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Post-Bloom CPPU Application Is Effective at Improving Fruit Set and Suppressing Coloration but Ineffective at Increasing Fruit Size in Litchi

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Abstract: This study aimed to provide a reference for the practical use of forchlorfenuron (CPPU) in the improvement of fruit set and quality (especially size) in litchi (*Litchi chinensis* Sonn.). CPPU at 5 mg/L was sprayed to fruit clusters of 'Feizixiao' at 2, 4, 6 and 8 weeks after female bloom (WAFB) in 2017 and at 2, 4, 6, 8, 9 or 10 WAFB in 2018, with spraying water as the control. The treatments were all effective at suppressing fruit abscission, resulting in higher fruit retention at harvest. Except for treatment at 2 WAFB, which caused stunted fruit and significantly reduced fruit weight, all the treatments had no significant influence on fruit weight and flesh (aril) weight, although CPPU tended to increase pericarp weight and strongly suppressed fruit coloration, and such effects were most significant in treatments at 4, 6 and 8 WAFB. Content of total soluble solids (TSS) in commercially ripe fruit (11WAFB) was not significantly influenced by CPPU applied at any of the stages. Ripe fruit continued to gain redness but lost TSS as it became overripe. CPPU suppressed redness gaining, the effect being stronger in later application. The treatments did not influence TSS loss, but treatments at 4, 6 and 8 WAFB significantly reduced the increase in fruit membrane leakage. In treatment at 6 WAFB, CPPU residue in the pericarp decreased sharply with time but relatively constant in the aril, where it maintained lower than 10 μg/kg, the maximal residue limitation in Australia and the United States. The results suggest that 5 mg/L CPPU is effective at improving fruit set and suppressing fruit pigmentation but has no significant effect on fruit size and TSS accumulation or maintenance in litchi.

Keywords: litchi; CPPU; fruit set; fruit maturation; quality

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1. Introduction

Fruit set and development, in most fruits, is preconditioned by pollination and fertilization, which stimulates biosynthesis of endogenous hormones, e.g., auxin, gibberellins (GAs) and cytokinins (CTKs) that play crucial role in fruit set and fruit growth in the early phase [1]. Production of large sized fruit is highly desirable by fruit growers as larger fruit are generally more appealing to consumers and thus of higher market value. Fruit size is determined by fruit cell number multiplied by cell division and cell volume realized by cell expansion [1]. In most fruits, cell number is more crucial for fruit size trait [2–4]. Plant growth regulators that promote cell division and/or cell enlargement are commonly applied to increase fruit set and enlarge fruit size. Among them, forchlorfenuron or N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU) is a regulator with strong CTK activity and has been used extensively in fruit production for improving fruit set and size. In China, CPPU has been registered for application in a number of crops including kiwifruits, table grapes, melons, loquat, mango, oranges and litchi [http://www.chinapesticide.org.cn/hysj/index.jhtml, accessed on 10 November 2022]. In kiwifruit, CPPU at 20 mg/L applied 2 weeks after full bloom (WAFB) increased fruit size and soluble sugars at harvest [5]. Yu et al. (2001) induced larger and parthenocarpic fruit of *Lagenaria leucantha* by applying 50 mg/L CPPU at anthesis compared with a pollinated control [6]. CPPU at 10–20 mg/L applied 2 weeks after full bloom increased fruit size in pears, but its effect diminished when applied 2 weeks

later [7]. In grapes, the size increasing effect of CPPU was shown to be more prominent in seedless varieties than in seeded varieties and the effect tended to be weakened when the application was delayed [8]. Treatment with 5–20 mg/L CPPU at 2 WAFB increased berry weight by 23.6–62.8% in 'Fujiminori' grape [9]. Greene (1991) found fruit thinning and size increasing effects of CPPU (7.5 or 15 mg/L) applied at petal fall and 19 days after petal fall in 'McIntosh' apple without influencing soluble solids and color at harvest, and flesh firmness was increased by CPPU applied 19 days after petal fall but was not influenced by CPPU applied at petal fall [10]. However, Curry and Greene (1993) reported no influence on fruit weight by CPPU (5–50 mg/L) applied at full bloom or 2 weeks later in 'Delicious' apple [11]. In 'McIntosh' apple, application at 4 to 6 mg/L when fruit was 6–16 mm in diameter increased fruit size without causing fruit asymmetry [10]. Stern et al. (2003) also reported 10 mg/L CPPU applied 2 weeks after full bloom significantly increased fruit size of 'Royal Gala' apple with no negative effect on fruit quality [12]. However, Kano (2000) found CPPU applied at anthesis accelerated fruit growth of watermelon but resulted in fruit with thicker rind [13]. Fruit growth of 'Kosui' Japanese pear could be suppressed by CPPU treatment [14]. CPPU application has been reported to delay coloration and fruit maturation in Japanese persimmon [15], grape [16,17] and blueberry [18], but in kiwifruit, it advances fruit ripening [5]. The available reports suggest that fruit response to CPPU treatment differs greatly among fruit species and application times.

