Polyphenol Oxidase Activity, Color Changes, and Dehydration in Table Grape Rachis during Development and Storage As Affected by *N*-(2-Chloro-4-pyridyl)-*N*-phenylurea

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Flame Seedless grapes were sprayed with *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU) at 0, 2.5, and 5.0 ppm to develop rachis resistant to browning and dehydration. Rachis polyphenol oxidase (PPO) activity was determined during cluster development. Cluster components were weighed at commercial (CM), and physiological maturity (PM). PPO activity, rachis color changes (L^* and a^*), and cluster weight loss were evaluated at 0 °C for 8, 16, 32, and 56 days. CPPU-treated rachis had a decrease of 36% in PPO activity and a week delay in peak activity. At PM, dry weight of CPPU-treated rachis increased by 3 g. Postharvest rachis PPO activity declined with CPPU application, and color changes followed the same pattern for CM and PM. After 32 days of storage, L^* and a^* in lateral branches were significantly superior in CPPU treatments. Weight losses below 2.1% were significantly lowest in CPPU-treated clusters for 16 days of storage regardless of cluster maturity.

Keywords: *Polyphenol oxidase; table grapes; rachis, darkening; dehydration; CPPU*

INTRODUCTION

Table grape exports from Mexico are focused toward early markets because of their better prices. Cultural practices used to accelerate ripening cause an asynchronic maturity rate between berries and rachis. The latter is the vegetative structure supporting the berries and functions as a contention and transport element (*1*).

Gibberellic acid is the main growth regulator used to increase berry size. However, the expenses associated with its multiple applications and high dosages demand the search for new products that are active at low concentrations. Recent studies report the use on fruit trees of a compound from the substituted phenylurea family, *N*-(2-chloro-4-pyridyl)-*N*-phenylurea (CPPU) (*2*−*9*). CPPU is a synthetic cytokinin, which also delays senescence (*10*).

Grape cluster postharvest limitations require specialized management; although its respiratory rates are low, susceptibility to dehydration and enzymatic browning dictate a fast and careful management. Grape clusters are composed by berries and rachis. Berries are protected by a thick epidermis with cuticular wax depositions acting as an important barrier against dehydration. Conversely, rachis lacks such protection and, therefore, are more prone to dehydration (*11*). At harvest, ∼96% of cluster fresh weight corresponds to berries and only 4% to rachis. Nonetheless, the accelerated senescence of the latter is one of the main conditions limiting cluster postharvest life (*1*).

Rachis enzymatic browning is one of the factors involved in diminishing table grape quality. Browning has been associated with the enzyme polyphenol oxidase (EC 1.10.3.1). The darkening capacity of several cultivars varies depending on the nature and quantity of the enzyme present in the product and the available substrate as well as cluster developmental stage (*12*−*15*).

Deterioration of table grape rachis, both by dehydration and tissue browning, affects cluster functionality and appearance and represents considerable losses.

MATERIALS AND METHODS

Plant Material. Flame Seedless grapes grown near Pesqueira, Sonora, were used. A total of three blocks of 30 vines each were sprayed with either a commercial dose of CPPU (2.5 ppm), a high dose (5.0 ppm), or water as a control (0 ppm). Application was done at berry set, as described under Experimental Design and Statistical Analysis.

Field Sampling. Weekly sampling included all developmental stages from berry set to physiological maturity. Sampled clusters were placed in polyethylene bags and immediately transported to the laboratory in an icebox. Storage studies included two maturity indices, one at commercial maturity (CM) and the second, 3 weeks later, close to physiological maturity (PM).

Sampling during Storage. CM and PM clusters were stored at 0 °C for 56 days. PPO activity, rachis color changes, and cluster weight loss were evaluated at 8, 16, 32, and 56 days.

Cluster Development. Rachis and berries fresh and dry weights were followed with a precision scale, 5000 + 1 g (Sartorius, Germany). Drying was done at 60 °C for 72 h according to the method of Crisosto et al. (*16*). Berry diameter was measured using a random selection of 10 berries per cluster. Berry total soluble solids (TSS) were quantified using a 0−32 °Brix hand refractometer (Atago ATC-1E).

Enzymatic Analysis. PPO activity was quantified according to a modified method from Martínez-Téllez and Lafuente (*17*). Enzyme activity was reported as PPO·min^{-1}·g^{-1} of fresh weight.

