TREATMENT OF GRAPEVINES WITH PROHEXADIONE CALCIUM AS A GROWTH REGULATOR. THE INFLUENCE ON PRODUCTION, WINEMAKING AND SENSORY CHARACTERISTICS OF WINES

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Abstract

Aims: The overall objective of this study was to investigate the effect of the growth regulator Prohexadione Calcium (ProCa) on the production of Tempranillo vines in La Rioja, the vinification and the sensory profile of wines obtained from treated grapes.

Methods and results: Prohexadione calcium was applied to Tempranillo vines from Rioja Baja at preblooming in years 2004 and 2005. A number of rows were not treated and they were used as controls. Vinification was carried out at an experimental winery. Oenological parameters were determined. A sensory analysis was performed on the obtained wines. The results showed that crop yield was reduced in treated vines. As regards the fermentation process, slight differences were observed in the case of the treated Tempranillo grapes in comparison to the control. The sensory analysis revealed different organoleptic characteristics in the wines obtained from treated grapes in comparison to those produced from the untreated ones.

Conclusion: The growth regulator Prohexadione calcium can be used as an alternative to bunch thinning. Improved sanitary conditions of grapes are observed. The treatment at preblooming produces a reduction in berry size and weight. An improvement in sensory characteristics and wine quality is observed, as a consequence of reduced production.

Significance and impact of study: ProCa as a growth regulator may be an option for a quality vitiviniculture. No previous studies have been published on applications of ProCa in grapevines in either Europe or in cv. Tempranillo. Additionally, studies with other varieties have not demonstrated sensory improvements in wines obtained from treated vines.

Key words: prohexadione calcium, grapes, Tempranillo *cv*., winemaking, sensory analysis

Résumé

Objectifs : L'objectif de cette étude a été de rechercher les effets de la prohexadione-calcium en tant que régulateur de la croissance sur la production de vins Tempranillo de La Rioja et son influence sur la vinification et les caractéristiques sensorielles des vins obtenus.

Méthode et résultats : On a appliqué de la prohexadione-calcium sur des vignes de Tempranillo en Rioja Baja à la préfloraison pendant deux années consécutives, 2004 et 2005. Quelques rangs n'ont pas été traités en tant que témoins. La vinification a eu lieu dans une cave expérimentale. On a déterminé les paramètres œnologiques. On a fait une analyse sensorielle des vins obtenus. Les résultats ont montré que le rendement des vins traités était inférieur à celui des non traités. Pendant la fermentation, des légères différences ont été notées pour les raisins du cépage Tempranillo traités, par rapport aux raisins non traités. L'analyse sensorielle a montré des caractéristiques organoleptiques différentes entre les vins issus des raisins traités et ceux issus des non traités.

Conclusions : La prohexadione-calcium peut être utilisée comme régulateur de la croissance à la place de l'éclaircissage. On a observé une amélioration des conditions sanitaires des raisins. Le traitement à la préfloraison diminue la taille et la forme des grappes. Les caractéristiques sensorielles et la qualité des vins sont améliorées à la suite de la réduction de la production.

Importance et intérêt de l'étude : La prohexadione-calcium comme régulateur de la croissance peut être un choix pour une viticulture de qualité. Il n`existe pas de publications sur l'application de la prohexadione-calcium sur les vignes en Europe ou sur le cépage Tempranillo. De plus, les études sur d'autres cépages n'ont pas démontré une amélioration des caractéristiques sensorielles des vins issus des raisins traités.

Mots clés : prohexadione-calcium, grappes, Tempranillo, vinification, analyse sensorielle

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INTRODUCTION

The production of good quality grapes requires the control of several and multiple parameters in the vineyard. Therefore, control of production yield is becoming increasingly more important for a quality vitiviniculture. At present, it seems undeniable that high crop yields give lower quality grapes than those obtained from low crop yields. Nowadays, bunch thinning is a very widely-used technique to reduce production in vigorous vines or in vines that have been subject to favorable weather conditions. Bunch thinning produces an increase in Color Intensity (CI), Total Phenolic Index (TPI) and anthocyanins (García Escudero *et al.*, 2004).