Litchi is a subtropical fruit distributed narrowly in the warm subtropical regions with chilling but not freezing winter. China is the largest producer of litchi in the world with a production of 2.016 million tons from 475,700 hectares in 2019 [19]. The fruit has a quite short period of development from bloom in early April to full maturity in late June in central Guangdong, China. Litchi is characterized by weak fruit set capacity [20] and plant growth regulators including CPPU are used to improve fruit set [21]. A study carried out by Stern et al. (2006) showed that CPPU applied before or at color break delayed fruit maturation by 2–3 weeks in litchi cv. 'Mauritius' [22]. Fahima et al. (2019) showed subtle differences in morphological response to CPPU applied at different stages [23]. Application at 4 WAFB resulted in the thickening of pericarp in all parts, while application at 7 WAFB induced cell multiplication and thus thickening only at the epidermis. CPPU at 10–20 mg/L had no significant effect on litchi fruit size, TSS and acidity regardless of application time although it significantly suppressed coloration [23,24]. However, our recent study showed that CPPU at 20 mg/L was effective at suppressing sugar decline and lipid peroxidation in overripe fruit [24]. The current study was carried out to examine the time effect of CPPU application at a lower concentration (5 mg/L) on fruit set and quality parameters in ripe and overripe fruits. Residue of CPPU applied at 6 WAFB in the pericarp and the aril were traced. The study aimed to understand the regulatory role of cytokinin in fruit development and senescence and to provide reference for CPPU application in fruit development regulation in litchi.

2. Materials and Methods

2.1. Materials and Treatments

The experiment was carried out in the fruiting seasons of 2017 and 2018 in Baili Orchard located in Rudong township, Yangxi County, Guangdong, China. A randomized block design was adopted with five single-tree biological replicates and fruit clusters in the same tree as the experimental units subjected to treatments with CPPU. The five 19- or 20-year-old litchi cv. Feizixiao trees on 'Heiye' rootstock for the experiments were similar in load and phenological status. As reported in grape [8,9] and apple [25], CPPU as low as 5 mg/L is effective to increase fruit size, we examined effect of CPPU at 5 mg/L applied at different stages on fruit development in litchi. In the season of 2017, five fruit clusters (panicles) bearing 20–30 fruitlets were selected from each of the four sides of the canopy of each tree at 2 weeks after female bloom (WAFB). They were tagged with taps with different colors and subjected to different treatments including spraying distilled water added with 0.01% tween-20 as the control, and spraying 5 mg/L CPPU added with 0.01% tween-20

at 2 (T1), 4 (T2), 6 (T3) or 8 (T4) WAFB. All the sprays were conducted until run-off. Fruit number in each cluster was recorded prior to treatment and biweekly. Therefore, biweekly relative fruit abscission could be calculated for each treatment. In 2018, the experiment was repeated with CPPU treatments carried out at 2 (T1), 4 (T2), 6 (T3), 8 (T4), 9 (T5) or 10 (T6) WAFB and fruit number in each cluster was recorded prior to treatment and at weekly or biweekly intervals until commercial harvest. Relative fruit drop was calculated as percentage of dropped fruit in a cluster during certain interval against the fruit number previously counted at the beginning of the interval.

The optimal ripeness for harvest occurred around 11 WAFB, when 3 fruit (12 fruit from 4 clusters per treatment per tree) from each cluster were sampled for measuring fruit and fruit part weights, total soluble solutes (TSS) and color parameters in both seasons. In the season of 2018, overripe fruit at 13 WAFB were also collected for the above analyses and also for fruit electrolyte leakage (membrane leakage). Because T1 induced production of stunted fruit in some fruit clusters, we collected only normal fruit from T1 for quality analysis.

2.2. Methods

Fruit color parameters including *a*, *b* and *L* values were collected using a CR-300 colorimeter (Minolta, Japan). Fruit and fruit tissue weights were individually collected using an electronic balance at an accuracy of 0.01 g. Content of TSS in the flesh was measured with a PAL-1 digital refractometer (Atago, Japan). Fruit membrane leakage was determined by measuring rate of electrolyte leakage from an intact fruit submerged in 50 mL of pure water in a 125 mL beaker after it was washed with pure water [26]. The electricity conductivity (EC) of the water in the beaker was measured using an AR-8011 conductivity meter (Smartsensor, China) immediately and 1 h after fruit was put into the beaker. An increase of 1 mS/cm in EC within one hour was arbitrarily used as one unit of fruit membrane leakage.

