Research Article

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# Applied $GA_5$, $GA_4$, and $GA_{4/7}$ increase berry number per bunch, yield, and grape quality for winemaking in *Vitis vinifera* L. cv. Malbec

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## Abstract

**BACKGROUND: The gibberellins (GAs) $GA_5$ (inhibitor of $GA_3$-oxidase), $GA_4$ (biologically active), $GA_{4/7}$ (commercially available mixture of $Ga_4$ and $GA_7$) prohexadione-calcium (ProCa, inhibitor of dioxygenases that render GAs bioactive, negative control), and $GA_3$ (positive control) were applied to bunches of *Vitis vinifera* cv. Malbec. Different techniques, doses, and timings were used in a 3-year field experiment. In year 1, $GA_5$, ProCa, and $GA_3$ were applied at 35, 20, and 0 days before veraison (DBV) by dipping bunches three times. In year 2, single applications of $GA_5$ and $GA_3$, also by immersion, were tested at 60, 45, and 30 DBV. In year 3, applications at 60 and 30 DBV of $GA_5$, $GA_4$, and a mixture of $GA_{4/7}$ were evaluated by dipping or spraying the bunches.**

**RESULTS: Vegetative growth, berry weight, and sugar content were unaffected by treatments. ProCa did not affect the yield with respect to water control, although it reduced the levels of phenolics in berry skins, an undesirable effect for winemaking. $GA_5$, in the dose range 5–50 mg $L^{-1}$, raised berry numbers, thereby augmenting bunch weight and skin phenolics at harvest, so increasing berry quality for winemaking. $GA_4$ and $GA_{4/7}$ produced similar benefits to $GA_5$, with similar doses.**

**CONCLUSION: The applications of $GA_5$, $GA_4$, and $GA_{4/7}$ to developing grape berry bunches, in a range of concentrations and by dipping or spraying, increased berry numbers per bunch at harvest. The method can be used as a viticultural practice to improve the production and quality of wine grapes.**

*Supporting information may be found in the online version of this article.*

**Keywords:** GAs; gibberellic acid; grapevine; Malbec; prohexadione-Ca; *Vitis vinifera* L.

## ABBREVIATIONS

| | |
|---|---|
| DBV | days before veraison |
| FW | dry weight |
| DW | fresh weight |
| GAs | gibberellins |
| $GA_3$ | gibberellin $A_3$ |
| $GA_4$ | gibberellin $A_4$ |
| $GA_{4/7}$ | mixture solutions of gibberellin $A_4$ and $A_7$ |
| $GA_5$ | gibberellin $A_5$ |
| LA | leaf area |
| ProCa | prohexadione-Ca |
| TPI | total polyphenols index |
| TSS | total soluble solids |

## INTRODUCTION

Gibberellins (GAs) are naturally occurring phytohormones that control or affect a wide range of processes in higher plants. More than 130 structurally different GAs have been characterized from plants, fungi, and bacteria, but only $GA_1$, $GA_3$, $GA_4$, $GA_7$, and $GA_{32}$ have shown intrinsic biological activity in higher plants, the rest being biosynthetic precursors or catabolites.[1] Pérez *et al.*[2] have shown that two GA biosynthesis pathways are present in early stages of berry development of the cultivar Sultana: the early C-13-hydroxylation leading to $GA_1$ biosynthesis, and the non-C-13-hydroxylation for $GA_4$ biosynthesis. The highest concentrations of biologically active $GA_1$ and $GA_4$ were detected very early, at berry set. Both $GA_1$ and $GA_4$ then diminished to low, baseline levels over the next 20–30 days.

Sugar accumulation has been observed in grape following application of the growth-active $GA_3$ (commercially known as gibberellic acid). Applied $GA_3$ targets the allocation and facilitates the transport of photoassimilates[3] by enhancing the phloem area and the expression of sugar transporters.[4] Current viticultural

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practices include the use of $GA_3$ applications, mostly on seedless table grapes, either at flowering stage (in order to reduce the fruit set and bunch compactness) or shortly before veraison to increase berry size.[5] In addition, $GA_3$ increased rachis length, making bunches looser and more aerated, so decreasing the incidence of cryptogamic bunch diseases.[6] Despite this, some potential detrimental effects have been found for the use of $GA_3$ in grape, such as decreased bud fruitfulness following an application.[7] Furthermore, applied $GA_3$ reduced grape berry color by impairing the biosynthesis of anthocyanins.[7]

