Effect of synthetic cytokinin and vapour pressure deficit on potassium and calcium content in table grape berry.

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Abstract.
The forchlorfenuron (CPPU) application is recommended in table-grape after fruit-set to boost berry sizing, albeit CPPU application during pre-flowering is gaining attention due to its effect in influencing berry Ca content. This study evaluated the effects of pre-flowering applications of the synthetic cytokinin CPPU and vapor pressure deficit (VPD) manipulation on potassium (K) and calcium (Ca) content in table grape berries (*Vitis vinifera* L.). The experiment was conducted in southern Italy (Bernalda, Basilicata) in a plastic covered vineyard of the cv 'Sugrathirteen'N. (Midnight Beauty ®). Forchlorfenuron treatments included single (S_CPPU; 100 inflorescences) and double (D_CPPU; 75 inflorescences) applications, while low VPD was obtained by enclosing, after fruit-set, 50 bunches in transparent PVC bags (VPD_{LOW}). Potassium accumulation in berries was unaffected by VPD and resulted to be synchronized with sugar accumulation, reflecting its predominant phloem-based transport. In contrast, Ca accumulation was significantly influenced by VPD reduction, indeed VPD_{LOW} bunches showed a reduction in both Ca content and berry fresh mass. CPPU treatments *per se* increased K concentration in the rachis but did not significantly affect Ca accumulation in berries. These findings suggest that VPD conditions around bunches rather then pre-flowering CPPU applications could be pivotal in determining berry fresh mass, Ca content and sugar accumulation.

Keywords CPPU, ripening, sugar content, titratable acidity, *Vitis vinifera* L.

1 Introduction

Plant growth regulators (PGRs) are widely used in table grape growing with the purpose of improving fruit quality and post-harvest potential[1, 2]. Among PGRs, the synthetic cytokinin forchlorfenuron (CPPU) is used after fruit-set to boost berry sizing[3].Recent studies investigated the role of pre-flowering CPPU that influence berry firmness by modulating cell wall composition, particularly polysaccharides and calcium (Ca) components [2].
Vitis vinifera L. is recognized as a potassium-loving species. Indeed, potassium (K) is particularly requested during berry ripening [4 and literature therein], and it is mainly phloem-delivered toward berries late in the season (for review see [5]).
In fleshy fruit such as *V. vinifera* berries, Ca is important for physical properties including firmness, crunchiness, and for cracking vulnerability (for review see [6]). Calcium is a xylem-mobile nutrient [7], and its transport is mainly driven by transpiration flow [4, 8].Among other, transpiration depends on a) vapor pressure deficit (VPD), and b) conductance of the transpiring organ [9, 10].Hence, VPD around cluster zone is supposed toplay a crucial role in driving berry Ca accumulation. Indeed, in cherry fruit [11], the role of VPD in the air surrounding fruit was reported to influence fruit transpiration and, consequently, Ca accumulation. However, the relationship between Ca accumulation and transpiration is not always straightforward[8]. Under certain conditions, such as highrelative humidity(RH), Ca accumulation can be uncoupled from fruit transpiration.
Table grape cultivation accounts for 43 k ha in Italy [12]. Nowadays, table grape vineyards are mainly grown under plastic cover to protect fruit from diseases and modulate harvest timing. Plastic covers modify microclimatic conditions, including temperature (T) and RH[13] and, in turn, VPD, potentially affecting nutrient content in berry.
However, the interplay between PGRs like CPPU and microclimatic factors such as VPD on berry mineral composition remains quite underexplored in table grape.
Based on this background, the present experiment tested the hypothesis that both pre-flowering CPPU application and VPD around the cluster influence K, Ca and qualitative traits in table grape berries. The results provide practical insights for growers to enhance Ca accumulation and berry quality, mainly by managing factors influencing VPD rather than applying PGRs.