2.3. CPPU Residue Analysis

Since T3 showed a strong effect on coloration, samples from this treatment in 2018 were used for CPPU residue analysis. They were collected at 0 (2 h after spray), 2 and 4 weeks after CPPU application (WACA). The procedures of sample preparation and analysis were previously reported by [24]. Briefly, 2.0 g of pericarp or aril tissue powder was transferred into a centrifuge tube containing 10 mL acetonitrile and vortexed for 30 min. A total of 1 g of sodium chloride was added to the mixture and vortexed for 5 min before centrifuge at 5000× *g* for 5 min. The supernatant was collected and forced through 0.22 µm microfiltration membrane. The filtrate was ready for HPLC-MS/MS analysis. The instrument used was an Agilent LC-1200 HPLC coupled with a triple quadrupole tandem mass spectrometer (AB Sciex 4000Q Trap) equipped with ESI ion source. The HPLC working conditions included an Agilent poroshel EC C18 column (150 mm × 3.0 mm, 2.7 µm) at 35 °C, a mobile phase of acetonitrile: 0.1% formic acid (4:1) at a flow rate of 0.50 mL/min, and a sample injection volume of 10 µL. The ion source parameters included an ionspray voltage of 5500 V and a source temperature of 550 °C. Multiple reaction monitor was used for detecting CPPU by monitoring ion transitions of *m/z* 248.0/129.0 and *m/z* 248.0/93.0. Analytical pure CPPU was used to prepare a series of gradient solutions in the range of 1–500 µg/L, which were used as the internal standard for construction of the standard curve separately for the pericarp and the aril. Both showed a perfect linear function ($r > 0.999$) between CPPU concentration and peak area.

2.4. Statistics

The above measurements and analyses were conducted with five biological replicates ($n = 5$). Data were processed using Excel, and analysis of variance, Duncan's multiple range test and t-tests were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results and Analysis

3.1. Effect on Relative Fruit Drop in Different Periods

In both seasons, relative fruit drop of 'Feizixiao' was high in the early stages of fruit development before 6 WAFB and highest during period of 2–4 WAFB. CPPU treatments at different stages significantly reduced the relative fruit drop rate (Figure 1). In the season of 2017, CPPU treatments at 2 and 4 WAFB (T1 and T2), which significantly suppressed fruit drop during the post-treatment 4 weeks, led to a higher drop in later stage in week 8 to 10 (Figure 1A). However, in the season of 2018, relative fruit drop remained constantly lower in CPPU treatments than in the control (Figure 1B). Final fruit retention was lowest in the control, which was significantly lower than all the CPPU treatments in both seasons (Figure 1C,D). CPPU applied at 2 WAFB (T1) had the highest fruit retention. However, it induced the production of stunted fruit together with normal fruit in the same cluster (Figure 2). The stunted fruit were empty with no seed and aril and thus no commercial value. The results suggest that 5 mg/L CPPU had a strong fruit retention effect but had inhibitory effect on fruit growth when applied at early stages e.g., within 2 WAFB.

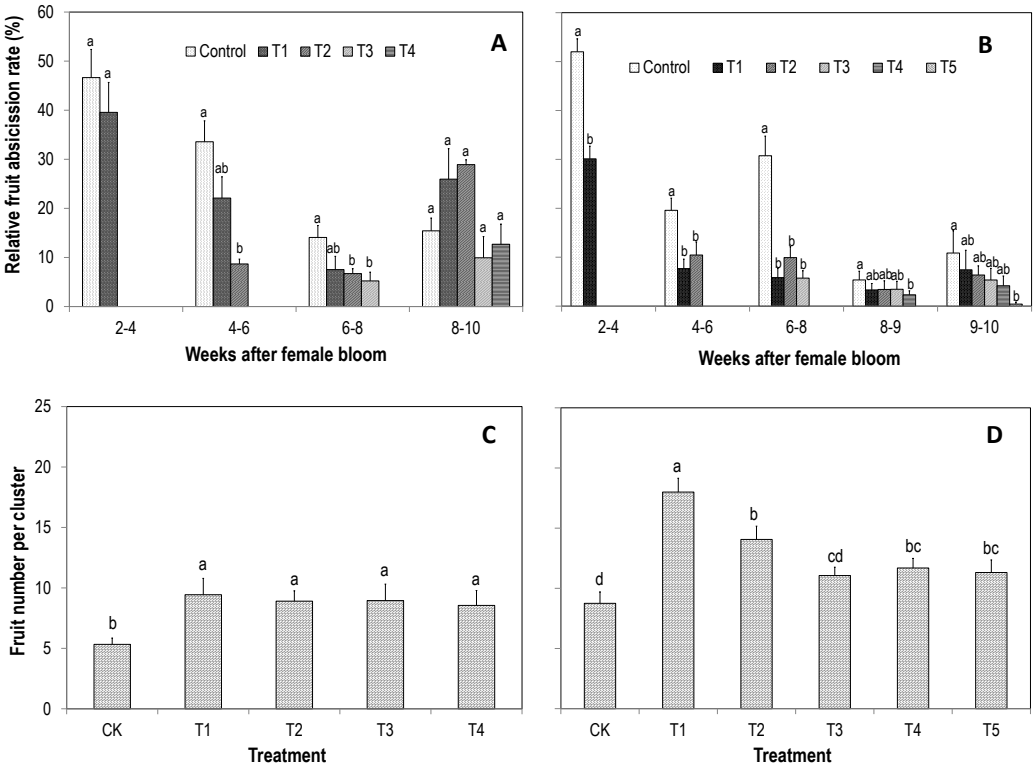

Figure 1. Effect of CPPU treatment at different stages on periodical fruit abscission rate (**A**,**B**) and final fruit retention at harvest (**C**,**D**). A and C, results of 2017; (**B**,**D**), results of 2018. CK represents the control, while T1, T2, T3, T4, T5 and T6 represent spraying 5 mg/L CPPU at 2, 4, 6, 8, 9 and 10 weeks after female bloom, respectively. Different letters above the bars indicate significant difference among treatments at the same duration in the same season at $p < 0.05$, Duncan's multiple range test.

