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A Multi-Year Investigation of Forchlorfenuron Effect on Fruit Quality in *A. chinensis* var. *chinensis*

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Abstract: CPPU (Forchlorfenuron (1-(2-chloropyridin-4-yl)-3-phenylurea), a synthetic cytokinin, is extensively used to enhance fruit size and quality size in several crops, including kiwifruit. This study investigates the effects of three different CPPU application strategies on two different crop loads on the main fruit quality parameters at harvest and on storage performances of fruit. Our results show that a two application of CPPU significantly increased fruit weight, especially under standard crop load conditions, due to a more efficient resource allocation. Fruit firmness improved with 2 and 3-times CPPU applications, likely due to enhanced cell wall development, and was consistently affected by crop load, with higher loads resulting in softer fruits. CPPU applied twice did not significantly influence soluble solids content, but significantly influenced by the crop load, with standard load fruit showing higher sugar accumulation. Fruit dry weight showed similar trends, with CPPU exhibiting greater impact effect under standard crop loads. Concerning postharvest performances, fruit from CPPU resulted in a minor effect on maintaining flesh firmness during the first 5 months of storage. CPPU proved to be a possible strategy to enhance fruit quality, however the overall efficacy is highly dependent on field management practices, which may interfere on the physiological response of the plant. Thus, it is crucial to control filed variables to ensure that the benefits of CPPU are fully realized.

Keywords: Brix; CPPU; fruit weight; firmness; kiwifruit; post-harvest

1. Introduction

In the current scenario of global population growth along with farming surfaces shrinking and soil fertility depletion, overall production itself is not the major challenge to food security anymore, while the quest for sustainable and efficient farming practices has never been more imperative. A broad shift from labour intensive cultural methods toward highly productive and renewable practices is instead demanded by many food production players (e.g., policy makers, farmers, and companies). Among the multiple solutions for crop management optimization, plant bioregulators, also known as plant growth regulators (PGRs) have emerged as a valuable innovation toward a more effective, resilient, and environmentally conscious food production [1,2].

The term refers to natural or artificial compounds, influencing plants' metabolic pathways ' by mimicking the corresponding natural hormones or influencing their biosynthesis [3]. PGRs are widely used in perennial fruits cultivation since they may promote bud break and flower development [4], fruit set [4], disease resistance [5,6], yield and fruit quality [6]. Furthermore, they can be also used in post-harvest to enhance post-harvest storability [7]. The PGRs' performance variability, regarding the plant hormonal balance, is not yet fully understood, nevertheless, factors like timing and rate of application, hormonal homeostasis, pollination, temperature, juvenility, nutrients availability and crop load are known as influencing factors. Nonetheless, if properly applied, PGRs could substantially promote fruit quality, extend harvest storability, reduce diseases' incidence, and regulate the ripening process. [1].

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Forchlorfenuron (1-(2-chloropyridin-4-yl)-3-phenylurea), also known as CPPU, is a synthetic cytokinin-based PGR that has been widely used to increase fruit size and quality traits in apple [8,9] cherry [10], grape [11] and it is largely used during preharvest treatments in kiwifruit [12,13]. CPPU action mechanism is complex and not yet fully elucidated [14]. Indeed, in kiwifruit, CPPU application resulted in an increase in the gibberellin and cytokinin biosynthesis and signalling, and in the repression of auxins and ABA (abscisic acid) pathway, thus, CPPU application enhance cell expansion of epidermal and parenchyma cells, promoting cell division of subepidermal cells [15]. Besides the multiple changes in fruit morphogenesis, carbohydrate metabolism and fruit ripening [16], several fruit parameters with a crucial impact on overall fruit quality are likely to be affected by CPPU. However, since CPPU mechanisms on fruit development primarily affects its hormonal balance and signaling, the results are often contradictory. For example, some studies showed that CPPU application after full bloom increased fruit size and increased sugar accumulation, reduced acidity, and lower firmness [17], whereas, others showed that CPPU application in kiwifruits significantly reduced soluble solid content (SSC) and titratable acids [18].

The variability of CPPU effects in kiwifruit can be explained by the influence of several factors such as the magnitude of pollination [19], pruning [20], or blooming stage when compound is applied [21]. Moreover, the effects of CPPU, especially at high doses, can also negatively affect fruit traits, histology and metabolites partitioning thus increasing softening, incidence of post-harvest disorders and reduce storability. Finally, CPPU application to increase kiwifruit size may led to residues accumulation, making fruit unmarketable [22]. In fact, despite CPPU toxicity to mammal is clinically proved to be low, this PGR is degraded in kiwifruit to 3-hydroxyphenyl forchlorfenuron (3-OH-CPPU) and 4-hydroxyphenyl forchlorfenuron (4-OH-CPPU), which have been shown to be cytotoxic [23].