2 Materials and Methods

2.1 Experimental vineyard.

The experiment was carried out in Basilicata (South Italy) (Bernalda, 40°23'38.0"N 16°44'39.6"E) in 2023 at

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a vineyard of the *V. vinifera* L. cv 'Sugrathirteen' N. (Midnight Beauty®) grafted onto 140Ru (2.70 × 2.50 m planting distance). The vineyard was covered using apolyethylene high density sheet (GEALAM; Geaplast, Massafra, Italy) for about 150 days (from soon after sprouting until harvest). Vines were drip-irrigated (mean annual irrigation volume 3,200 m^3 ha^{-1}) and trained at *tendone* and cane-pruned (about 95,000 buds ha^{-1}). The vineyard canopy was uniformly managed by means of shoot thinning, positioning, leaf-plucking, and bunch hanging after fruit-set (berry diameter around 8-10 mm). During the experimental season, agronomic and phytosanitary practices were applied in the vineyard according to national integrated production regulations.

2.2 Experimental design and treatments.

A total of 23 homogenous vines was selected within the vineyard and sub-groups (2-3 contiguous vines each) represented the experimental unit randomly replicated (× 3-4) and assigned to the following treatments.

2.2.1 VPD treatment.

At 28 days after anthesis (DAA; full anthesis on May 23rd)a low *VPD* (VPD_{LOW}) treatment was imposed on 50 bunches (berry diameter approx. 12mm). These bunches were bagged using transparent PVC bags (34 × 52 cm)in order to keep the RH of the air surrounding the bunches near saturation [8]. The VPD_{LOW} bunches were distributed in three vines per replicate (tot.nine vines). Transparent bags were chosen for their limited impact on bunch shading, as in [8].

2.2.2 CPPU treatment.

The CPPU was sprayed on a total of 175 inflorescences grouped in S_CPPU (100inflorescences) and D_CPPU (75inflorescences) receiving the CPPU one or two times, respectively.

Both S_CPPU (×9 vines) and D_CPPU (×6 vines) groups were sprayed (2.25 mg CPPU L^{-1}a.s.) when the inflorescences were clear (E-L 12/ BBCH 53phenological code, [14]; 32 days before anthesis).

The D_CPPU vines were sprayed again at single flowers separated (E-L 17/ BBCH 57; 15 days before anthesis) at the same concentration. The CPPU was sprayed using the commercial Sitofex® (7.5 g L^{-1}a.s.; Alzchem, Trostberg, Germany). The application rate has been chosen based on previous trial aimed at enhancing bunch stem thickness (unpublished data).

Additional 100 bunches from nine vines were neither bagged nor sprayed with CPPU and served as control (CTRL; ×8 vines).

2.3 Stomatal conductance.

During the vegetative season, at 8, 22, 34, 50 e 76 DAA the stomatal conductance (g_s, mol m^{-2} s^{-1}) of mature leaves (70 -100 records per each date) was measured during clear days using a portable porometer Li-600 (Li-COR, Lincoln, NE, USA). Measurements were carried out between 11:00 am and 1:00 pm.

2.4 Meteorological data.

Air temperature (T) and RH outside the vineyard (× 1 sensor), inside the vineyard (× 3 sensors) and inside bagged bunches (× 2 sensors) were measured by the means of a digital probe (mod. CS215, Campbell Scientific Inc., Utah, USA) connected to a datalogger (CR10X, Campbell Scientific Inc., Utah, USA). The logger was programmed to record at 60 s intervals and to compute and store averages at 15 min intervals. The probe inside the bags was placed inside a falcon open at the bottom to avoid any direct contact with condensed water drops. The VPD was calculated according to [15] considering T and RH.

2.5 Mineral analysis.

Mineral concentrations of K and Ca were measured in inflorescences at full anthesis (0 DAA), and separately in berries and rachis at 30, 47 and 76 DAA. In each sampling point, four bulk samples (10 berries and 10 rachis each) were sampled per treatment. Samples were placed in a refrigerated bag, carried in the laboratory and both fresh and dry weigh were assessed. The dry samples (~ 1 g) were then used for mineral concentration determinations by performing ash acid digestion (HCl 1 M) and ICP-OES determination as reported in [8]. The mass of mineral per fruit was determined considering the dry matter of the berry and related concentration.