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Table 1. Physical Properties of Structural Components from Flame Seedless Clusters at Different Cluster Maturities and CPPU Doses Applied[a]

CPPU (ppm)	cluster wt (g)	rachis wt (g) fresh	rachis wt (g) dry	berry wt (g) fresh	berry wt (g) dry	berry diameter (cm)	TSS (°Brix)
			Commerical Maturity				
0	587[b]	17[ns]	3.5[ns]	388[b]	56[ns]	1.4[b]	17.7[ns]
2.5	757[a]	18	3.5	405[a]	59	1.5[a]	16.8
5.0	731[a]	19	3.8	404[a]	53	1.5[a]	15.9
			Physiological Maturity				
0	590[b]	18[b]	3.6[b]	390[b]	76[b]	1.5[b]	21.4[ns]
2.5	776[a]	20[a]	4.6[a]	420[a]	89[a]	1.9[a]	20.3
5.0	789[a]	21[a]	4.7[a]	424[a]	93[a]	1.9[a]	19.4

[a] Mean separation by Tukey ($p \leq 0.05$), six replicates. Values labeled with different letters are statistically different. ns, not significant. Comparisons are valid only within each maturity index.

Preparation of Acetone Powder. Berries were eliminated from clusters, and the rachis tissue was homogenized with acetone at −20 °C (1:8, w/v). After filtration, the solvent was eliminated and the rachis dry weights recorded.

PPO Extraction. Phosphate buffer solution (0.05 M, pH 7.2 with 1.0 M KCl) was used. Polyvinylpolypyrrolidone (PVPP) was added to acetone powder to eliminate phenols. After constant stirring at 4 °C for 20 min, the mix was centrifuged at 12500 rpm at 4 °C for 30 min. Supernatant was used as PPO extract.

Enzymatic Assay. The reaction mixture consisted of 25 μL of enzymatic extract added to 1250 μL of 0.03 M caffeic acid as substrate (phosphate buffer, 0.05 M at pH 7.2). Reaction temperature was 30 °C. Enzymatic activity was followed for 2 min at $\lambda = 410$ nm using a UV−vis spectrophotometer model Lambda 3A (Perkin-Elmer).

Color Measurements. Color determinations were carried out with a tristimulus colorimeter Minolta model CR300. Variables determined were luminosity (L^*) and chromaticity (a^*). The evaluation of rachis central axis, lateral branches, and pedicels was done individually because they showed different browning susceptibilities.

Experimental Design and Statistical Analysis. The design used was completely randomized with six replications of 30 vines each with a factorial arrangement for treatments and sampling dates. Data analysis was done by an ANOVA, whereas mean comparison and grouping were done by Tukey ($p \leq 0.05$). SAS version 6.08 (SAS Institute Inc., 1992) was employed for data analysis.

RESULTS AND DISCUSSION

Field Study. *Cluster Development.* As shown in Table 1, significant increases in cluster weight, berry diameter, and fresh weight were found in CPPU-treated clusters at both harvest indices. Berry dry weight was significant only at PM. Thus, the larger berry weight was due to larger size and water content rather than increases in dry weight. Considering that cytokinins promote both elongation and cellular division (*18*), these results suggest that the CPPU effect on Flame Seedless berries was mostly due to cell elongation rather than division.

On the other hand, rachis fresh and dry weights of CPPU-treated clusters were significantly higher than controls only at PM. Nelson (*11*) reported that well-matured rachis are more lignified and their water content is lower than in younger ones, a condition that confers an increased resistance to deterioration during postharvest handling. According with results reported elsewhere (*4*, *6*, *8*−*10*) CPPU increased table grape cluster weights and berry size and length as well as rachis dry weight.