Figure 2. Effect of CPPU treated at different stages on fruit appearance in 'Feizixiao'. CK represents the control, while T1, T2, T3, T4, T5 and T6 represent spraying 5 mg/L CPPU at 2, 4, 6, 8, 9 and 10 weeks after female bloom, respectively. Photos were obtained at 11 WAFB (result of 2018).

3.2. Effect on Appearance and Quality at Harvest

Fruit weight, fruit tissue weight and color parameters at commercial maturity (11 WAFB) were compared among treatments (Tables 1 and 2). Fruit weight was lowest for the treatment of T1 in both seasons. In the season of 2018, fruit weight was significantly reduced by T1 compared with the control, while there was no significant difference among the other treatments and the control. Flesh weight displayed a similar pattern with the fruit weight, and flesh recovery had no significant difference among all treatments. Pericarp weight tended to be increased by CPPU treatments at different stages in both season. TSS content in the aril was not affected by all CPPU treatments (Tables 1 and 2). However, color parameters were strongly influenced by the treatments (Table 1, Figure 3). At commercial maturity, the value *a* reflecting redness was significantly reduced by all the CPPU treatments at different degrees (Table 1 and Figure 3), but the earliest (T1) and the latest (T6) treatments seemed less effective (Figure 3). The effect of T3, T4 and T5 were similar and significantly stronger than T1 and T6, suggesting that CPPU treatment during 6 to 9 WAFB was effective to suppress reddening of fruit. The color value *b*, reflecting yellowness, was significantly increased by all CPPU treatments except for T1. *L* value reflecting brightness was significantly increased by T2, T3 and T4, while T1 had an *L* value similar to the control (Table 1). The colorimeter data agreed well with the appearance of fruit shown in Figures 2 and 3.

Table 1. Effect of CPPU applied at different stages on fruit appearance and quality at commercial harvest (result of 2017).

Treatment	Fruit Weight (g)	Pericarp Weight (g)	Flesh Weight (g)	Flesh Recovery (%)	TSS (%)	Color Parameters		
						a	*b*	*L*
Control	26.4 ± 1.45 ab	4.82 ± 0.30 ab	20.7 ± 1.07 ab	78.7 ± 0.50 a	17.1 ± 0.31 a	4.4 ± 0.91 a	18.3 ± 0.53 b	40.8 ± 0.90 b
T1	24.5 ± 1.08 b	4.71 ± 0.17 b	19.0 ± 0.81 b	77.5 ± 0.26 a	16.8 ± 0.22 a	2.5 ± 1.35 a	18.8 ± 0.58 b	41.2 ± 0.87 b
T2	27.2 ± 1.71 ab	5.52 ± 0.38 a	20.9 ± 1.33 ab	76.9 ± 0.51 a	17.0 ± 0.25 a	3.9 ± 0.89 b	21.4 ± 0.40 a	44.3 ± 0.71 a
T3	25.6 ± 1.22 ab	5.18 ± 0.20 a	19.7 ± 1.11 ab	76.8 ± 1.02 a	16.9 ± 0.20 a	−8.0 ± 0.38 c	22.0 ± 0.29 a	44.2 ± 0.63 a
T4	28.1 ± 1.20 a	5.53 ± 0.29 a	21.6 ± 0.89 a	76.8 ± 0.31 a	17.4 ± 0.33 a	−5.5 ± 0.54 b	21.7 ± 0.17 a	44.2 ± 0.41 a

CK represents the control, while T1, T2, T3 and T4 represent spraying 5 mg/L CPPU at 2, 4, 6 and 8 weeks after female bloom, respectively. Different letters indicate significant difference among treatments within the same season at $p < 0.05$, Duncan's multiple range test.

Table 2. Effect of CPPU applied at different stages on fruit quality parameters at commercial ripe (11 WAFB) and overripe (13 WAFB) stages (result of 2018).

