Effects of the application of forchlorfenuron (CPPU) on the composition of verdejo grapes

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Abstract. The application of cytokinins such as forchlorfenuron (CPPU) has been widely used in table grape varieties to increase yield and berry size. However, the potential interest of these phytoregulators in wine grapes have been scarcely analyzed. The objective of this study has been to evaluate the influence of CPPU treatment on the agronomic performance and composition of Verdejo grapes. The trial was conducted in 2021, in the Protected Designation of Origin "Rueda" (Spain). CPPU was applied using a concentration of 15 mg/L, by spraying the bunches when the berries were 5-6 mm in diameter. The photosynthesis rates and the stem water potential, measured after the application, tended to decrease in the treated plants without modify the values of vine yield and vigour. The treatment significantly affected the content of soluble solids and total polyphenols of the grape must, detecting increases of 15.4% and 7%, respectively, compared to the controls. Preliminary results suggest that the application of CPPU on the bunches could improve the quality of the Verdejo grapes. The treatment would be interesting to apply in cultivation conditions where the harvest has difficulties to reach an adequate level of maturity, such as excessive vigour or too cold climate.

1. Introduction

Plant growth regulators are substances derived from natural or synthetic sources, which modulate and control physiological changes by interfering the plant´s hormone system, besides being able of improving phenolic composition, aromatic profile and another quality compounds [1, 2]. Some growth regulators exogenously applied produce molecular and physiological changes during ripening [3, 4] which can help to reach maturity faster and improve the quality of the must [5, 6] in vineyards under conditions of excessive vigour or cool regions, where heat units for maturing fruit are not enough.

The cytokinins are particularly used for berry enlargement in grape varieties. They can be applied with gibberellins to stimulate cell division and elongation as much as delaying leaf senescence. Cytokinins are thought to be involved in fruit set and early growth [7, 8]. High concentrations of cytokinins have been found in Kyoho berries during berry flesh in early development [9]. Moreover, other authors have reported that certain cytokinins have an accelerated increase at veraison in grapes and kiwi fruit, remaining in a high concentration during ripening [10, 11]. Foliar treatments with compounds which acts like cytokinins such as forchlorfenuron (CPPU) have been widely used in table grapes in order to increase berry size and improve yield [12, 13]. However, the effect of these product on wine grapes has been scarcely studied [12]. Polyphenol increases have been reported by the application of CPPU in different seedless grapevine cultivars, finding significant differences depending on the variety, doses and time of treatment application [13-15].

The aim of the present study was to determine how CPPU treatment on the clusters, after fruit set, influences the agronomic performance and the composition of white wine grapes cv. Verdejo.

2. Materials and Methods

2.1 Experimental design

The experiment was carried out in 2021 in La Seca (Valladolid, Spain), within Rueda Designation of Origin. The vineyard corresponds to Verdejo variety, grafted on 110-Richter rootstock. Vines are conducted on double cordon, in a planting frame 3.0 x 1.5 m (2222 vines/ha) with a load of about 35,000 buds/ha. The vineyard was irrigated, receiving globally throughout the cycle an average water supply of around 30% of the reference evapotranspiration.

The experiments were performed in a randomized complete block design with four replications. The elementary plots were made up of 8 plants for treated (T) and control vines (C), leaving a border plant between each two plots. Before treatments, a light defoliation was carried out manually in the cluster area in all plants. Clusters were sprayed 21 days after full bloom to full wetness with an aqueous solution of 15 mg/L forchlorfenuron (CPPU, Zhengzhou Farm Reaching Biochemical, Co Ltd, Henan, China), when the berries were 5-6 mm in diameter. The solution included 0.05 % Agral (Syngenta Agro, Madrid, Spain), a nonionic surfactant. The control plants were sprayed with water plus Agral in the same date. All treatments were applied with manual sprayers, on both sides of the trellis.

2.2 Field data collection

Water status measurements of the plants, as leaf stem water potential, were tested at 9, 30, 41 and 61 days after treatment (DAT) and photosynthetic activity was checked at 9, 31, 48 and 63 DAT. Stem water potentials were measured between 11 and 13 h (solar time) in adult leaves, on the shaded side of the trellis, previously covered with aluminum bags for 1.5 hours before measurement, using a Scholander-type pressure chamber (Solfranc Technologies SL, Spain). Net assimilation (µmol $CO_2/m^2/s$), leaf and ambient temperature, and chlorophyll fluorescence parameters were determined with a LI-Cor 6400 portable infrared gas analyzer (IRGA) equipped with a 6400-40 leaf chamber pulse width modulation fluorometer (Li-Cor, Inc. Lincoln, Nebr., USA). The fluorescence parameters measured were: minimum (F0) and maximum fluorescence, variable and steady-state fluorescence, efficiency and maximum efficiency of photosystem II, apparent electron transport rate and photochemical and non-photochemical (NPQ) quenching. Photosynthesis measurements were taken between 11 and 13 h (solar time) on the interveinal space of the right main lobe of exposed leaves of the middle zone of the shoot, on a sample of two leaves in each elemental plot. The airflow rate through the leaf chamber was kept at 500 µmol/s.