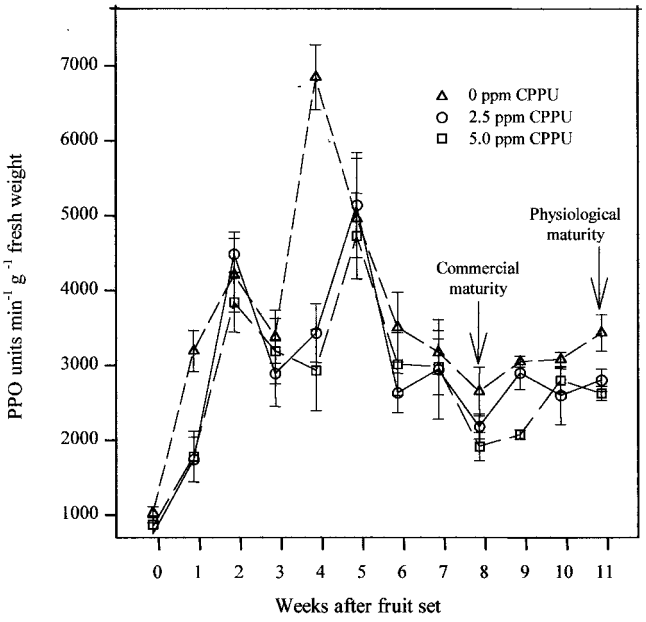

Figure 1. PPO activity pattern in Flame Seedless rachis as a function of cluster development and ripening and CPPU dose. Standard error = 153 PPO units.

It is important to point out that CPPU delayed maturity, with berries developing a less intense and uniform coloration. Nevertheless, total soluble solids (TSS) values were within the low threshold required for export (15.5 °Brix). The latter may be attributed to a dilution effect caused by an increased size. Table 1 shows berry TSS values higher at PM than at CM. This delay in cluster maturation was reported before in Thompson Seedless and Flame Seedless (*4*, *8*, *9*). However, no significant differences were found between treatments at CM and PM.

PPO Activity. A significant interaction ($p < 0.05$) between cluster development and CPPU dosage determined the pattern of PPO activity (Figure 1). No differences were found between concentrations of 2.5 and 5 ppm.

Two activity peaks were found, the first during the second week after berry set when PPO activity increased up to 4203, 4482, and 3841 PPO units for 0, 2.5, and 5 ppm of CPPU, respectively. The second increase appeared during the fourth and fifth weeks for control and treatments, respectively. In this second peak, CPPU-treated samples showed not only a decreased activity but also a week delay in reaching such peak. Activities in this second peak were 6848, 5141, and 4727 PPO units for 0, 2.5, and 5.0 ppm of CPPU, respectively.

This second activity peak coincided with véraison, an event characterized by a high phenolic synthesis, primarily for anthocyanin and tannin formation (*19*). Although rachis does not show a berry-like pigmentation, it participates in phenol metabolism; therefore, given the phenolic nature of the substrates required the increase in PPO activity might be related to this event. Sánchez-Ferrer et al. (*20*) reported a similar increase in PPO activity in Monastrell grapes.

After the maximum peak date, a steady decrease in enzyme activity was observed as clusters matured until reaching values of 2650, 2181, and 1914 PPO units for 0, 2.5, and 5.0 ppm of CPPU, respectively, during the eighth week, corresponding to CM. This same behavior as a function of fruit maturity was reported earlier by Kindron et al. (*21*) in Clairette grapes, by Sapis et al.

Table 2. Rachis PPO Activity during Storage at 0 °C As Affected by CPPU Dose and Cluster Maturity[a]

	PPO activity (PPO units min^{-1} g^{-1} fresh of wt)					
	commercial maturity			physiological maturity		
day	0 ppm of CPPU	2.5 ppm of CPPU	5.0 ppm of CPPU	0 ppm of CPPU	2.5 ppm of CPPU	5.0 ppm of CPPU
0	2650	2181	1914	3430	2621	2800
8	3076	2795	2592	3480	2669	2651
16	3530	3076	2601	3668	2788	2668
32	3568	3080	2619	3731	2944	2931
56	3760	3096	2878	3772	3161	2972

[a] Standard errors of 152 and 191 PPO units at CM and PM. ($p \leq 0.05$), averages of six replicates.

(*22*) in red and white berries of different cultivars, by Murata et al. (*13*) in apples, by Amiot et al. (*14*) in pear fruit, and by Hernández et al. (*15*) in sweet cherry. During the three weeks following CM, an increase in enzymatic activity was observed again, with significant differences of 3430, 2621, and 2800 PPO units for 0, 2.5, and 5.0 ppm of CPPU, respectively.