This study aims at investigating the potential benefit from three different strategies of CPPU applications on commercial kiwifruit orchards. For this reason, field trials have been performed in four consecutive years, on two different commercial orchards, subjected to two different crop loads (standard and high). The present research focuses on the fruit quality parameters impacting on final-price formation: fresh weight, flesh firmness, soluble solid content, and dry matter. Finally, the effect of CPPU application was evaluated on fruit storability assessing fruits' ripening changing along the storage period.

2. Results

The effects of the CPPU on fruit quality (A1-3) are presented following to the application strategy (A1-3) and results are displayed according to the year, field and crop load (HCL: high crop load, SCL: standard crop load) of the trial. Measures were: Fresh fruit wight (g)- FW, flesh firmness (kgf)- FF, SSC (Brix) and dry weight (%) – DM of fruits from treated (TT) (A1) and, not-treated (NT) plants of Field 1 and Field 2, crop load: standard and high, Years: 2020, 2021.

2.1. Application Strategy A1

Strategy A1 consisted in a single application of CPPU (0.25 ml/L, volume of application: 500L/Hectare) performed at budbreak. In 2020, the highest fruit weight was observed in field 2 on both crop loads, regardless the treatment (CPPU application – no statistical impact on the weight) (Figure 1). Again, in 2021, field, and crop load had an influence on fruit weight more significant than CPPU treatment. Furthermore, there was a statistical effect in the interaction field × crop load, field × treatment, and year × crop load × treatment (Figure 1 and Table 1).

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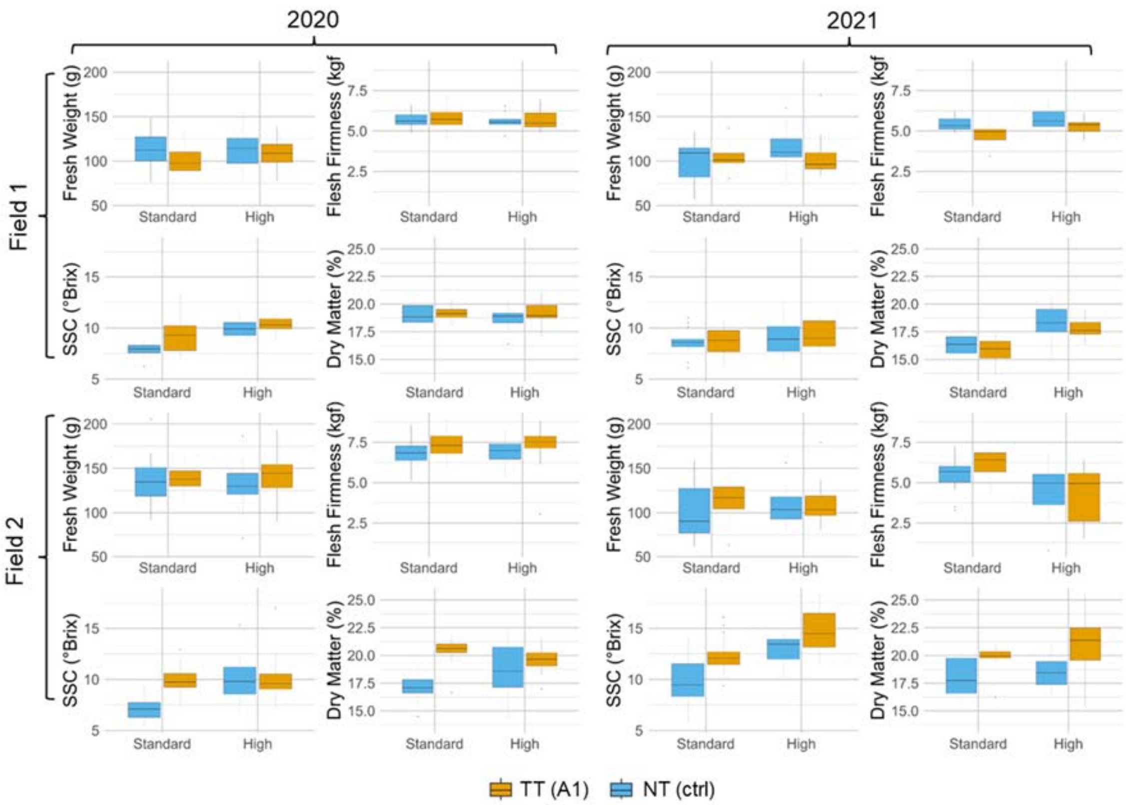

Figure 1. Fresh fruit wight (g), flesh firmness(kgf), SSC (° Brix) and dry weight (%) of fruits from treated (TT) (A1) and, not-treated (NT) plants of Field 1 and Field 2, crop load: standard and high, Years: 2020, 2021.