Treatment	Fruit Weight (g)		Pericarp Weight (g)		Flesh Weight (g)		Flesh Recovery (%)		TSS (%)	
	11WAFB	13 WAFB	11WAFB	13WAFB	11WAFB	13WAFB	11WAFB	13WAFB	11WAFB	13WAFB
Control	25.1 ± 0.56 a	29.3 ± 0.25 a	5.00 ± 0.20 b	5.97 ± 0.04 a	19.0 ± 0.41 a	22.4 ± 0.27 a	75.7 ± 0.84 a	76.4 ± 0.34 a	18.3 ± 0.30 a	17.2 ± 0.35 a
T1	22.7 ± 0.60 b	27.8 ± 0.33 b	5.26 ± 0.29 ab	6.40 ± 0.46 a	16.4 ± 0.54 b	20.3 ± 0.18 b	72.2 ± 1.12 a	73.2 ± 0.37 b	18.5 ± 0.22 a	16.7 ± 0.37 a
T2	24.2 ± 0.81 ab	28.6 ± 0.80 ab	5.52 ± 0.13 a	6.07 ± 0.27 a	17.9 ± 0.57 ab	21.7 ± 0.62 ab	73.8 ± 0.29 a	76.1 ± 0.64 a	18.1 ± 0.29 a	16.7 ± 0.18 a
T3	25.6 ± 0.50 a	30.6 ± 1.10 a	5.08 ± 0.18 b	6.38 ± 0.23 a	19.3 ± 0.28 a	23.3 ± 0.98 a	75.4 ± 0.68 a	76.1 ± 0.73 a	18.1 ± 0.45 a	16.5 ± 0.29 a
T4	24.8 ± 0.36 ab	29.2 ± 0.75 ab	5.27 ± 0.17 ab	6.16 ± 0.21 a	18.8 ± 0.25 a	22.0 ± 0.60 a	75.9 ± 0.12 a	75.5 ± 0.25 a	18.3 ± 0.39 a	16.8 ± 0.23 a
T5	25.3 ± 0.91 a	30.0 ± 0.72 a	5.27 ± 0.22 ab	5.93 ± 0.21 a	18.8 ± 0.91 a	21.3 ± 0.52 ab	76.2 ± 0.40 a	76.1 ± 0.79 a	18.0 ± 0.20 a	16.6 ± 0.34 a
T6	26.2 ± 0.24 a	29.4 ± 0.60 a	5.03 ± 0.14 b	6.11 ± 0.16 a	19.5 ± 0.20 a	22.4 ± 0.41 a	74.5 ± 0.41 a	76.1 ± 0.26 a	18.0 ± 0.24 a	16.5 ± 0.26 a

CK represents the control, while T1, T2, T3, T4, T5 and T6 represent spraying 5 mg/L CPPU at 2, 4, 6, 8, 9 and 10 weeks after female bloom, respectively. Different letters indicate significant difference among treatments within the same season at $p < 0.05$, Duncan's multiple range test.

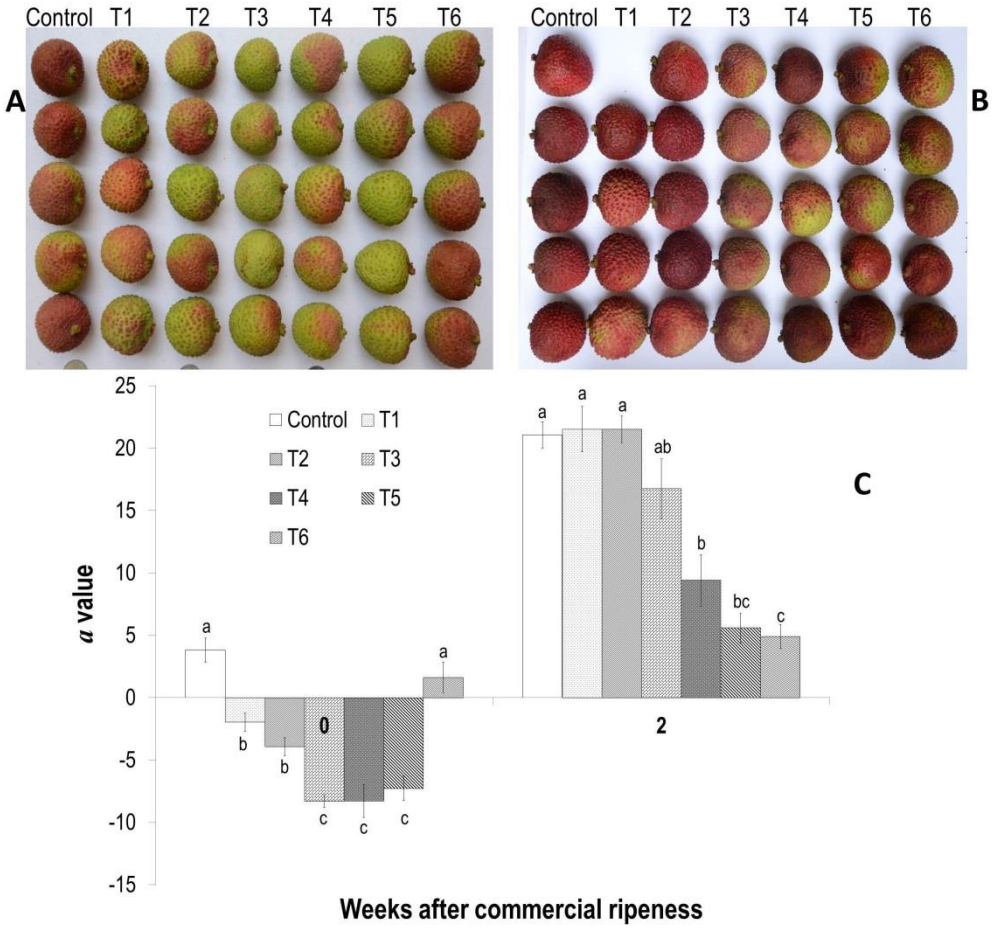

Figure 3. Effect of CPPU treatments at different stages on color and a value as fruit became overripe on tree. (**A**). Fruit appearance at optimal commercial ripeness; (**B**). Fruit appearance at 2 weeks after optimal commercial ripeness; (**C**). Changes in *a* value. CK represents the control, while T1, T2, T3, T4, T5 and T6 represent spraying 5 mg/L CPPU at 2, 4, 6, 8, 9 and 10 weeks after female bloom, respectively. Different letters indicate significant difference among treatments at the same sampling time at $p < 0.05$, Duncan's multiple range test.