An alternative method for controlling production is the use of plant growth regulators. Prohexadione calcium (Pro-Ca) (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate) is a gibberelin biosynthesis inhibitor with limited persistence (Owens and Stover, 1999). ProCa operates by blocking two oxoglutarate-dependant dioxygenases, which catalyse the later steps in the biosynthetic sequence. The 3-hydroxylation of GA20 (inactive) into GA1 (biologically active) is especially inhibited, resulting in a reduction of longitudinal shoot growth in plants (Evans *et al.*, 1999; Graebe, 1987). ProCa is easily applied by spraying and constitutes no apparent risk for consumers or the environment (Rademacher and Kober, 2003). ProCa has been reported to be absorbed completely within eight hours and to degrade in plants with a half-life of a few weeks and in soil with a half-life of less than one week, without producing toxic metabolites (Ilias and Rajapakse, 2005). ProCa has been registered and used on apples as Apogee® (27.5 % ProCa) in North America and as Regalis® (10 % ProCa) in Europe (Miller and Tworkoski, 2003) by BASF. ProCa has been applied in fruit trees with satisfactory results (Basak and Rademacher, 2000; Bazzi *et al.*, 2003; Gosch *et al.*, 2003; Smit *et al.*, 2005).

Several studies have revealed that the application of Prohexadione calcium also reduces the incidence and severity of fire blight caused by the bacterium *Erwinia amylovora* (Fernando and Jones, 1999; Momol *et al.*, 1999), although the compound does not have bactericidal activity. The first studies of ProCa on grapevines were performed by the researchers Lo Giudice *et al.* (2003, 2004). These authors studied the impact of the application of ProCa in the vigorous *Vitis vinifera* grapevines, especially in *cv.* Cabernet Franc, Cabernet-Sauvignon, Chardonnay and Seyval.

Significant compounds for the quality of red wines such as phenols (anthocyanins, flavonols, etc) are found in grape skins. These compounds are responsible for color, astringency, bitterness and body of wines (Ribéreau-Gayon *et al.*, 2002). Prohexadione calcium produces a decrease in berry size. This fact leads to an increase in surface-to-volume ratio, theoretically increasing the proportion of these flavor and aroma precursors in the must and wine and potentially increasing wine quality (Lo Giudice *et al.*, 2004). Moreover, a reduction in berry size and the production of less compact bunches may diminish the incidence and severity of *Botrytis cinerea* and other bunch rots, especially in humid environments (Vail and Marois, 1991).

In *cv.* Cabernet-Sauvignon, the reduction of berry weight is correlated with an increase in color intensity (absorbance at 420 and 520 nm), total anthocyanins and total phenols. The observed effects on grape composition were generally positive, but the effect on the quality and the organoleptic characteristics of the final wine are still unknown (Lo Giudice *et al.*, 2004).

The aim of this study is to examine the effect on the production, the fermentative process, the oenological parameters and the sensory characteristics of the wine obtained from Tempranillo grapevines (major variety in DOCa Rioja) treated with Prohexadione calcium.

MATERIALS AND METHODS

1. Field trials

Prohexadione calcium was applied during the years 2004 and 2005 in the same plot in Rioja Alta. Tempranillo vines were 13 years old and presented constant conditions of vegetative growth and development, under similar conditions of fertilization and irrigation. Vines were trained and spur-pruned on a wire trellis system for support and the canopy was vertically shoot-positioned. The row and vine spacing was of 2.5 and 1.2 m, respectively. Shoot density was adjusted to 12 shoots per vine.

ProCa was applied as Regalis®, 10% ProCa, (BASF, Germany) at a dose of 3 kg ha^{-1} at preblooming (BBCH 57) (12 June 2004 and 11 June 2005). Treatments were applied to both sides of the canopy, wetting the entire shoots. The product was sprayed with a HARDI® atomizator (Denmark). Applications were carried out when there was no rain predicted for at least 24 hours. A number of rows were not treated and were used as a control plot.

2. Vinification process

Mature grapes were harvested on 11 October 2004 and 7 October 2005. Four experimental vinifications for control and treated grapes were carried out in the experimental winery of the University of La Rioja, two for the control crop and two for treated grapes. All wines were obtained using the traditional winemaking method. Grapes were destemmed, crushed and fermented into

100 L stainless steel tanks; 100 mg kg^{-1} of potassium metabisulfite was added. Crushed grapes were inoculated with commercial yeast strain VRB *Sacharomyces cerevisiae* (30 g hL^{-1}) from Lallemand (Australia). The prefermentation process lasted 24 hours.