2.6 Berry quality traits.

Berry fresh mass (g, FW), dry matter (g, DM) and sugar content per berry (mg $berry^{-1}$), were assessed during berry growth. A sample of 35 berries was collected from each replicate of CTRL, VPD_{LOW}, S_CPPU and D_CPPU. Berries were randomly picked from the vines of each replicate, enclosed in a plastic bag, stored in a portable refrigerator and transported to the laboratory. A first subgroup of 25 berries per each replicate was used for the berry fresh mass determination by measuring one by one berry through a precision scale (0.0001 g; Mettler AE200, Columbus, USA). The same berries were then oven dried (80°C) until reaching constant dry mass. A second subgroup of 5 berries per replicate was used to obtain the sugar content per berry. Each berry was weighed and then squeezed to extract the juice on which the total soluble solid concentrations (TSS) was determined using a digital refractometer (ATAGO, DBX-55; Tokyo, Japan). The sugar content per berry (S) was calculated according to equation 1:

$$S = \left(\frac{TSS \times berry\ FW}{100}\right) \times 1000 \qquad [\text{mg berry}^{-1}] \qquad (1)$$

A third subgroup of 5 berries per replicate was crushed to extract about 5 mL juicefor titratable acidity determination (TA; g tartaric acid equiv. L^{-1} of juice)

according to OIV (Compendium of International Methods of Wine and Must Analysis. *Red*, *2*, 0-0097).Briefly, 5 mL of juice were diluted with 95 mL of distilled water, the solution was than stirred and titrated to a pH endpoint of 7.00 with NaOH N/10.

2.7 Statistical analysis

Per each treatment, the data obtained from each replicate were averaged and ± standard error (SE) was calculated. To assess the differences between the means the one-way ANOVA was run followed by the post-hoc Student-Newman-Keuls test, *p* values lower than 0.05 were considered significant. The ANOVA assumptions were checked using the Shapiro-Wilk (normality) and Levene's (equal variance) tests. In case of failure of the tests, the Kruskal-Wallis was used. All statistical analysis and charts were obtained by using SigmaPlot 12.3 (Systat Software Inc., San José, California, United States).

3 Results and Discussions.

The results showed that CPPU application influenced K content in berries and rachis, while Ca accumulation wasnot influenced by CPPU and was mainly driven by VPD.

3.1 Stomatal conductance.

The application of pre-flowering CPPU did not significantl yaffect g_s which ranged from 0.283 ±0.03 to0.084 ± 0.01 mol $m^{-2}s^{-1}$during the experimental period. The plastic cover, due to the shading effect on the canopy, likely masked any potential effects of CPPU. Indeed, leaves under plastic cover exhibited a g_s reduction of approx. 40–85% compared to full exposed leaves.

3.2 Berry mineral composition.

The K content per berry (Fig. 1 A) increased from 30 to 76 DAA across all treatments. However, at last sampling point (76 DAA), VPD_{LOW} berries showed a significantly lower 16% K content per berry. Considering that K is a phloem mobile nutrient[7,5],that reduction in mass may be linked to the lower fresh mass of VPD_{LOW} berries rather than to VPD manipulation. Indeed, when normalized to dry mass (inset of Fig 1 A), VPD_{LOW} berries exhibited a 23% higher K mass relative to CTRL, consistently with the idea that K delivery to the berries is independent from their transpiration [4,11]. That is, the K content showed to increase linearly (r = 0.97)with berry dry mass (Fig. 2 A), according to its phloem water transport[4,11].Potassium accumulation in the berry plays a crucial role in establishing and maintaining an osmotic gradient between the leaves (source) and the berry (sink), thereby ensuring phloem flow and facilitating sugar transport, which predominantly determines berry dry matter [16]. This mechanism supports the relationship between K and DM, as well as between K and carbon content of the DM [11]. Furthermore, since water supply through the xylem is impeded in the post-veraison stage (see discussion below), K accumulation in the berry would be usefulfor water uptakethrough the phloem [4].