The postharvest effect of growth regulators on PPO activity is not yet elucidated. However, Schwimmer (*23*) reported that gibberellic acid and ethephon delay postharvest browning by diminishing phenolase biosynthesis. On the other hand, the displacement of PPO activity in CPPU-treated rachis might be related to maturity delay. Given that compounds with cytokinin-like activity delay senescence, Reynolds et al. (*10*) attributed this effect to the reduction in anthocyanin content, TSS, and pH in table grapes. The decrease in berry pigmentation may be explained by the decrease in PPO activity because color is defined by anthocyanins and phenolic compounds act as direct substrates. In agreement with Boss et al. (*24*), anthocyanin synthesis in grapevines starts 10 weeks after bloom and remains steady throughout maturity. The same authors remarked that this biosynthesis is controlled by a gene regulation strict mechanism. In this manner, CPPU could diminish berry coloration by a simple "dilution" by increasing berry diameter or by affecting the genetic regulation of anthocyanin biosynthesis. However, demonstrating the latter requires further studies.

Postharvest Study. *PPO Activity.* Rachis PPO activity during cold storage was independently evaluated in clusters harvested at CM and PM. Responses were similar, because in both cases activity depended upon a highly significant interaction between CPPU dosage and storage period. As storage proceeded, enzyme activity increased, regardless of maturity index, although rachis PPO at CM showed higher increases in activity during storage as compared to PM. At the end of the storage period, CPPU (5.0 ppm) applied at PM registered the smallest increase in activity (Table 2). There are earlier reports on the effect of growth regulators on table grapes' enzymatic browning. Pool and Weaver (*25*) reported a minor PPO activity on Thompson Seedless berries treated with chlorophenoxyacetic acid (CPA) or GA_3. In addition, they found that girdling these vines caused a synergistic effect in diminishing browning. Thus, grapevines that did not receive any of the treatments showed a significantly higher enzymatic activity. In our study, grapevines were submitted to regional common agronomic practices; therefore, their individual effects were not considered on the enzyme activity pattern. Table 2 also shows how the activity of the enzyme increased with storage period. Pool and Weaver (*25*) also indicated that internal browning, as well as PPO activity in berries of Thompson Seedless, increased with the storage period at 0 °C.

Effects on Rachis Color. Flame Seedless rachis are very susceptible to browning, according to former reports (*1*, *16*) made on subjective measurements. PPO activity and rachis color changes are objective measurements allowing quantification of such a process. Luminosity (L^*) and green color loss (a^*) were the variables that best described these events (see Figures 2 and 3).

Color change determinations on structural components of rachis (central axis, CA; lateral branches, LB; and pedicels, PD) permitted us to differentiate their susceptibilities to browning. CA was the structural component less susceptible to browning, and no statistical significance between CPPU treatments and storage period was found for L^* or for a^* (data not shown). LB showed a higher susceptibility to browning than CA but less than PD. CPPU-treated LB maintained higher L^* and a^* values than controls during 32 days at 0 °C. The browning process on PD was very accelerated, and only after 56 days in storage CPPU (5.0 ppm) kept luminosity (L^*) at its highest value, as compared with all treatments at both harvest indices. The high susceptibility of PD to deterioration may be attributed to a major amount of enzyme, substrate, or both, although oxygen exposure may play a more significant role, explained by gas exchange through stomata and lenticels, by the falling off of berries by dehydration or just by simple splitting of the cluster (*11*).

Earlier research (*16*) reported differences in browning development along rachis structure in Flame Seedless grapes. It occurred first on pedicels, followed by LB and finally on CA. On the basis of a subjective scale used in such study, at the end of the 84 days of storage at 0 °C, the whole rachis structure was completely brown. Relating browning to rachis dehydration, the evidence suggests that, with the same water loss, the rachis of this cultivar showed severe symptoms of browning, whereas Thompson Seedless was less affected. This suggests that, besides dehydration, there are other factors involved such as genotype. As noted by Nelson (*11*), rachis browning appears as a symptom second to dehydration, although he considers the browning rate to be a function of temperature, more than dehydration itself, which emphasizes the enzymatic nature of this process. The browning of the rachis increased significantly with storage period for the two maturity indices, the three applied treatments, and structural components studied.