The cap was punched down during the maceration-fermentation process (pigeage) and pumping-over extractions were also performed during fermentation (remontage). The wines were drawn off and then inoculated with commercial lactic bacteria strain Alpha *Oenoccus oeni* (1 g hL^{-1}) from Lallemand (Australia) in 30 L stainless steel tanks. After malolactic fermentation, wines were racked and 90 mg L^{-1} of potassium metabilsulfite were added. Wines were clarified by settling at 4 ºC for a week and then bottled. The wines were stored at 4 ºC in order to prevent spoilage.

3. Determination of usual oenological parameters

In order to evaluate berry size, 100 berries were placed in trays with different diameter holes (18, 16, 14, 12 and 10 mm). Thus, berries passed through from higher diameter trays to lower ones. Berries were crushed. Then, solid was separated and weighed and the liquid volume was measured. Conventional oenological parameters (density, ethanol concentration, pH, reducing sugars, titratable and volatile acidities, total and free SO_2) were determined in accordance with official OIV practices. L-malic was determined by an enzymatic method in accordance with official AOAC analysis methods. Color Intensity (CI) was calculated as the sum of absorbances at 420, 520, and 620 nm, and the hue of the wine was calculated as the ratio of absorbance at 420 nm and 520 nm. Total Polyphenol Index (TPI) was estimated as absorbance at 280 nm. Tannins were determined using the method described by Ribéreau-Gayon and Stonestreet (1996). Amount of laccase was determined using the method described by Dubourdieu *et al.* (1984). All determinations were carried out in triplicate.

4. Sensory analysis

In February 2005 and 2006, sensory analyses were performed by a panel formed by experts from the Asociación de Enólogos de La Rioja and Enology graduates from the University of La Rioja. The panel had 25 and 55 members in 2004 and 2005, respectively. Two sessions in different days were carried out. In the first session, the aim of the sensory evaluation was to determine whether the wine obtained from treated vines was significantly different from the control. Since this is a classical application of a discrimination test, a duo-trio test was chosen (Meilgard *et al.*, 2007). Three samples were presented to the experts, one of which was identified as the reference. One of the other two was identical to the reference. The panelists were asked to state which product most closely resembled the reference. Since the question was « Which sample matched the reference sample? » the one-tailed binomial test was used. Panelists considered visual, odor, taste and mouth-feel perceptions.

In the second session, a descriptive sensory analysis was carried out. Panelists were provided with evaluation sheets containing a list of different terms for appearance, aroma, attributes in mouth, and aftertaste. The panelists chose the terms characterizing the wines. A chi-square (χ^2) analysis performed on the average citation frequency of each term in the two samples was carried out in order to identify statistical differences. The citation frequency method was performed as a successful tool for conventional descriptive analysis (Campo *et al.*, 2008).

RESULTS

1. Assay in 2004

a. Grapes

Table 1 shows some yield parameters. The average reduction in the weight of berries from treated vines indicated a reduction in the crop yield of 25%. The

Table 1 - Several yield components

Samples	Average berry weight (g)	Berry size*				% Solid / liquid (m /v)
		10-12 mm	12-14 mm	14-16 mm	16-18 mm	
Control 2004	2.38 ± 0.06^a	8.1 ± 0.3^a	54.3 ± 7.2^a	31.8 ± 7.5^a	5.7 ± 1.1^a	42.3 ± 2.4^a
ProCa 2004	1.78 ± 0.03^b	24.2 ± 2.3^b	57.5 ± 4.6^a	15.1 ± 2.7^b	3.2 ± 1.5^b	57.1 ± 4.3^b
Control 2005	1.57 ± 0.02^a	0.3 ± 0.6^a	14.7 ± 1.5^a	75.4 ± 1.1^a	9.6 ± 1.1^a	50.4 ± 3.7^a
ProCa 2005	1.41 ± 0.02^b	5.7 ± 1.5^b	17.7 ± 0.6^b	68.0 ± 1.7^b	8.5 ± 0.6^a	55.6 ± 2.5^a

* % of berries for each diameter; a,b Different letters in same column and year means significant differences at 5 % level.