Regarding Ca content, a roughly large variance was detected in CTRL berries (Fig. 1B). This behaviour is likely due to the spatial distribution of bunches under the tendone canopy, which may create heterogeneous VPD conditions around them, thereby influencing Ca accumulation. Furthermore, Ca content in berries was not significantly affected by both CPPU treatments (Fig. 1 B). In contrast, berries grown under VPD_{LOW} conditions exhibited significantly lower Ca content (0.39 ±0.04 mg $berry^{-1}$)compared to CTRL. The VPD of the air surrounding the CTRL and both CPPU treatments ranged from 0.3 to 2.5 kPa whereas in VPD_{LOW} it had a narrow variation from 0.1 to 0.5 kPa. This result confirms that Ca accumulation, being xylem-dependent, is more influenced by environmental factors[8, 11] than by pre-flowering CPPU treatments (S_CPPU and D_CPPU). Conversely, [2] reported a positive effect of pre-flowering CPPU application (× 2 times) on berry Ca content measured at harvest in cv. 'Thompson seedless' B. grown without plastic cover. Divergent results might be attributed to a) differences in CPPU concentrations applied in the experiments, with 6 mg L^{-1} applied twice in [2]; and b) different growing conditions (plastic-covered *vs* uncovered vineyard).

Unlike K, the Ca content did not exhibit a linear relationship with DM (r = 0.84) (Fig. 2B). An initial lag phase was observed up to 900 mg $berry^{-1}$ DM, during which Ca content did not increase proportionally with berry DM. However, between 900 and 1600 mg $berry^{-1}$ DM, the relationship between Ca content and DM followed a linear regression (r = 0.86, p< 0.001; data not shown), indicating a steady accumulation of Ca as dry matter increases (hence ripening progression), as observed in grapevine by [17,18].

Although this study did not consider measuring berry transpiration, the relationship between Ca content and DM presented in Fig. 1B for some extent aligns with Ca content to cumulative kiwifruit transpiration relationship [8] However, it is important to recognize a transpiration-independent pathway for Ca accumulation in fruit, as discussed in [8], although this topic is beyond the scope of the present study. Finally, the Ca concentration (mg g^{-1} DM) decreased between 30 and 76 days after anthesis (DAA) (inset in Fig. 1B), consistent with trends reported in grapevine [4], apple [19], kiwifruit [20], and cherry [11]. This decrease may suggest a reduction in the xylem contribution to the berry water budget after post-veraison, as inferred and discussed by [4]. Therefore, reduced transpiration and xylem inflow into the post-veraison berries lead to a decrease in the flow of water containing Ca that can accumulate in the berry. However, while the xylem flow disruption/restriction in post-veraison *Vitis vinifera* berries is plausible and partially confirmed by these results, it remains debated (see [21]).