The number of bunches per vine, average bunch weight, and total production per plant were determined at harvest. Berry weight was obtained from a sample of 100 berries randomly collected from each elemental plot. Vigour was estimated as mean pruning weight.

2.3 Grape composition analysis

The harvest was carried out until the average value of the total soluble solids content of the must samples reached 21.5 ºBrix. The must obtained from a 100 berry sample in each elementary plot was used to determine TSS, pH, titratable acidity, malic and tartaric acid contents, yeast assimilable nitrogen and potassium content, total polyphenol index and color parameters (CIELAB) established by the OIV [16].

2.5 Statistical analysis

Analysis of variance and Student's t-test were applied to evaluate the effects of the treatment with CPPU on different variables studied. Data analysis was performed with version 9.2 of the SAS software package (SAS Institute Inc., Cary NC, USA).

3. Results and Discussion

3.1 Photosynthetic activity and water status

The values of stem water potential in treated plants were significantly lower than untreated ones at 41 and 61 DAT (Figure 1), showing that CPPU treatment would cause stomatal closure, worsening the water status in the plant. The normal ranges of water potential in grapevines are between -0.3 and -2.0 MPa [17]. Therefore, the data obtained in this study of -0.3 and -0.8 MPa showed normal ranges.

Environmental and physiological characteristics such as light, CO_2 concentration, water status and abscisic acid (ABA) affects directly the plant stomatal opening [18]. Davies et al. [19] have reported the importance of ABA accumulation on the stomatal closure modulation. Leaf turgor loss have been correlated with stomatal closure [20-22], showing that leaves with more negative values of water potential are able to maintain stomatal and hydraulic conductance and growth under drought conditions [23].

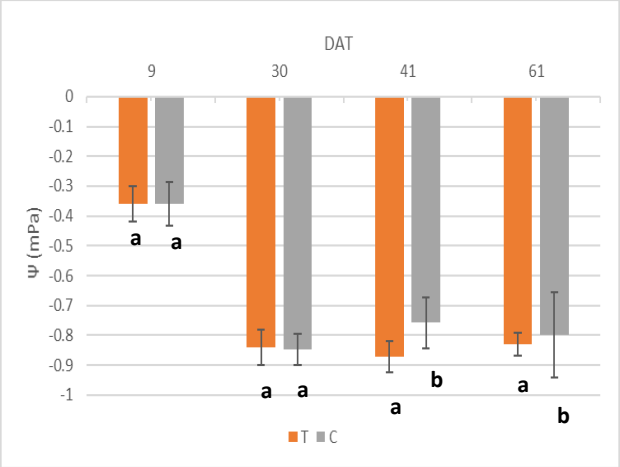

Figure 1. Water potential at different days after treatment (DAT) in plants without treatment (C) and treated with CPPU (T). Different letters denote significant differences (Student's T test, $p<0.05$).

The values of net photosynthetic CO_2 assimilation showed a tendency to decrease in treated plants at 48 DAT ($F= 3.77$, $p= 0.06$). This might be a consequence of the stomatal closure caused by the worsening water status after CPPU application. To close the stomata, the treatment might have increased the ABA levels in the vines. Several studies have implied that the stomatal closure is produced as a response of water deficit, inducing a decline in the photosynthetic activity by the restriction of CO_2 assimilation in the leaf mesophyll [18, 25]. There was a significant decrease of F0 at 48 DAT (Figure 2), in agreement with Hailemichael et al. [24], who reported a positive correlation between leaf water potential and F0.

Several studies have implied that the stomatal closure is produced as a response of water deficit, inducing a decline in the photosynthetic activity by the restriction of CO_2 assimilation in the leaf mesophyll [18, 25]. According to the above, the results obtained in this work suggested that the decrease in the stem water potential in the plants with CPPU treatment could be caused by the increase of ABA, inducing a stomatal closure and by consequence a decline in the photosynthetic activity.

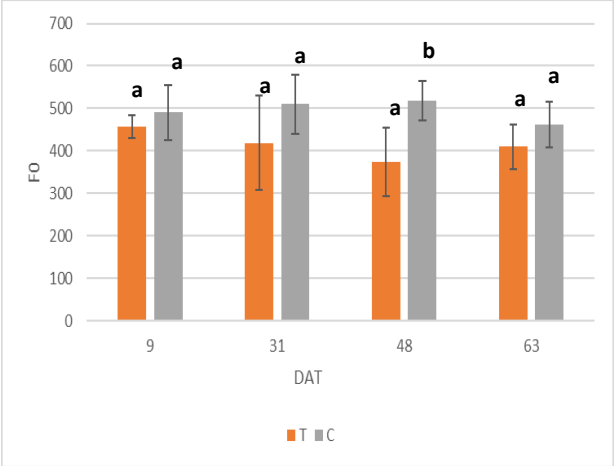

Figure 2. Comparison of means of minimum chlorophyll fluorescence (F0) measured at different days after the CPPU treatment (DAT) in untreated (C) and treated plants (T). Different letters denote significant differences (Student's T test, $p<0.05$).