presence of « shoulders » in bunches was reduced by 90%. The size of individual berries from treated vines were different from those of control vines. Clusters from treated vines had a higher number of small berries (10-14 mm diameter) and a lower number of berries with 14-18 mm than control grapes. Solid /liquid relation was higher in treated grapes.

b. Alcoholic and malolactic fermentations

Averages of oenological parameters for control and « ProCa » musts and wines are shown in table 2. Must from treated grapes gave higher values for Brix degree, pH and titratable acidity. Wine produced from these grapes had a higher alcoholic degree. CI, TPI, hue, titratable acidity, and tannins were higher in wines obtained from treated grapes. Laccase values were notably low due to good sanitary conditions.

With regard to the fermentative process, differences between control and « ProCa » were observed. The evolution of sugars (%) is shown in figure 1. The fermentation of treated grapes was quicker and « dryness » (reducing sugars < 2 g L^{-1}) was achieved earlier. Malolactic fermentation had a duration of 23 days for both control and « ProCa » wines.

c. Sensory analysis

The Duo-Trio test performed to check whether wines produced were different showed that wines did not differ at a significance level of 5% because number of correct responses was 17 and the critical number for significance is 18. These results suggested a certain differentiation that was not significant enough at this level. Actually, *p* was 0.10 .

The results for the descriptive analysis are shown in figure 2. The spider webs represent the citation frequency in a maximum of 25.

For appearance or visual perceptions, depth was different ($p<0.05$) for « ProCa » wine because it was considered higher than the control. Color was also different in « ProCa » wine. This wine was considered with more purple, biguerrau cherry and blackish colors ($p<0.05$) than the control one, while the shades found in the control wine were cherry red and deep red. Some differences were observed in terms of aroma and odor perceptions. Intensity and aromas were similar for both control and « ProCa » wines. Fruity aromas were present in both wines. ProCa wine also had spicy aromas and were globally considered more complex ($p<0.05$) than the control wine. Tastes were more balanced ($p<0.05$) in wine from treated grapes. Mouthfeel was mild and light ($p<0.05$) for the control wine. In contrast, « ProCa wine » presented higher astringency and stickiness. Aftertaste was very fruity for both control and « ProCa » wines; the latter also presented

Table 2 - Conventional oenological parameters at the beginning and end of fermentation in 2004 and 2005

Must

Samples	º Brix	pH	Titratable Acidity[c]	*Laccase* (nmol mL^{-1})
Control (2004)	20.6 ± 0.2[a]	3.49 ± 0.01[a]	4.35 ± 0.02[a]	0.231 ± 0.014[a]
ProCa (2004)	24.4 ± 0.1[b]	3.78 ± 0.01[b]	5.34 ± 0.01[b]	0.046 ± 0.010[b]
Control (2005)	22.2 ± 0.1[a]	3.55 ± 0.02[a]	4.65 ± 0.01[a]	nd
ProCa (2005)	20.2 ± 0.1[b]	3.49 ± 0.01[b]	4.70 ± 0.02[b]	nd

Wine

Samples	Ethanol (v/v)	pH	Titratable Acidity[c]	Volatile Acidity[d]
Control (2004)	13.0 ± 0.4[a]	3.68 ± 0.01[a]	4.12 ± 0.11[a]	0.21 ± 0.01[a]
ProCa (2004)	13.8 ± 0.3[b]	4.04 ± 0.04[b]	4.72 ± 0.09[b]	0.21 ± 0.01[a]
Control (2005)	13.4 ± 0.1[a]	3.72 ± 0.01[a]	4.78 ± 0.18[a]	0.37 ± 0.01[a]
ProCa (2005)	13.6 ± 0.1[a]	3.68 ± 0.04[a]	4.42 ± 0.47[a]	0.27 ± 0.07[b]

Wine

Samples	TPI[e]	CI[f]	Tannins	Hue
Control (2004)	44.3 ± 3.3[a]	12.7 ± 0.9[a]	3.10 ± 0.16[a]	0.42 ± 0.01[a]
ProCa (2004)	61.6 ± 0.6[b]	14.6 ± 0.4[b]	4.31 ± 0.24[b]	0.54 ± 0.02[b]
Control (2005)	39.5 ± 1.3[a]	12.7 ± 0.2[a]	2.76 ± 0.09[a]	0.59 ± 0.03[a]
ProCa (2005)	44.5 ± 2.0[b]	13.2 ± 0.2[b]	3.12 ± 0.11[b]	0.55 ± 0.04[a]