Concerning fruit quality characteristics, in both study-years, the firmness showed the highest value in field 2, regardless CPPU treatment, in the standard crop load. Year, field, and crop load statistically influenced this parameter (the treatment did not). Yet, a statistical interaction was found between: year × field, year × crop load, year × treatment, field × treatment, and year × field × crop load. The highest values were found in 2021 (Figure 2 and Table 1). Moving to the Brix, in 2020 the highest Brix level was found in the TT, only for the high crop load, in both study-fields. Regarding the 2021, the highest values were recorder in field 2, for the TT under the two crop loads. Again, year, field, and crop load statistically influenced this parameter, together with the treatment. As interactions, year × treatment, field × crop load, crop load × treatment, year × field × crop load, year × field × treatment, and year crop load per treatment influenced the Brix level (Figure 2 and Table 1). Lastly, concerning the fruit dry weight, in the 2020 study year, the highest value was found out for the TT in both testing fields, in the situation of high crop load, whilst in 2021 the highest recordings of Brix was up to TT, in field 2, for both crop loads. Here, every factor and linked interactions had an effect on this value, apart from year × treatment, crop load × treatment, and year × field × treatment (Figure 2 and Table 1).

Table 1. Multifactorial Analysis of Variance for CPPU 1 application.

	Weight	Firmness	Brix	Dry Weight
Year	***	***	***	***

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Field	***	***	***	***
Crop load	**	***	***	***
Treatment			***	***
Year × Field	***	***	***	***
Year × Crop load		***		***
Field × Crop load	*	***	**	*
Year × Treatment		*		
Field × Treatment	***	•	***	***
Crop load × Treatment		***	***	
Year × Field × Crop load			***	•
Year × Field × Treatment			*	***
Year × Crop load × Treatment	*		*	*
Field × Crop load × Treatment		•	•	*
Year × Field × Crop load × Treatment				

Significant codes: *** = p<0.001, ** = p<0.01, * = p<0.05, **.** = p<0.1

2.2. Application Strategy A2

The second strategy A2 consisted in two application of CPPU. The first (150ml/ha) performed at budbreak was equal to A1. The following application (0.33 ml/L, volume of application: 500L/hectare) was performed at 7-10 days before flowering.

Considering this strategy, and firstly investigating the fruit weight, in 2020 the TT of the standard crop load showed the highest value. For the first study year the trend was of having higher values in TT compared to NT, especially for the standard crop load. Overall, field, crop load and treatment had a statistical effect on this parameter, as well as the interaction year × field, field × treatment, year × field × treatment (Figure 3 and Table 2).

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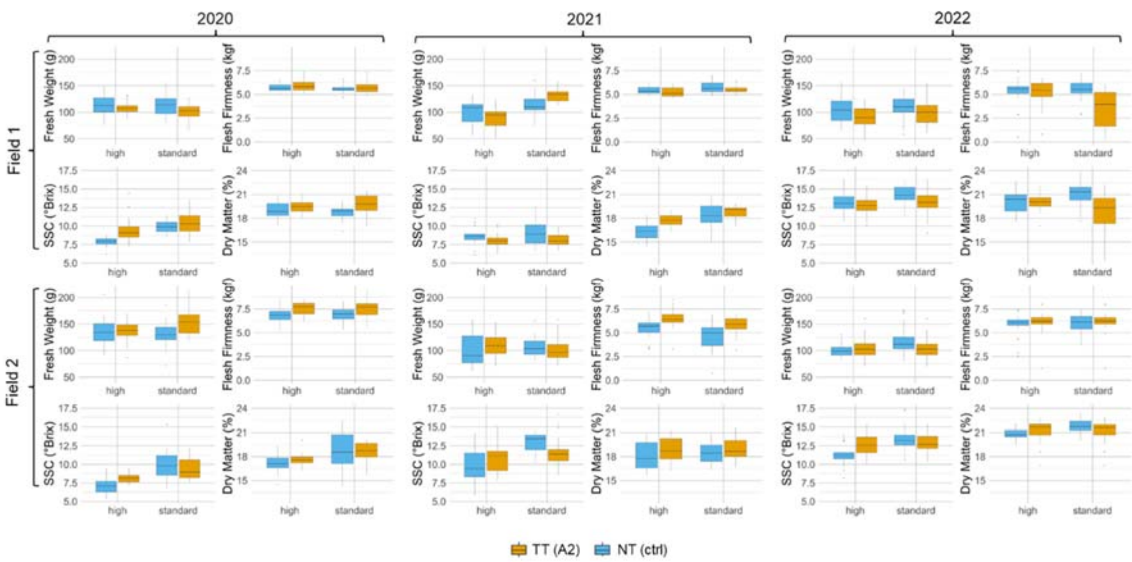

Figure 3. Fresh fruit wight (g), flesh firmness(kgf), SSC (° Brix) and dry weight (%) of fruits from treated (TT) (A2) and, not-treated (NT) plants of Field 1 and Field 2, crop load: standard and high, Years: 2020, 2021, 2022.