Effects on Weight Loss. Considering the low respiration rate and the nonclimateric nature of grapes, weight loss is defined primarily by dehydration (*1*), which is the most important physiological disorder affecting the shelf life of table grapes. As rachis mature, they become more lignified and their water content is lowered. Rachis ripening is characterized by increasing lignin and suberin and decreasing water content, which makes them less susceptible to dehydration. It also increases

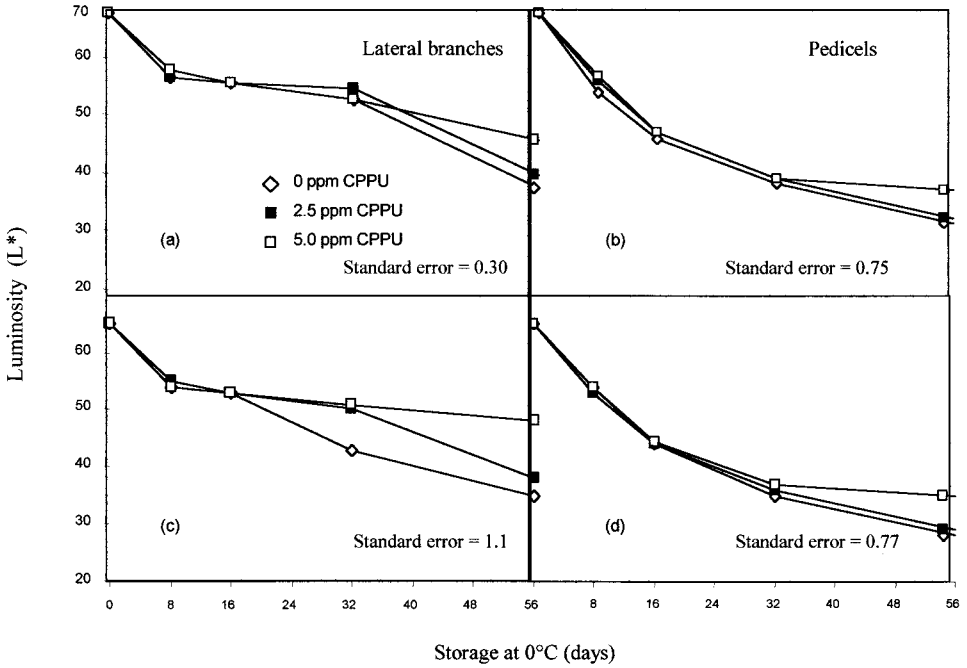

Figure 2. Browning development on structural components of Flame Seedless rachis during storage at 0 °C as a function of CPPU dose and commercial (a, b) or physiological (c, d) maturity index.

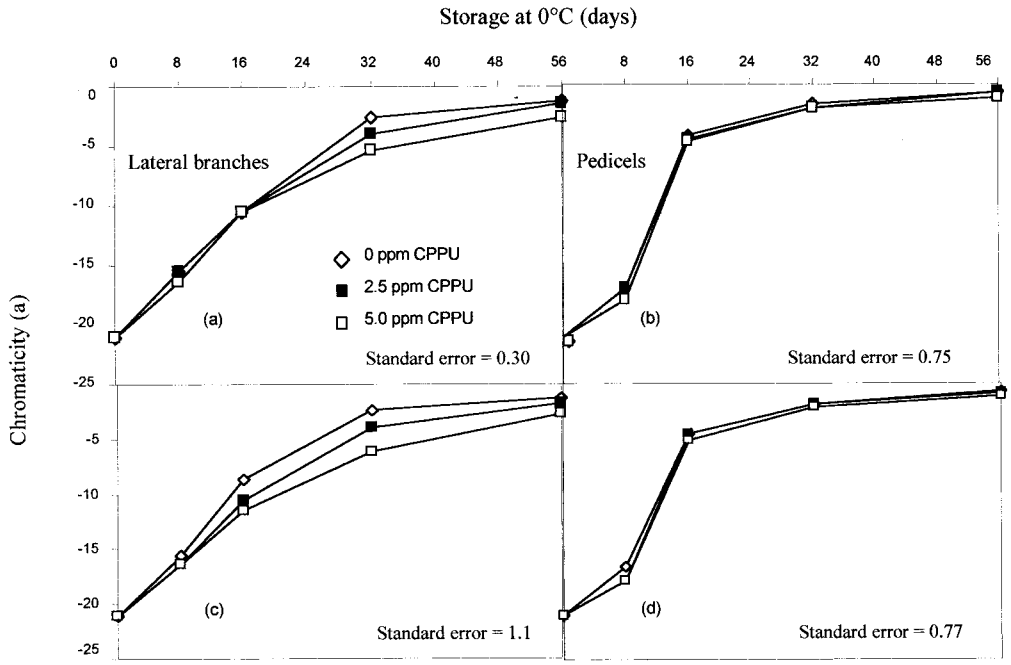

Figure 3. Loss of green color (chromaticity) of structural components of Flame Seedless rachis during storage at 0 °C as a function of CPPU dose and commercial (a, b) or physiological (c, d) maturity index.