[a,b] Different letters in same column and year means significant differences at 5 % level.
[c] expressed as g tartaric acid per litre
[d] expressed as g acetic acid per litre
[e] Total Polyphenol Index (absorbance at 280 nm)
[f] Color Intensity (sum of aborbances at 420, 520 and 620 nm)
nd = non detected

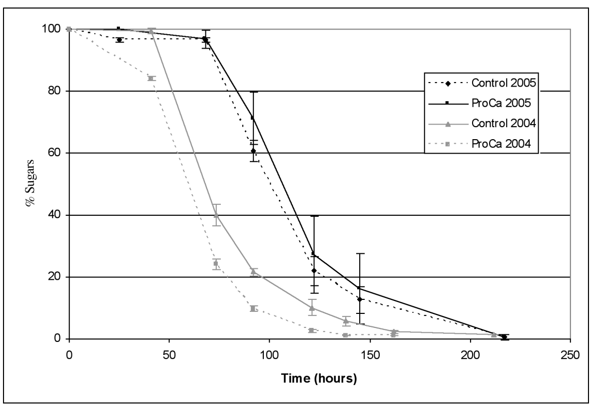

Figure 1 - Evolution of the % of sugar during fermentation in each sample

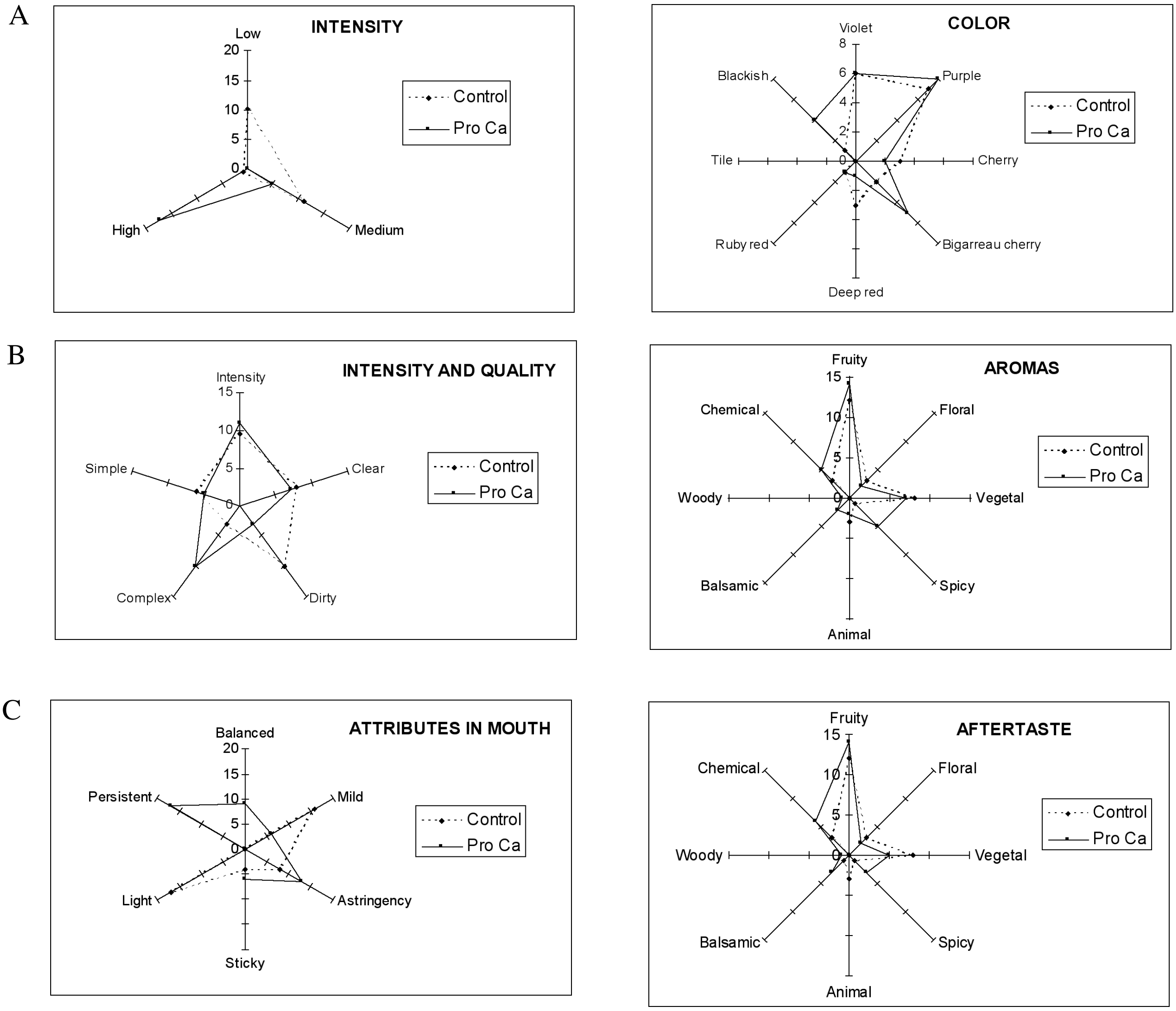

Figure 2 - Spiderweb diagrams for sensory analysis in 2004.
A) Appearance; B) Odor; C) Attributes in mouth and aftertaste

spicy and balsamic attributes. « ProCa » wine had more persistence than the control (p<0.05).

2. Assay in 2005

a. Grapes

In this year, the application of « ProCa » had a less marked effect than in 2004. The maturation and vegetative growth of treated vines were similar to the control vines. For treated vines, the presence of shoulders was reduced by 30%, and yield production decreased by 10%. There was a higher number of small berries in treated grapes, with 10-12 mm and 12-14 mm diameters (see table 1).

b. Alcoholic and malolactic fermentation

Usual oenological parameters for control and « ProCa » wines are shown in table 2. In the winemaking of treated grapes, Brix degree was slightly lower than in controls. There were no differences in pH and titratable acidity for both control and « ProCa » musts. Wine from treated grapes also gave lower values for titratable acidity and pH. Significant differences in ethanol were not observed in the control and « ProCa » wines. TPI, tannins, and CI were higher for wines obtained from treated grapes. Hue was similar in both control and « ProCa » wine. Good sanitary conditions were observed as shown by the absence of laccase.