Regarding the fruit firmness, the year, field, crop load and CPPU application (Treatment) showed a statistical significance in influencing the considered parameter. Overall, the values of TT tended towards being higher compared to NT, in both study years and fields. Moreover, the interactions field × treatment and year × treatment was statistically significant in determining firmness maintenance, the same occurred for the year × field (Figure 4 and Table 2). Indeed, considering the fruit Brix, the highest values were found for the standard crop load in each study year, without considering the Treatment factor (as it was not significant), especially in field2. Thus, the year, field, and crop load showed a statistical effect on this parameter. Likewise, Year × Crop load, Field × Crop load, Year × Treatment, and Crop load × Treatment statistically influenced the sugar level (Figure 4 and Table 2). Taking into account the fruit dry weight, year, field and crop load, once again, affected this value, together with the interactions as follow: year × field, year × crop load, year × treatment and crop load × treatment. Finally, since also in this case there was not an effect made by the application of CPPU, the highest values were recorded for the high crop load, in 2022 study year, in the field2 (Figure 4 and Table 2).

Table 2. Multifactorial Analysis of Variance for CPPU 2 applications.

	Weight	Firmness	Brix	Dry Weight
Year		***	***	***
Field	*	***	***	***
Crop load	**	***	***	**
Treatment	*	*		
Year × Field	***			***
Year × Crop load		***	*	*
Field × Crop load			**	

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Year × Treatment			**	**
Field × Treatment	**	***		
Crop load × Treatment		***	***	*
Year × Field × Crop load				
Year × Field × Treatment	**			
Year × Crop load × Treatment			*	
Field × Crop load × Treatment			*	
Year × Field × Crop load × Treatment	**		•	

Significant codes: *** = p<0.001, ** = p<0.01, * = p<0.05, **.** = p<0.1

2.3. Application Strategy A3

As last strategy, three-time CPPU (A3) application has been evaluated in one study year (0.50 ml/L, volume: 500L/Hectare).

Taking into account the fruit weight, the overall highest value was found in field2, under the high crop load. At any rate, each variable did not show a statistical impact on the weight, whereas only the interactions crop load × field, and field × crop load × treatment had a statistical impact on this parameter (Figure 5 and Table 3).

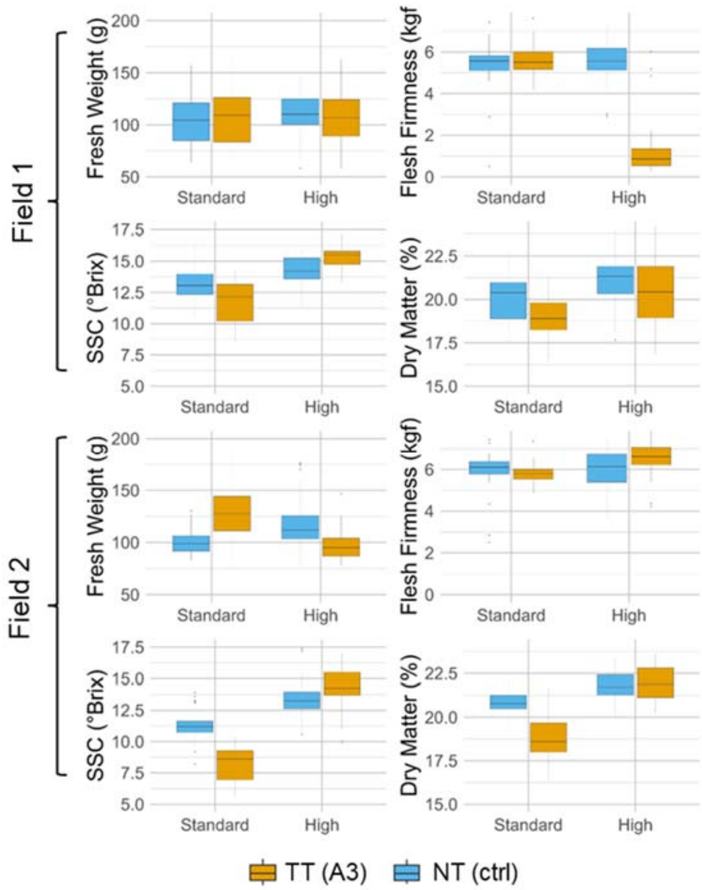

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Figure 5. Fresh fruit wight (g), flesh firmness(kgf), SSC (° Brix) and dry weight (%) of fruits from treated (TT) (A3) and, not-treated (NT) plants of Field 1 and Field 2, crop load: standard and high, Years: 2022.