The values of NPQ showed a significant decrease in treated plants at 9 DAT with respect to control ones ($F= 6.3$, $p<0.05$) with no differences on the other control dates. NPQ represents the thermal dissipation of absorbed light energy in the photosystem II by alternative mechanisms [26, 27] and has a strong connection with water status [28]. Hailemichael et al. [24] reported a significant relationship between pre-dawn leaf water potential and NPQ at preveraison and veraison, disagreeing with Alves et al. [29] who indicated an increase of NPQ values when drought was intensified. The results of the analysis of variance showed that CPPU treatment modified the values of F0 and NPQ but not the other fluorescence parameters measured.

3.2 Vine yield and vigour

The treatment applied did not significantly modify the yield (Table 1), although it tended to be lower in treated plants than untreated ($F= 3.23$, $p= 0.08$). This is consistent with the worsening water status and the tendency to decrease of net assimilation values in treated plants, as commented above.

Zabadal et al. [11] indicated that the application of CPPU at different concentrations (5, 10 and 15 mg/L) on the clusters of "Himrod" vines, before bloom, increased berry diameter and mass at maturity while the CPPU concentration increased. These authors also analyzed the CPPU response at different times of application (4, 5, 7 and 9-mm berry diameter), observing a reduction of berries per cluster and cluster mass, when the product was applied at 5- and 7-mm berry diameter, in comparison with the treatment applied at 4 mm, but equal to the control. These results agreed with the yield decrease tendency observed in our work. Other studies have reported a significant increase of yield by the application of CPPU when the treatment was applied to a berry diameter of 11-12 mm in cv. Italia [30] and 7-8 mm in Flame Seedless grapes [31].

The treatment applied did not produce significant differences in the 100-berry weight between treated (181.4 g) and control vines (186.1 g). In any case, the effect of CPPU on yield and berry size are strongly influenced by application time and grape variety.

The vigour in treated and untreated plants did not show significant differences (Table 1). These results indicated that CPPU treatment could be able to maintain an appropriate balance between vegetative and reproductive development regardless its effects on grape quality.

Table 1. Mean values of yield and vigour and parameters of grape composition in plants without treatment (C) and treated with CPPU (T).

	Treatment		
Parameters	C	T	Sign.
Yield and vigour			
Yield (kg/vine)	4.7	3.9	
Average bunch weight (g)	140.4	129.2	
Pruning weight (kg)	1.28	1.24	
100 berry weight (g)	186.1	181.4	
Must Composition			
Total soluble solids (°Brix)	21.1	21.6	*
pH	3,5	3,5	
Total acidity (g/L)	6.1	6.0	
Total Polyphenol Index	24.2	25.9	*
Malic acid (g/L)	1.7	1.9	
Tartaric acid (g/L)	5.5	5.5	
Potassium (mg/L)	953.8	1258.0	
Assimilable Nitrogen (mg/L)	177.5	186.9	
Color Parameters (CIELAB)			
L*	76.7	75.8	
a*	1.3	2.2	
b*	25.9	25.9	
C*	25.9	26.0	
h*	87.2	86.8	

*Means are significantly different (Student's t-test, $p<0.05$)

3.3 Grape composition

In Table 1 are presented the must composition data of control and treated vines. The results showed that the application of CPPU caused a significant influence on TSS and the total polyphenol index, without effects on other parameters. The treatment increased the concentration of sugars and polyphenols, consequently, induced a faster ripening on the Verdejo grapes. It is possible that earlier ripening occurred in treated plants is related to an increase of ABA biosynthesis caused by CPPU application, the same one that would have generated the lowering of the stem water potential (SEE SECTION 3.1). The ripening process of non-climacteric fruits such as grapes is mainly regulated by ABA. It has been demonstrated that cytokinins play a key role in the regulation of environmental stress responses having strong interactions with ABA [32, 33]. Previous studies have also reported a significant increase of TSS in Crimson seedless [12] and Ruiduhongyu grapes [34] by the exogenous application of ABA, inducing an accelerated sugar accumulation that occurs mainly in the late ripening period.

Tyagi et al. [15] registered an increase of proanthocyanidins and flavan-3-ols levels in grapes by the application of CPPU in Sangiovese grapes (5 ppm, 8.7 mm berry diameter), but decreasing TSS content. In the same line, other studies [11, 13] have shown that CPPU treatment can cause a significant delay in sugar accumulation depending on the dose and time of application.

In summary, CPPU treatment applied in this study influenced the phenolic and technological grape maturity, increasing their values without modify the yield and vigour of Verdejo grapes. These results suggest that CPPU treatment could be useful to improve fruit quality in zones where the grape cultivar has difficulties to reach an adequate level of maturity at harvest, either due to excessive vigour or too cold climate.

4 Acknowledgments

The present work has been carried out within the framework of a collaboration agreement between the University of Valladolid and Bodega Cuatro Rayas S. Coop. This communication has been presented thanks to the support provided by the mobility of doctoral students UVa 2022.

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