Figure 1 shows the evolution of sugar content (%) in the fermentative process of control and « ProCa » grapes. The kinetics were similar for both samples. No significant differences in the evolution of fermentation were identified for both samples. Both fermentations had the same duration, with reducing sugars < 2 g L^{-1}. Malolactic fermentation lasted 20 days for both wines. Malic acid was below 0.1 g L^{-1}.

c. Sensory analysis

The Duo-Trio test performed to check whether wines produced were different showed that wines did not differ at a significance level of 5%. Sensory panelist could not distinguish between the wines because correct responses were 31 and the critical number for significance is 34.

In the subsequent descriptive analysis, certain differences were observed. Spider webs represent the citation frequency in a maximum of 55 (Figure 3). As regards appearance, differences in depth or intensity and color were shown. « ProCa » wine presented a higher intensity than the control ($p<0.05$); control wine showed a higher deep red ($p<0.05$) and violet ($p<0.05$) colors and purple hues were also observed in « ProCa » wine. As regards odor perceptions, wine from treated grapes had a higher intensity ($p<0.05$) with a clear and complex quality ($p<0.05$). Fruity aromas were observed in both control and « ProCa » wines. Tastes were more balanced in wine obtained from treated grapes than wine obtained from untreated grapes ($p<0.05$). For control wine, mouthfeel was perceived as mild and light in contrast to the higher stickiness of « ProCa » wine. Aftertaste was very fruity for both control and « ProCa » wines. These wines also had spicy and balsamic attributes. Wine from grapes treated with ProCa was more persistent than the control wine.