Considering the firmness, the highest values were recorded for the standard crop load, and the CPPU-treated plants showed higher values compared to the not-treated, under both crop loads and fields, apart from the then year 2022, standard TT. Furthermore, each factor and interaction showed a statistical significance on this parameter (Figure 6 and Table 3). Regarding to overall Brix, it was found the highest value in the standard crop load, of both fields, when CPPU is applied (TT). Yet almost every factor and interaction had a statistical significance onto this parameter, apart from field × crop load × treatment (Figure 6 and Table 3). Lastly, evaluating the fruit dry weight, also in this case almost each factor and variable had a statistical influence on the measured parameter, with the sole exception of field × treatment. Specifically, the values were always higher in TT compared to NT. Values were generally higher in field2 (Figure 6 and Table 3).

Table 3. Multifactorial Analysis of Variance for CPPU 3 applications.

	Weight	Firmness	Brix	Dry Weight
Field		***	***	***
Crop load		***	***	***
Treatment		***	**	***
Field × Crop load		***	***	*
Field × Treatment		***	*	
Crop load × Treatment	***	***	***	***
Field × Crop load × Treatment	***	***		**

Significant codes: *** = p<0.001, ** = p<0.01, * = p<0.05, **.** = p<0.1

2.5. Post-Harvest Performances

Quality parameters were analyzed along storage of kiwifruit at 45 days intervals up to 180 days, labeled as T_0, T_1, T_2, T_3, T_4. Among the different quality parameters analyzed (FW, FF, SSC, DM), flesh firmness was chosen as main evaluation parameter. Indeed, FF is the most important determinant of storage length, since fruit reaching FF lower than 0.7 Kgf are considered unmarkable. Flesh firmness was influenced by the year, crop yield and field. Despite at harvest crop load had no influence on FF, during storage, fruit from HCL tended to have a faster softening. However, in all cases, FF values generally dropped in the first 90 days of storage (Table 4). In 2020, in field 1, independently from crop load, CPPU application resulted in slightly differences when considering the time point at which various treatments achieved the firmness marketability threshold. Overall, T2 (90 days after harvest) is the timepoint at which most of the stored fruits reach values of firmness around the limit and differences at this point indicate the most relevant effect of forchlorfenuron.

CPPU treatments generally exhibit a less steep decline over time, indicating a potential positive effect of the CPPU treatments in maintaining higher values. Among the CPPU treatments, CPPU A2 in Field 1 of 2020 shows a more gradual decline, particularly for the HCL crop load, despite there is no statistical significance. In 2021, CPPU A2 consistently demonstrates favorable outcomes across both fields, again particularly under High Crop Load (HCL) conditions. This treatment shows higher measurements than control and A1 across multiple time points (T0-T4) of storage, although some values were not significant.

Finally, in 2022, kiwifruit storage performances were rather poor, and most of the fruit scored an FF around 0.7Kgf starting from 45 days of storage. In field 1, fruit from the vines that has received the double application of CPPU tended to have the highest FF values independently from crop load. In field 2, both application strategies resulted in slightly higher value of FF along storage an effect of CPPU was visible in comparison with the control on both filed 1 and 2, however, high significancy values were not highlighted. Overall, the major boost of firmness derived from the A2 strategy rather than A3, as possible evidence of CPPU saturation of plant physiology pathways.

Table 4. Flesh firmness (kg/f) of fruits from control or CPPU treated vines in the three years of experiment, field and crop load. ctrl: non-treated (NT), SCL: standard crop load, HCL: high crop load). Heatmap express the relative differences expressed in a single field in each year. For each field separately, different letters within a column indicate significant differences according to Tukey's HSD test (P ≤ 0.05).

