al.*, 2005). There is different susceptibility to greening infection in citrus species with reports that pummelo is more resistance than other citrus species (Chang and Petersen, 2004). These may result to the different symptoms in pummelo compared to other citrus species.

From observations in this study, in the central region of Thailand there is the possibility that greening disease results in the pre-harvest fruit drop called “the yellow fruit calyx”. In an infected pummelo tree *Candidatus Liberibacter* spp. causes greening disease through phloem-limited bacterium, attacking the vascular system of the plant and so limiting the carbohydrate and plant nutrient translocation from leaf to fruit. This in turn can effect abnormal fruit growth and the auxin metabolism in the infected fruit. The disease infection may result in low carbohydrate, IAA, and plant nutrient concentrations found in the dropped fruit and leaves from the pre-harvests dropped fruit trees. From the exploration of the soil chemical properties it was found that there is no significant difference in the soil chemical property between the pre-harvest normal and dropped fruit trees. This ruled out soil property as the cause of pre-harvest fruit drop in this study.

5. Conclusion

The study of the causes and physiological changes of pre-harvest fruit drop in the pummelo cultivars *Thong Dee* and *Khao Nam Phueng* had found that greening disease infection was the most likely cause of the pre-harvest fruit drop called “yellow fruit calyx” in pummelo in the central area of Thailand. The pre-harvest dropped fruits had lower carbohydrate and IAA concentrations than the normal fruit in both cultivars. Nonetheless, there were significant differences in some plant nutrient concentrations in the fruit between normal and pre-harvest dropped fruit and between their leaves.

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