In grapevine, Giacomelli *et al.*[8] showed that $GA_4$ is the main bioactive GA after anthesis, although information related to the effects of $GA_4$ application is lacking. There are chemical similarities between $GA_4$ and $GA_7$, which predisposes their separation at commercial level to be difficult (and expensive); thus, the $GA_7$ content in different commercially available preparations usually reaches up to 40%.[9] As an example, products with a mixture of $GA_4$ and $GA_7$ (referred to as $GA_{4/7}$) are used to reduce fruit reddishness in apple.[9] Moreover, this mixture is used to induce fruit set and increase fruit size, as observed for pear[10] and maize;[11] and $GA_{4/7}$ applied a few weeks after full bloom reduced the drop of peach fruits.[12]

$GA_5$ is highly florigenic, and ring D-modified variants of $GA_5$ have been 'devised' that were exceptionally florigenic;[13] these include C-16,17-dihydro $GA_5$, which operates as a competitive substrate inhibitor of $GA_3$-oxidase,[14] thereby reducing the biosynthesis of the biologically active $GA_1$ and $GA_4$ (from biologically inactive $GA_{20}$ and $GA_9$ respectively). In fact, $GA_5$ *per se* is able to function in this manner in a cell-free system.[15] However, $GA_5$ is known to be converted to $GA_3$ via $GA_6$ by some higher plants,[16] both of which can be growth active.[1] Speculatively, an excess of $GA_5$ might lead to increases of endogenous $GA_3$, so fine-tuned doses may be necessary.

Prohexadione-calcium (ProCa; calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate) is a biologically active GA biosynthesis inhibitor that affects $GA_3$-oxidase activity. ProCa, as a structural mimic of 2-oxoglutarate (an important co-factor of dioxygenase enzymes), interferes with 3-$\beta$-hydroxylation of C-3-deoxy GAs, such as $GA_{20}$ to $GA_1$, or $GA_9$ to $GA_4$.[17] Notwithstanding, ProCa may also impair $GA_1$ and $GA_4$ catabolism by competing with $GA_2$-oxidase. An additional point that is important for wine grape cultivars is that ProCa treatment may alter the metabolism of phenolics in grapevine tissues, thereby reducing flavonoid biosynthesis.[18]

Taking into account the complex role of bioactive GA after anthesis, we hypothesized that applications of GAs to developing grape berry bunches affect the allocation of photoassimilates, and thereby berry growth and the drop of berry fruits. In the present field trials, several experiments were carried out to evaluate the effect of $GA_4$, $GA_{4/7}$, $GA_5$, $GA_3$ (positive control), and ProCa (negative control) applied to bunches at different timings, concentrations, and modes (bunch immersion or spray) on important agronomic traits of Malbec (the most important grape cultivar for red wine in Argentine viticulture). We analyzed shoot growth above the treated bunches, fruit yield, and berry characteristics for 3 years and fruitfulness for 1 year. The experiments were performed along three consecutive years and in the same vineyard.

## MATERIALS AND METHODS

### Plant material and experimental design

The experiments were carried out during the 2010–2011 (year 1), 2012–2013 (year 2), and 2014–2015 (year 3) flowering and fruiting seasons in a commercial vineyard (1450 m above sea level, 69° 15′37″ W and 33°23′51″ S, Gualtallary, Mendoza, Argentina). The grapevines were a selected clone of *Vitis vinifera* L. cv. Malbec, planted in 1997 on their own roots, trained on a vertical trellis system, arranged in rows, oriented north–south, spaced 2 m apart, with 1.20 m between plants in a row. The grapevines were maintained with no soil water restriction during the whole experiment using a drip irrigation system, and the fruiting vines were protected with anti-hail nets (black polyethylene). The grapevines were cane pruned and shoot-thinned to eight shoots when these shoots reached 10 cm long, and at flowering two bunches per shoot were left. The experimental unit consisted of one plant selected as being homogeneously 'typical' among six consecutive plants in the row, and a randomized complete block design with five blocks was used ($n = 5$).

*Year 1 experiment*

Treatments were performed by submerging all the bunches of a plant (experimental unit) in aqueous solutions containing 5, 50, and 250 mg $L^{-1}$ $GA_5$, 50, 250, and 500 mg $L^{-1}$ ProCa (BASF 125 10 W – 10% active ingredient, negative control), 5, 50, and 250 mg $L^{-1}$ $GA_3$ (positive control), or water ('natural' control). The $GA_5$ used in this study was synthesized from $GA_3$ as detailed in Fairweather *et al.*[19] and kindly provided by Professor Lewis N. Mander. ProCa was purchased from BASF (Buenos Aires, Argentina), and $GA_3$ from Sigma-Aldrich Chem Co. (St Louis, MO, USA). All the solutions contained 0.1% v/v of Triton X-100 as a surfactant, and a minimal amount of 96% aqueous ethanol was used to initially dissolve the GAs (the BASF 125 10 W is formulated as a very water-soluble powder). The treatments were applied three times, at 35, 20, and 0 days before veraison (DBV), so testing multiple applications.