Year	Field	Treatment	Crop Load	45 days		90 days		135 days		180 days	
2020	FIELD 1	ctrl	SCL	4,6		1,6		0,9		1,1	
			HCL	4,0		2,6		1,2		0,9	
		CPPU A1	SCL	3,8	ns	2,3	*	1,4	ns	1,4	*
			HCL	2,3	*	2,0	*	1,9	*	1,2	*
		CPPU A2	SCL	4,1	ns	2,5	ns	1,3	ns	1,5	*
			HCL	4,5	*	2,9	ns	1,7	*	0,8	ns
2020	FIELD 2	ctrl	SCL	3,3		1,0		0,8		0,6	
			HCL	1,3		0,6		0,5		0,6	
		CPPU A1	SCL	2,1	*	0,8	*	0,6	*	0,6	ns
			HCL	1,9	*	0,6	ns	0,7	*	0,5	*
		CPPU A2	SCL	2,0	ns	0,8	*	0,7	*	0,6	ns
			HCL	1,6	ns	0,7	ns	0,5	ns	0,5	*
2021	FIELD 1	ctrl	SCL	3,1		0,7		0,5		0,6	
			HCL	2,4		0,6		0,5		0,5	
		CPPU A1	SCL	2,5	ns	0,7	ns	0,5	ns	0,5	ns
			HCL	2,5	ns	0,6	ns	0,4	ns	0,4	ns
		CPPU A2	SCL	2,4	*	0,7	ns	0,6	ns	0,5	ns
			HCL	2,5	ns	0,8	*	0,5	ns	0,5	ns
2021	FIELD 2	ctrl	SCL	1,9		0,8		0,6		0,6	
			HCL	3,5		1,4		0,8		0,7	
		CPPU A1	SCL	2,1	ns	1,0	ns	0,6	ns	0,6	ns
			HCL	3,1	ns	1,0	*	0,7	ns	0,7	ns
		CPPU A2	SCL	2,5	ns	1,2	*	0,6	ns	0,7	ns
			HCL	2,7	ns	1,3	ns	0,6	ns	0,8	ns
2022	FIELD 1	ctrl	SCL	0,8		0,5		0,5		0,5	
			HCL	0,7		0,5		0,5		0,4	
		CPPU A2	SCL	1,0	ns	0,6	ns	0,5	ns	0,6	ns
			HCL	0,8	ns	0,5	ns	0,4	*	0,5	ns
		CPPU A3	SCL	0,8	ns	0,6	ns	0,5	ns	0,3	*
			HCL	1,0	ns	0,8	*	0,5	ns	0,5	ns
2022	FIELD 2	ctrl	SCL	0,7		0,6		0,5		0,5	
			HCL	1,0		0,5		0,6		0,3	
		CPPU A2	SCL	0,8	ns	0,6	ns	0,6	ns	0,5	ns
			HCL	0,9	ns	0,8	ns	0,4	ns	0,4	ns
		CPPU A3	SCL	0,8	ns	0,6	ns	0,6	*	0,5	ns
			HCL	0,8	ns	0,6	ns	0,6	*	0,5	*

3. Discussion

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CPPU, a synthetic cytokinin, is widely used for increasing fruit size e in different crops [24–28]. Moreover, of CPPU application can efficiently maintained fruit quality and delayed ripening [29,30]. In kiwifruit, the use of CPPU has been investigated primarily on *A. chinensis* var. *deliciosa* 'Hayward'. Thus, we aimed at optimizing the use of CPPU in *A. chinensis* var. *chinensis* taking in consideration its effects both at harvest and during post-harvest storage. In agricultural research, using multiple experimental fields and multiple years of study is crucial to account for the natural variability in environmental conditions, such as soil quality, climate, and pest pressures. This approach ensures that research findings are robust, reliable, and broadly applicable, making them useful across diverse agricultural settings. Observing year and field effects is normal and expected, reflecting real-world conditions and enhancing the overall resilience and adaptability of agricultural recommendations. In our study, the effect of CPPU on fruit weight was erratic and highly dependent both on the year and orchard (Table 1). This result highlights the principle that seasonal changes in climatic conditions and agronomic practices can significantly affect plant physiology. In fact, variations in temperature, light, and humidity within the orchard directly influence the synthesis and degradation of plant hormones, disrupting their balance and thereby modifying the effectiveness of external inputs such as bioregulators. Moreover, biotic stresses, such as diseases and pests, can be indirect consequences of suboptimal growing conditions, further interfering with normal hormone function. Not only, the exogenous application of hormones to plants can significantly disrupt the balance of their endogenous hormone homeostasis. Plants naturally regulate their growth and development through a complex network of hormone signaling pathways, maintaining homeostasis to respond optimally to environmental stimuli. However, when external hormones are introduced, this balance is often perturbed, leading to physiological changes that may not align with the plant's developmental needs. For instance, excess or deficient levels of a particular hormone can interfere with the plant's ability to regulate processes such as cell division, elongation, or stress responses. As a result, there could be an altered reproductive development.