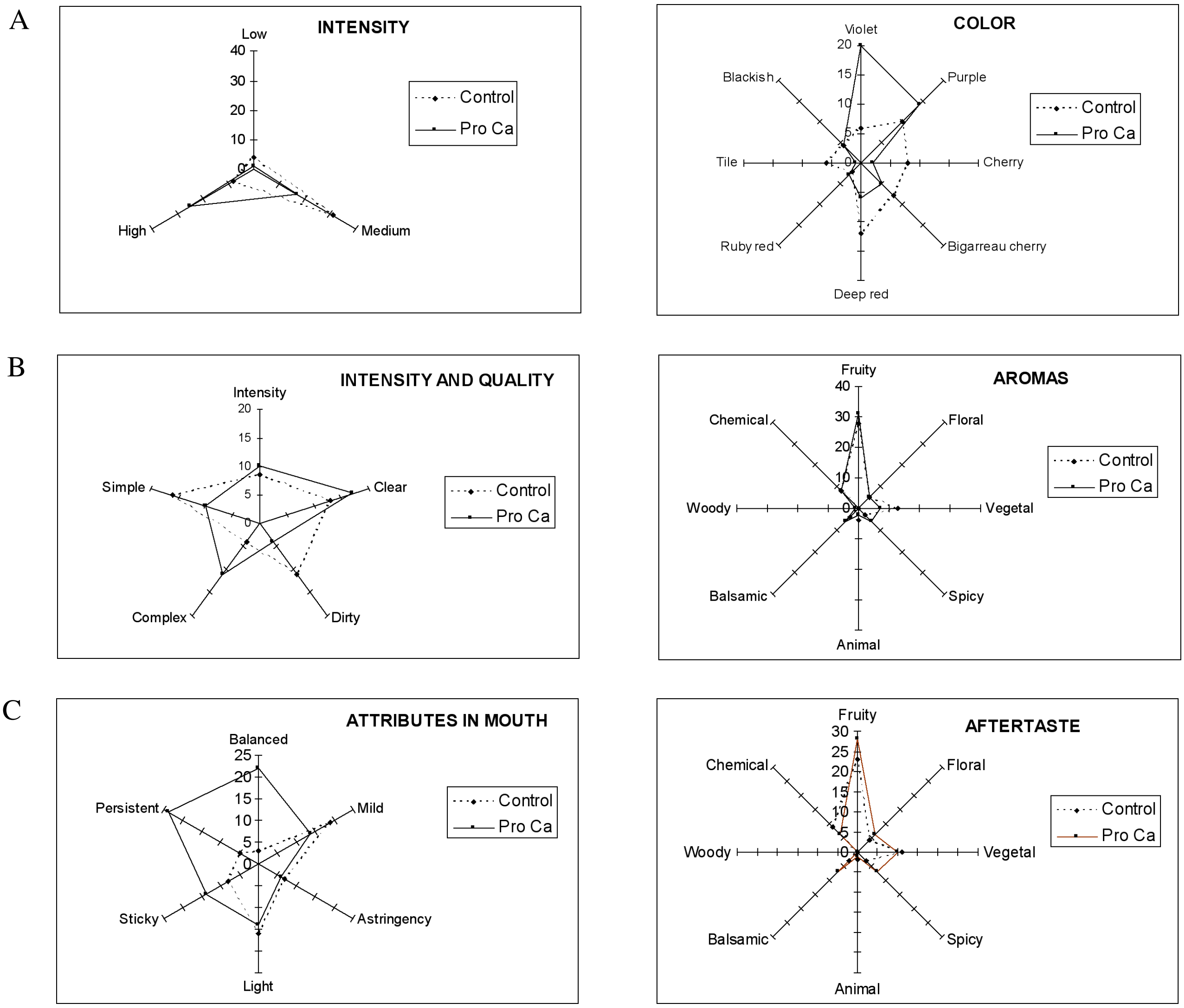

Figure 3 - Spiderweb diagrams for sensory analysis in 2005.
A) Appearance; B) Odor; C) Attributes in mouth and aftertaste

DISCUSSION

An improvement in the sanitary conditions of grapes from vines treated with ProCa was observed as revealed by the laccase values. This result confirmed the theory of Vail and Marois (1991). The smaller size of berries and less compact clusters with reduced shoulders number diminish the incidence of *Botrytis cinerea*. Prohexadione calcium produced a reduction in crop yield. This effect was the result of a reduction in fruit set. As Lo Giudice (2004) described, Prohexadione calcium has the potential to reduce fruit set, particularly at preblooming. The prebloom application of ProCa caused browning and abscission of flowers. ProCa inhibits the conversion of aminocyclopropanecarboxylic acid into ethylene, and its application reduces ethylene-induced senescence in several plant systems (Rademacher *et al.*, 1992).