3.3. Effect on Quality and Membrane Leakage in Overripe Fruit

As shown in Table 2, ripe fruit in all treatments continued to gain fresh weight as they became overripe on tree. T1 remained the lowest and significantly lower than the control, while there was no significant difference among the other treatments. Fruit weight increment was highest in T4 and lowest in T5 and T6, but statistically, there was no significant difference among all treatments (Table 3).

Table 3. Effect on CPPU treatments on increments in fruit weight, TSS, redness value and membrane leakage during 2 weeks from commercial ripeness.

	Control	T1	T2	T3	T4	T5	T6
Fruit weight (g)	4.23 ± 0.60 a	5.09 ± 0.97 a	4.18 ± 0.84 a	4.90 ± 0.96 a	5.53 ± 1.07 a	3.63 ± 0.37 a	3.56 ± 0.66 a
TSS (%)	−1.16 ± 0.46 a	−1.80 ± 0.31 a	−1.35 ± 0.19 a	−1.56 ± 0.25 a	−1.24 ± 0.20 a	−1.54 ± 0.26 a	−1.42 ± 0.23 a
a value	16.6 ± 1.75 b	23.5 ± 2.19 a	25.2 ± 1.85 a	25.9 ± 2.72 a	18.0 ± 2.58 ab	12.2 ± 1.70 b	3.29 ± 0.80 c
Membrane leakage (unit)	4.29 ± 0.18 b	3.83 ± 0.20 bc	1.51 ± 0.20 d	3.15 ± 0.24 c	3.13 ± 0.24 c	3.53 ± 0.19 bc	5.6 ± 0.08 a

CK represents the control, while T1, T2, T3, T4, T5 and T6 represent spraying 5 mg/L CPPU at 2, 4, 6, 8, 9 and 10 weeks after female bloom, respectively. Different letters indicate significant difference among treatments at $p < 0.05$, Duncan's multiple range test.

TSS content in all treatments decreased as fruit became overripe. There was no significant difference in TSS content among all treatments in both the ripe and overripe fruits (Table 2). The decrement in TSS had no significant difference among all the treatments during the 2 weeks of 'hanging life' on tree (Table 3).

The redness as well as *a* value of the fruit continued to increase in all treatments as ripe fruit became overripe on tree (Figure 3, Table 3). T1, T2 and T3 had similar *a* value with the control. Later treatment of CPPU showed a stronger suppression effect on redness gaining and *a* value increase (Table 3). Therefore, the increment in *a* value was smallest in T6 followed by T5.

The fruit membrane leakage increased as fruit became overripe in all treatments and had no significant difference among treatments in ripe and overripe fruits (Figure 4). However, the increment in fruit membrane leakage differed significantly among treatments (Table 3). The highest increment was found in T6, followed by the control, T1 and T5, and the lowest in T2, followed by T3 and T4.

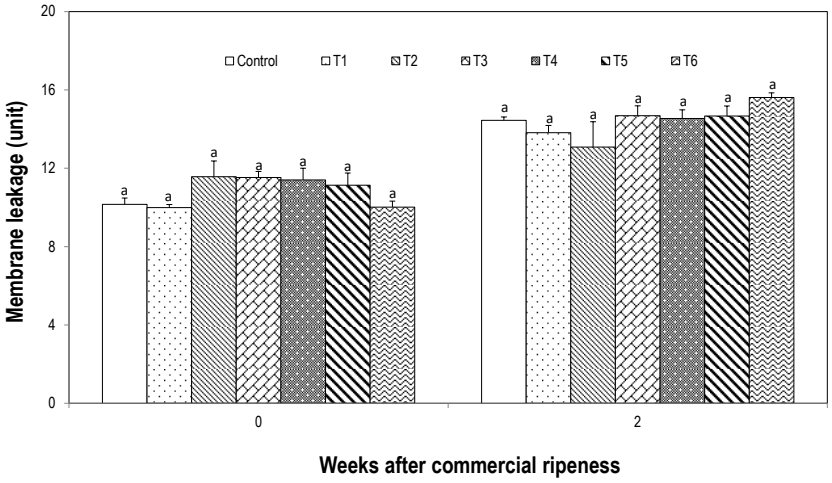

Figure 4. Effect of CPPU treatments at different stages on fruit membrane leakage as fruit became overripe on tree. CK represents the control, while T1, T2, T3, T4, T5 and T6 represent spraying 5 mg/L CPPU at 2, 4, 6, 8, 9 and 10 weeks after female bloom. Different letters indicate significant difference among treatments at $p < 0.05$, Duncan's multiple range test.