In our research, CPPU positively influenced the fruit weight only with a double application (A2) and primarily in standard crop load. In high-crop load, the triple (A3) application of CPPU tended to increase fruit size in comparison to control suggesting that a balanced crop load is key for fruit production. The lower effect of the double application of CPPU in high crop load could have been related to an excessive number of fruits competing for limited resources [33,34]. In this condition, only a third application (A3) may hold positive results. This result suggests that the effect of biostimulants may also help counteract the negative effects of high crop loads if the application strategy is tailored on the fruit number per vine and the orchard management. In fact, a high crop load, may reduce fruit quality, alter flower differentiation and increase the susceptibility to post-harvest disorders [31]. On the other hand, a low crop load requires lower irrigation rates than a high crop load [32], yet fruit production depends directly on the vegetative growth of the preceding season because fruits develop from buds located in shoots of the previous year [33]. The carbon demand from fruits in a year of high crop load limits vegetative growth, thus restricting the potential yield of the following year [34]. Hence, it is the competition between vegetative and reproductive growth that is at the root of the alternate bearing behaviour [35].

In standard crop load, the maximum effect on fruit weight was observed in the double (A2) application and not in the triple (A3) suggesting a cumulative, yet saturating effect reached with just two applications. Since CPPU is a synthetic cytokinin that promotes cell division and fruit growth, it acts primarily on meristematic cells, which are responsible for cell division, and is most effective during the early stages of fruit development, particularly right after fruit set. During this phase, cell division is highly active, allowing CPPU to enhance fruit size by increasing the number of cells [36]. The observation that the maximum effect on fruit weight was seen with two applications (A2) rather than three (A3) suggests a cumulative yet saturating response. After two applications, the hormonal response could have reached a physiological limit where additional applications no longer increase fruit size. This is likely due to receptor saturation or the natural limit of cell division capacity. Therefore, beyond two applications, further CPPU treatments do not significantly enhance growth.

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Considering the fruit firmness, this parameter was positively and statistically influenced only by the 2 and 3-times CPPU applications. Previous studies performed on *A. chinensis* car. *deliciosa* 'Hayward' reported that CPPU reduce flesh firmness at harvest, and, at the same time, increasing sugar content and levering acidity, thus suggesting that CPPU effect was mainly due to promotion of ripening [37]. In 'Harward', different studies reported that CPPU increased the volume and number of small isodiametric parenchyma cells in the outer pericarp thus enlarging his depth [38,39] In grapes, CPPU modulates hemicellulose and pectin cell wall composition increasing calcium content in cell wall and, thus resulting in higher berry firmness [40]. Similar effects were observed also in apple where CPPU influenced hemicellulose in cell wall [41]. In our case, CPPU did not anticipate ripening, and we can hypothesize that the increase of firmness can be due to changes in the cell wall structure and to the depth and structure or outer pericarp. However, further studies aiming at investigating the cytological and histological changes induced by CPPU on fruit are needed to confirm this hypothesis. In our study, crop load was the factor primarily influencing berry firmness. This result is in accordance to previous studies that showed that crop load and canopy size may influence firmness and have an effect on endogenous concentration of Calcium inside fruit [42]. Similarly to other fruits, such as apple, endogenous Calcium concentration, *per se*, does not directly correlate to firmness increase, however, calcium partitioning in the different tissues and cell compartments and exogenous $CaCl_2$ or CaO application have a positive effect on firmness [42–44]. Furthermore, in high crop load conditions, resource competition could result in softer fruits due to limited availability of other nutrients, in addition to Calcium, for individual fruits [45,46].

Taking into account fruit sugar content, the single and double applications of CPPU showed the trend of increasing SSC primarily in hight crop load, despite the results were not always statistically significant. Also in this case, crop load was the main driver of sugar content in the standard crop load, also, fruits had higher SSC valued than the one in high crop load. In the end, for the application 1: Brix was increased in both high and standard crop load, whereas the application strategy 2 showed only a trend towards an increment of the Brix due to application and for application 3 no differences arose. This outcome could be valuable for orchard management practices, indicating that CPPU application may be particularly beneficial to enhance fruit, regardless the crop load. However, again, there is an effect of environment (field), to be taken into account by farmers.

Dry weight at harvest is a key parameter directly correlating with storability and consumer acceptance since it affects fruit sweetness and flavour at consumption[47]. The single application of CPPU showed the trend to increase dry weight at least in one of the two orchards (Field 1) included in this study, specifically, the dry weight tended towards showing higher parameters in both crop loads, under the TT treatment. As for the previous analysis, CPPU application had varying effects on fruit dry weight depending on application frequency. This variability might stem from the differential regulation of fruit growth and development processes in response to both CPPU and environment factors (i.e., study-fields).