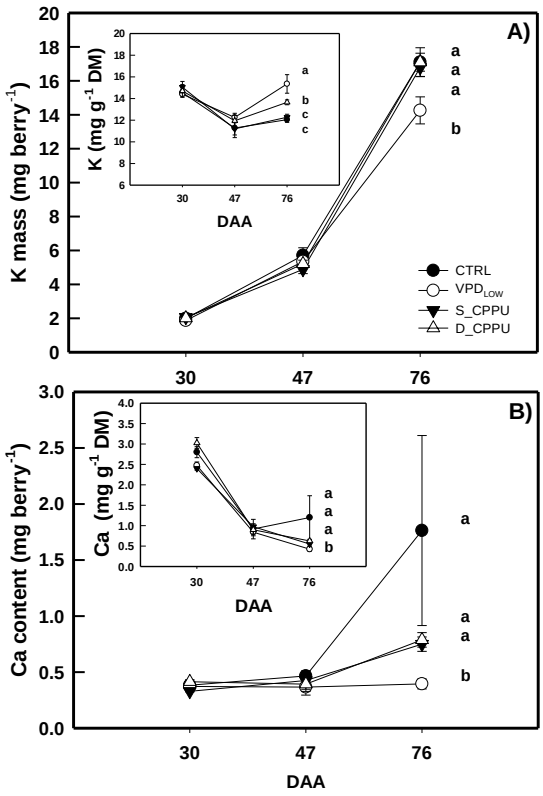

Fig. 1.A) K and B) Ca content per berry. Insets in A) and B)indicates K and Ca concentration (DM basis), respectively.Values are means ± SE bars. DAA = days after anthesis; DM = dry matter.Different letters indicate significant differences (p< 0.05, Student-Newman-Keuls test).

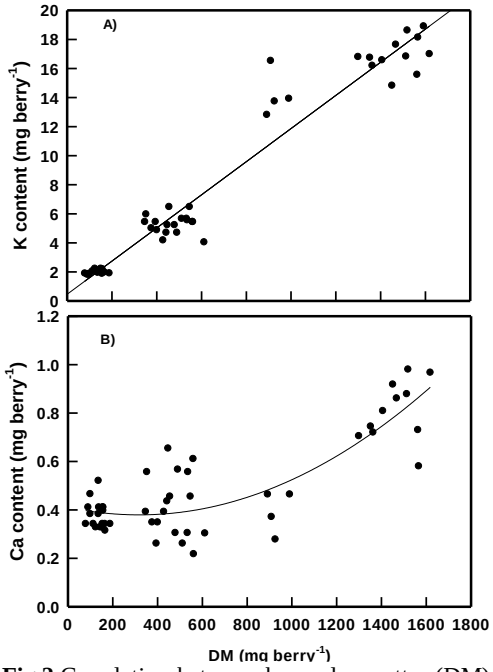

Fig.2. Correlation between berry dry matter (DM) and mineral content: A) K(r= 0.95; p< 0.0001) and B) Ca (r = 0.84; p< 0.0001). Regressions were obtained pooling all data obtained within treatments. Lines are illustrative only.

The K content in the rachis (Fig. 3) increased from an average of 23.77 mg g^{-1} DW at full bloom (0 DAA) to55.37 ± 0.99 mg g^{-1} DM at 76 DAA in S_CPPU, which was significantly 1.2-fold higher than CTRL.This increase is probably associated to a thicker rachis induced by pre-flowering CPPU application, although this aspect is still under investigation.Conversely, Ca content in rachis did not vary significantly among treatments (data not shown), although a trend toward low values under VPD_{LOW} conditions was observed. Notably, the+ 30% (as mg g^{-1} DM) increase in rachis Ca concentrationbetween 0 and 30 DAA was followed by only a + 6% increase from30 to 76 DAA. Considering the importance of Ca in post-harvest rachis quality [22], this finding highlights the importance of the temporal window between fruit-set and first stage of berry growth in optimizing the finalrachis Ca content.

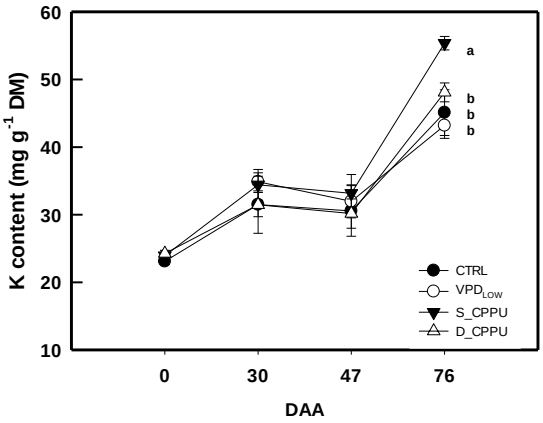

Fig. 3. RachisK content during bunch development. Values are means ± SE bars. DAA = days after anthesis; DM = dry matter. Different letters indicate significant differences (p< 0.05, Student-Newman-Keuls test).