the postharvest life of table grape (*11*). In this research CPPU doses were statistically significant only when clusters were harvested at CM (Figure 4), whereas storage period was significant for clusters harvested at both CM and PM (Table 3). According to Crisosto et al. (*16*) weight losses of 2.1% negatively affect the appearance of Flame Seedless clusters. At CM such a threshold was reached by untreated controls after 16 days at 0 °C, whereas CPPU-treated clusters were below that level (Figure 4). This effect was not significant at PM. Conversely, Reynolds et al. (*10*) did not report a significant effect of CPPU on table grape weight loss during storage at 2 °C for 90 days. Nonetheless, in such a study fruit maturity degree was not considered, which in this study was found to be a major factor in the behavior of the treatments employed.

Considering both the physiological and biochemical approaches of this work, the information presented here contributes to the description of the postharvest behavior of Flame Seedless. Our results indicate that variables such as CPPU application and cluster PM may delay decay symptoms during storage. According to this, CPPU-treated clusters harvested at CM have little chance to compete in early markets, but conversely CPPU-treated clusters harvested at PM may have important advantages because they could tolerate a longer storage period with a significantly diminished decay by browning and dehydration.

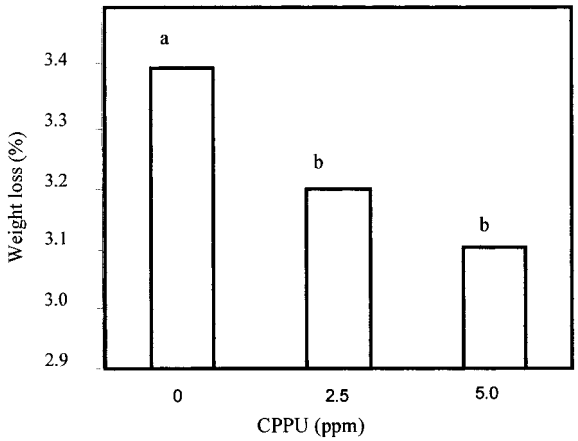

Figure 4. Effect of CPPU dose on Flame Seedless weight loss at commercial maturity as an average of 56 days of storage at 0 °C. Means separation performed by Tukey ($p \leq 0.05$), six replicates.

Table 3. Effect of Cluster Maturity on the Weight Loss of Flame Seedless Table Grapes during Storage at 0 °C[a]

	wt loss (%)	
day	commercial maturity	physiological maturity
8	1.2[d]	1.4[d]
16	2.0[c]	2.2[c]
32	3.6[b]	3.5[b]
56	6.2[a]	5.8[a]

[a] Average separation by Tukey ($p \leq 0.05$), six replicates. Comparisons are valid within each maturity stage.

One of the reasons for introducing the use of CPPU in viticulture is to substitute or at least lower the application of gibberellic acid (GA_3), because of expenses and labor involved and undesirable side effects such as decreased bud differentiation (*7*). It is important to remark that the high physiological activity of CPPU permitted the use of doses of 2.5 ppm with a similar effect to doses of 5.0 ppm. Dookozlian et al. (*3*) reported a CPPU application of 20 g/ha with an effect similar to two GA_3 applications of 200 g/ha.

No reports on deleterious effects of CPPU have been published. Dookozlian et al. (*3*) did not find a decrease on berry set a year after application; however, they recommended further investigations into this matter be made.

The quality diminishment on Flame Seedless by rachis darkening and weight loss during storage may be lessened by the combined effect of a CPPU application and harvesting at a more advanced maturity. According to our findings CPPU is a good alternative on the basis of its physiological and biochemical effects. However, ripening delay may represent a limitation for its use on grapes intended for the early-season export market, whereas a more mature rachis is an advantage for a crop requiring a more prolonged storage life.

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