3.4. Dynamics of CPPU Residue in the Pericarp and Aril

Since T3 showed a significant effect in suppressing fruit coloration and membrane leakage, we traced the CPPU residue in the pericarp and the aril in samples of this treatment biweekly (Figure 5). Concentration of CPPU residue in the pericarp constantly decreased with time and the decrease was more drastic in the first fortnight than in the second fortnight. CPPU concentration in the aril was 4 to 33 times lower than that in the pericarp. It decreased by nearly half in the first 2 weeks but was maintained as relatively stable in the second fortnight. The change pattern of CPPU residue in the pericarp and aril was similar to that generated by applying CPPU at a higher concentration of 20 mg/L [24].

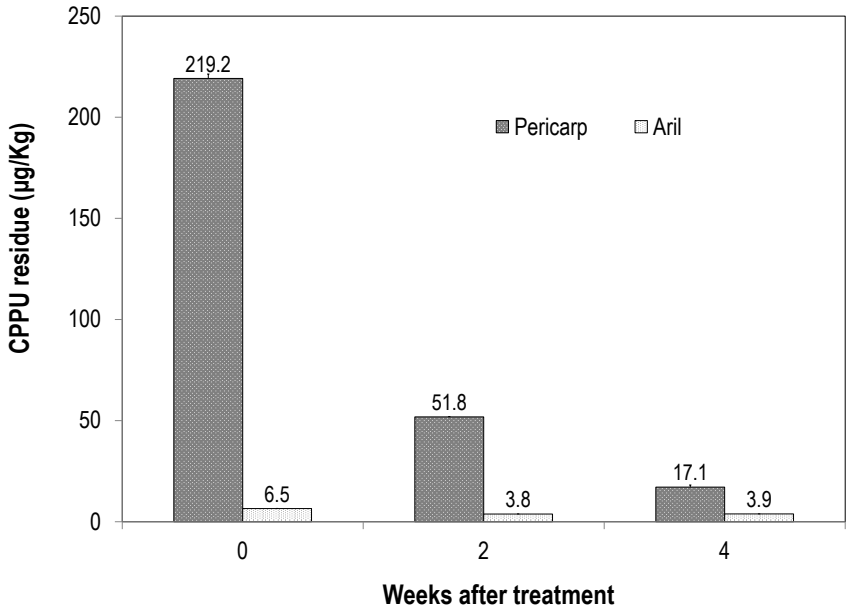

Figure 5. Dynamics of CPPU residue in the pericarp and the aril after 5 mg/L CPPU was applied to fruit 6 weeks after female bloom. Samples at 0 weeks after treatment were collected 2 h after treatment.

4. Discussion

4.1. CPPU Increases Fruit Set but Not Fruit Size in Litchi

As a plant growth regulator with strong cytokinin activity, CPPU has been widely used to promote fruit set and fruit growth. To realize this effect, CPPU is mostly applied at the early stage of fruit development during cell division, which is crucial for fruit size [2–4]. CPPU applied at full bloom could replace pollination to induce fruit set in some fruits such as water melon [27], melon (*Cucumis melo*) [28], sweet cherry [29], pear [30], cucumber [31] and bottle gourd (*Lagenaria leucantha*) [6]. CPPU applied at full bloom and at the beginning of fruit set suppressed fruit drop in avocado [32]. However, the application of CPPU may have a fruit thinning effect in highbush blueberries [33] and 'McIntosh' apple [10]. In litchi, CPPU at 40 mg/L reduced preharvest drop in a seedless cultivar [21]. The present study showed that CPPU at 5 mg/L applied at different stages significantly reduced fruit drop in 'Feizixiao' litchi with an aborted seed, the effect being stronger when applied earlier. Therefore, CPPU may serve as a strong fruit retention chemical for litchi.

Although the effect of CPPU in increasing fruit size has been widely reported in different types of fruits, including some drupes [29], berries [5,8,15] and pomes [7,12,25], its effect on fruit size differs greatly among species and time of application. In some sweet cherry varieties (e.g., 'Bing' and 'Tieton'), 15 mg/L CPPU applied at full bloom showed no effect on fruit size, while in another variety ('PC 8011–3'), it significantly increased fruit size [29]. In grapes, the size increasing effect of CPPU was shown to be more prominent in seedless varieties than in seeded varieties and the effect tended to be weakened when application was delayed [8]. In a seeded litchi cv. 'Mauritius' and seed-aborted cv. 'Feizixiao', CPPU up to 20 mg/L applied at 4 or 7 weeks after female bloom had no significant effect on fruit size [23,24]. The present study showed that application of 5 mg/L CPPU at different stages of fruit development did not show a fruit size/weight increasing effect in the seed-aborted 'Feizixiao' litchi. On the contrary, treatment at 2 WAFB (T1) caused significant reduction in fruit size. Fruit growth of 'Kosui' Japanese pear was also reported to be suppressed by CPPU treatment [13]. This could be partially explained by the significant increase in fruit retention. However, the treatment induced production of empty stunted litchi fruit with no commercial value. The mechanisms causing stunted fruit need to be clarified. Possibly, CPPU interferes with the early development of seed in young litchi fruit by inducing abnormal cell division in the pericarp resulting thickening of the