The use of CPPU has yielded promising results, especially with regards to the Brix and Dry Weight, across the years and applications. The efficacy of CPPU lies in its ability to serve as a simple and cost-effective strategy for bolstering overall fruit quality and yield. By stimulating the development and maturation processes, CPPU leads to fruits that boast enhanced size, sugar content, and structural integrity. In addition to that, CPPU provides an important outcome in enhancing the overall storability of fruits, specifically at 45 and 90 days. In the remaining cases, it did not bring any effect, nevertheless, the storability was not negative influenced.

4. Conclusions

The major effect related to an increase in the number of applications was recorded in high crop load condition. Indeed, the proper CPPU application strategy needs to be tailored to the orchard cultivation strategies to avoid any overuse or saturation of the product and maximize its effectiveness. The investigation on preserving the firmness during long storage supports the suggestion that CPPU could be employed to extend the number of days that fruits firmness stay above the marketability threshold

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5. Materials and Methods

5.1. Experimental Design

The experiment was carried out in for four consecutive years from 2020 to 2024 (from this point onward referred as 2020, 2021, 2022 and 2023), in the province of Latina (Italy). Two kiwifruit orchards with a uniform growth and vigour were identified, and cultivated following general management practices of pollination, soil fertilization, pruning and irrigation. Kiwifruit plants belong to the variety 'Zespri® Sungold' (*Actinidia chinensis* var. *chinensis*) grafted onto 'Hayward' and were trained to either the 'pergola' or 'tendone' system, and trees are 5m spaced between the rows and 2.5 or 3.5 on the rows.

The experiment was laid out in a randomized complete block design of 12 replicates (4 trees x 3 blocks, plus control) and CPPU (Sitofex® a.i1.0%; AlzChem Trostberg GmbH, Germany) was sprayed by foliar fertilization. Three different CPPU applications strategies and two crop loads (CL) have been tested along the seasons. The first application strategy (A1) includes a single spray of 500L/Hectare @ 0.25ml/L when orchard reaches the bud breaking phenological stage. The second strategy (A2) combines A1 with a second spray of 500L/Hectare @ 0.33 ml/L performed 7-10 days before flowering and thinning of lateral branches was completed. Finally, the third application strategy (A3) adds a 500L/Hectare spray @ 0.50 ml/L when fruits reach nut-size (approximately 35 days after full bloom). The first crop load (CL), namely high CL (HCL), imposed a yield of around 45 Mg/ha with 45 fruits per cane. Whilst the second crop load, described as standard CL (SCL), imposed a yield of around 60 Mg/ha with 60 fruits per cane.

5.2. Fruit Analysis and Storage Conditions

Harvest was performed main harvest, that was estimated for each field through a combination of fruit ripening parameters and growth index. In every commercial orchard, a total of 25 kiwifruit sample without visible defects or deformations were randomly picked at different positions of the vines for each treatment and crop load. Kiwifruits were stored in international trays, at 1°C, 90% RH in normal atmosphere. Furthermore, additionally 50 fruits per time point were collected for the storability assessment.

The samples were placed at 2°C overnight to be processed within 24hours. The biometric parameters under investigation were fruit weight (FW), dry matter (DM), soluble solid content (SSC) and firmness (FF), which are known to be some of the most relevant parameters in fruit market pricing and orchard productivity. Fruit weight was measured by using a digital scale (Max=1200g; d=0.01g; Kern, KB Precision Balance, UK). Dry Matter was measured by making a difference between fresh weight and dry weight. A 2-3 mm equatorial slice was cut for each fruit. Fruit slices was singularly placed to dry in an oven at a constant temperature 60-64°C for 24 hours. Soluble solids content (°Brix) was measured by equatorially cutting the fruit and 1-3 juice drops were squeezed on the prism surface of a digital refractometer (Atago, PAL-1). Fruit firmness was measured using a Fruit Texture Analyser (FTA Guss, Ravenna, IT) fitted with a 7.9 mm penetrometer probe after removal of skin and flesh to a depth of approximately 1 mm. The probe was driven into the flesh at 5 mm/s to a depth of 7.9 mm, and the maximum force recorded as the firmness value. Firmness was measured twice at the equator of each fruit, with the two measurements taken at 90° to each other and averaged. Firmness was measured as Kgf. Long storage assessment of fruit storability was carried out by monitoring the fruit firmness of 25 fruits per treatment every 40 days from the harvest time to 180 days after, following the same methodology of fruit quality analysis at harvest.

5.3. Statistical Analysis

Averages and standard errors were calculated using the data from all repetition samples. Normality was carried out applying the Shapiro normality test. The statistical significance of differences among treatments was assessed *via* multifactorial Analysis of Variance (ANOVA), followed by Tukey HSD test with *p* < 0.001, 0.01, 0.05, and 0.1. Box plots were created using R software, v. 2024, library ggplot2.

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