Differences between treated and control grapes were marked in 2004, although in 2005, a reduction in yield and shoulders number was also observed, and excellent sanitary conditions were obtained. The reason why this treatment had a less marked effect is uncertain. Vines could not be sensitive to the product due to the climatological and environmental conditions.

Data at harvest for oenological parameters revealed complete and early ripening for grapes treated with ProCa in 2004. These grapes were significantly different from the controls. Fermentation was faster in treated grapes. However, the application of prohexadione calcium did not have a marked effect on grapes in 2005, with slight differences in maturation. Therefore, there was no difference in alcoholic fermentation of treated and control grapes. Malolactic fermentation was not affected in either years.

There was a important increase in CI, TPI, and tannins in wines obtained from treated grapes with respect to those obtained from control grapes. This effect was observed in both years. The reduction of yield and berry size, and the increase in solid/liquid relation led to a higher concentration of phenolic compounds in Tempranillo grapes, as described by LoGiudice (2004) in other varieties. The application of prohexadione calcium produced better phenolic ripening during the two years.

Duo-trio tests showed that there were no statistical differences between control and « ProCa » wines in either 2004 or 2005. However, descriptive analysis revealed differences in color, aromas and mouthfeel. Due to the reduction in yield and berry size in treated vines, color-related polyphenols were concentrated so intensity was classified higher than in the control wine. Moreover, the application of Prohexadione calcium caused a higher CI (with higher absorbance at 420, 520 and 620 nm), and provided violet and purple colors in « ProCa » wines. These facts could be explained by a higher anthocyanin concentration or an increase in copigmentation (Gutiérrez *et al.*, 2005). Apart from the obvious fact that an increase in surface-to-volume ratio results in an increase in anthocyanins and flavonoids (Pérez-Magariño *et al.*, 2004), there is a second hypothesis. It has been shown that ProCa directly affects the biosynthetic pathway of anthocyanins and other flavonoids. This fact is possibly due to the role of 2-oxoglutarate-dependent dioxygenases (Awad and de Jagger, 2002; Heller and Forkmann, 1994; Rademacher *et al.*, 2003; Roemmelt *et al.*, 2003).

Flavors and aroma precursors also increased as a result of smaller berry size. Sensory analysis showed a high aromatic intensity for « ProCa » wine in 2005. Moreover, the aroma of « ProCa » wine was globally classified as more complex and clearer. Spicy aromas (licorice, clave, black pepper) were also observed in wine from treated vines. Tempranillo grapes develop intense varietal aroma and flavor nuances reminiscent of licorice, truffle (Jackson, 2002) which were clearly apparent in wines obtained from treated vines.

The astringency, body and stickiness achieved in « ProCa » wines were due to a higher content of organic acids and flavonoids, such us flavonols, flavan-3-ols and their polymers (procyanidins and condensed tannins), which were extracted from the seeds and skins of grapes. This finding correlated with a high TPI and tannins values found in these wines. High persistence in wines from treated vines was also a consequence of the concentration of flavourants and tastants produced by a reduction in small berry size.

Spicy aftertaste was also reported in « ProCa » wines. This fact confirms the odor profile obtained for those wines.

CONCLUSIONS

The preblooming application of Prohexadione calcium produced a reduction in yield, clusters and berry size in *cv.* Tempranillo. Moreover, better and earlier grape ripening was achieved with a high Brix degree. This effect was less marked in 2005.

During fermentation, there was a higher extraction of phenolic compounds as a result of an increase in surface-to-volume ratio. An increase in TPI, tannins and CI was observed in both years. Fermentative processes (alcoholic and malolactic fermentations) were not affected. The amount of laccase determined in 2004 was significantly lower in grapes from vines treated with ProCa.

The Duo-trio tests showed that significant differences were not observed at a level of 5 %, but results suggested a certain differentiation. The application of Prohexadione calcium modified the sensory characteristics of wines obtained from treated grapevines. Typical sensory attributes in Tempranillo wines were enhanced.

The treatment of grapevines with Prohexadione calcium can be used as a new tool for controlling production and therefore improving wine quality for *cv.* Tempranillo.

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