pericarp [23] and empty small fruit. The result suggests that the application of CPPU at the early stage of fruit development should be very cautious. The ineffectiveness of CPPU in increasing fruit size in litchi is very likely related to its unique fruit structure. Litchi is an arillate fruit, whose pulp tissue or aril initiates from the funicle around 5 WAFB and development of the pericarp (peel) precedes that of the pulp [34]. The pericarp and the aril are basically separated, with tissue connection only at the base. It is assumed that CPPU applied on litchi fruit surface has a very low diffusion to the aril, which might result in a very low concentration in the aril Figure 5 of [24]. This explains why CPPU treatment was effective in increasing pericarp thickness and weight, but not in increasing aril size and thus the final fruit size Table 1 of [23,24].

4.2. CPPU Treatments Strongly Suppressed Fruit Coloration but Had No Effect on TSS Accumulation and Maintenance

Cytokinin is generally considered as a fruit ripening inhibitor. CPPU applied at full bloom or early stages of fruit development has been shown to have a long-term effect on fruit ripening. It delays coloration and fruit ripening in Japanese persimmon [15], grape [16,17] and blueberry [18] (NeSmith, 2002), but in kiwifruit, CPPU advances fruit ripening events including fruit softening, sugar accumulation and coloration in the flesh, although it has resulted in higher chlorophyll contents in the fruit [5,35]. Studies on litchi showed that CPPU strongly suppressed fruit coloration that involves both chlorophyll degradation and anthocyanin biosynthesis [20,23,24,36–38]. Our study showed that 5 mg/L CPPU applied during 4 to 9 WAFB was effective at suppressing the coloration of litchi, and only application at 2 WAFB showed an insignificant effect. As fruit became overripe, litchi continued to gain redness with an increase in *a* value, which was also suppressed by CPPU treatments. However, the suppressing effect diminished as the CPPU application took place earlier (Table 2). The diminishing effect might be related to the degradation of CPPU residue in the pericarp as it decreased dramatically with time Figiure 5 of [24]. In addition, application of 5 mg/L CPPU at various stages did not significantly influence TSS accumulation, which agrees with our previous reports [23,24]. It seems that CPPU suppresses the ripening events in the peel but not in the flesh (aril) in litchi, which is different from some berries, whose ripening events in the flesh tissue are influenced by CPPU [5,17]. Again, this unique response of litchi to CPPU might be related to the unique structure of arillate fruit, whose flesh tissue is basically separated from the peel with poor diffusion of CPPU from peep to the flesh. Wang et al. (2007) reported that the spray 6-BA to fruit surface inhibited coloration but failed to influence sugar accumulation while the transfusion of 6-BA to fruit through peduncle significantly inhibited on sugar accumulation in the aril but had no effect on coloration of litchi fruit [38], suggesting that the effect of exogenous cytokinins depends on where they are applied.

Ripe litchi is highly perishable even on trees, giving it a very short 'hanging life'. As fruit becomes overripe on trees, litchi fruit loses quality with the loss of sugars [24,39] and accumulation of anaerobic products, e.g., alcohol and acetaldehyde [24,40]. Results of the present study (Table 2) suggested that 5 mg/L CPPU applied at different stages had no significant effect on loss of soluble solids as litchi fruit became overripe. The integrity of membrane loss reflected by electrolyte leakage (membrane leakage) increase occurred as fruit became overripe. CPPU treatment during 4 to 8 WAFB significantly reduced the increment in membrane leakage, while treatment earlier or later had no such effect. Therefore, CPPU application at appropriate stages helps to maintain membrane integrity, which may contribute to its effect in improving storability in addition to the formation of thickener of a pericarp and cuticle layer that prevent water loss during storage [23].

Results from this study (Figure 5) and a previous study [24] suggest that CPPU residue degrades rapidly in the pericarp but slowly in the aril. In treatments with 5 mg/L (Figure 5) and 20 mg/L [24], the CPPU residue concentrations in the aril maintained lower than the maximal residue limitation (10 μg/kg) in Australia and the United States [21]. Therefore, in terms residue limitation, preharvest application of CPPU within 20 mg/L for

the improvement of fruit set or postharvest performance is acceptable. As CPPU residue concentration decreases in the fruit with time, earlier spay will give lower residue in fruit at harvest.

5. Conclusions

Based on the available reports as well as our study, the effect of CPPU on fruit development differs greatly among fruit types and application times. In the case of litchi, 5 mg/L CPPU is effective in improving fruit set and suppress fruit pigmentation but ineffective to increase fruit size and TSS accumulation or maintenance in litchi. Its effect seems limited in the pericarp, possibly due to poor diffusion of CPPU from the pericarp to the aril. At the early stages (within 2 WAFB), application of CPPU may cause stunted fruit in litchi. Although CPPU produces both desirable and undesirable effects on litchi fruit, it can serve as a useful fruit set enhancer for crops.

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