3.3 Berry mass and ripening.

Berries from D_CPPU-treated vines exhibited a 9% lower fresh mass compared to the CTRL and a 14% reduction relative to S_CPPU-treated berries at harvest (76 DAA) (Fig. 4). Furthermore, D_CPPU-treated berries showed approximately 12% lower S (mg $berry^{-1}$) compared to CTRL and S_CPPU-treated ones (Fig.5), along with significantly higher TA (5.07 ± 0.11 g L^{-1}) compared to CTRL (4.77 ± 0.04 g L^{-1}) and S_CPPU(4.72 ± 0.07 g L^{-1}).

Berries grown under VPD_{LOW} conditions showed significantly reduced growth, with fresh and dry mass being 26% and 40% lower, than those in CTRL,respectively (Fig.4). This growth limitation also affected S accumulation, with approx. a 40% reduction in S per berry compared to CTRL (Fig. 5). However, when expressed as the S toDM ratio (data not shown), S content was unaffected by VPD manipulationshowing, for some extent, the S independencyfrom transpiration, as for K. However, further investigation is needed.

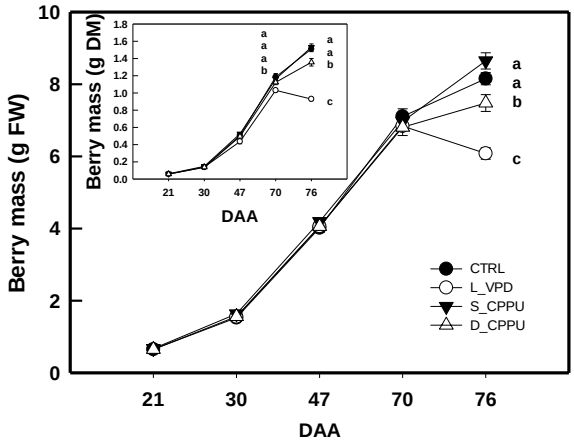

Fig.4 Berry fresh mass and dry matter (inset) evolution over berry growth. Values are means ± SE bars. DAA = days after anthesis; different letters indicate significant differences (p< 0.05, Student-Newman-Keuls test).

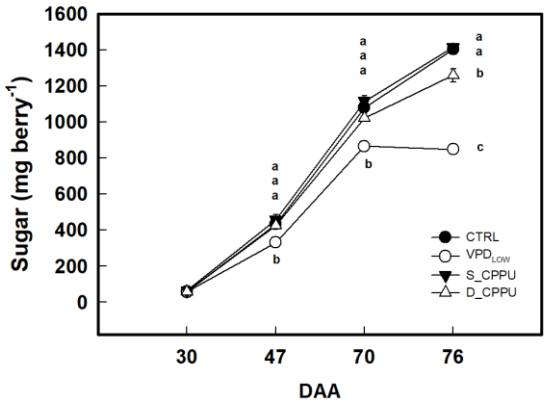

Fig.5 Sugar mass per berry evolution over berry growth. Values are means ± SE bars. DAA = days after anthesis; different letters indicate significant differences (p< 0.05, Student-Newman-Keuls test).

4. Conclusions

This study examined the role of a plant growth regulator (CPPU) and VPD in the air surrounding bunches in affecting mineral (K and Ca) contents and some fruit qualitative traits (berry mass, sugar and titratable acidity) in table grapes. Therefore, considering the CPPU rate used in the present study, VPD manipulation appears to be a more reliable strategy for increasing Ca content in berries. In table grape vineyards, specific strategies to enhance VPD around the clusters include a) increasing planting distances both within and between rows; b) adopting trellises that increase the distance between the canopy and the overhead cover; c) widening the space between covers in the inter-row and d) implementing shoot thinning and leaf plucking to reduce canopy density.

Based on the present findings, future research should focus on the role of VPD and CPPU in preventing physiological disorders in table grapes (*e.g.*, cracking, bunch stem necrosis, etc.). based on Ca and K accumulation dynamics and their balance.

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