*Year 2 experiment*

$GA_5$ and $GA_3$ were evaluated again by submerging all the bunches of a plant, but increasing the concentration range assayed (5, 20, 50, and 250 mg $L^{-1}$) and testing a single application at three different developmental stages: 60 DBV (berries pea size), and 45 and 30 DBV (beginning of bunch closure).

*Year 3 experiment*

$GA_5$ was re-evaluated, incorporating treatments with pure $GA_4$ and a mixture of $GA_{4/7}$. Application at different developmental stages (60 and 30 DBV) and doses (10 and 50 mg $L^{-1}$) was tested by a single application. Also, application mode was assayed, by submerging or by spraying until run-off all the bunches of a plant (experimental unit). $GA_4$ and $GA_{4/7}$ used were from the Richard P. Pharis collection at the Biology Department, The University of Calgary.

A schematic representation of the experimental design is shown in Fig. 1.

### Assessment of bunch weights, berry numbers, berry total soluble solids, phenolic contents, and vegetative growth

At harvest (24 °Bx), two bunches per experimental unit were collected in nylon bags, fresh mass weighed, and then the number of berries per bunch counted. Samples of 25 berries per experimental unit were randomly collected at harvest in nylon bags (taken from five bunches). Berries were kept on dry ice to prevent enzyme activity variation and dehydration and quickly taken to the laboratory where berry fresh weight (FW) was determined before storage at −20 °C. Then, 15 berries per experimental unit

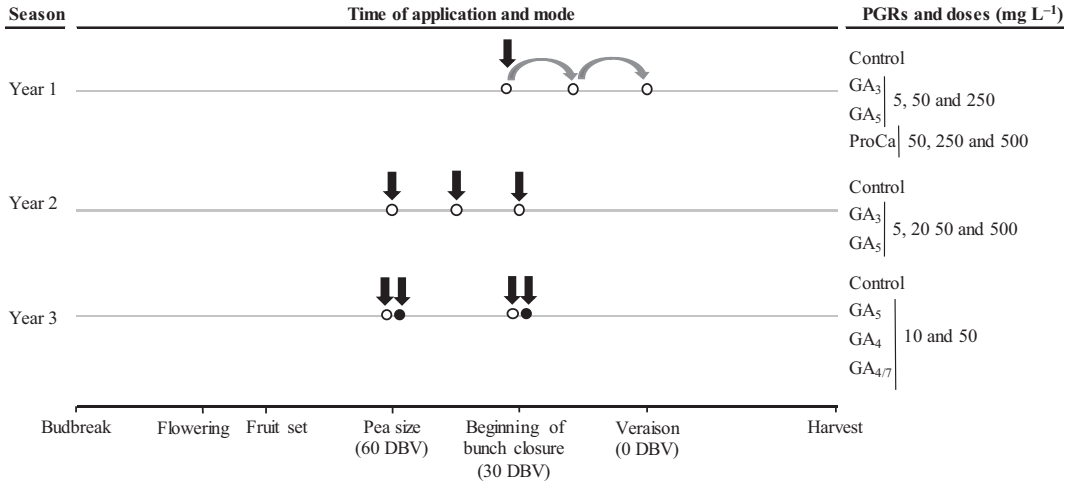


**Figure 1.** Schematic representation of experimental design. Black arrow: one application; grey arrow: the same plants with one (or two) doses, receive another application; white point: dipping; black point: spraying. DVB: days before veraison; GA, gibberellin; PGRs: plant growth regulators; ProCa: prohexadione-calcium.

were defrosted at room temperature and manually peeled. The total soluble solids (TSS, degrees Brix) and TSS on a per berry basis (TSS abs) were determined from berry mesocarp according to Berli *et al*[20]

For anthocyanins and total polyphenol index (TPI), berry skins were extracted with 15 mL of an aqueous ethanolic solution (12% ethanol, 6 g $L^{-1}$ tartaric acid and pH 3.2) at 70 °C for 3 h in darkness. Then, the liquid fraction was separated by decanting; this was maintained at 4 °C for 24 h and then centrifuged for 10 min at 10 000 × *g* to eliminate tartrates and other sediments. Finally, the supernatant was collected and stored at −20 °C. Five berries per experimental unit were defrosted at room temperature and used to assess berry dry weight (DW) by dehydration at 40 °C to a constant weight.

Anthocyanin and TPI were determined spectrophotometrically on the berry skin extraction solution as described in Berli *et al.*[21] and calculated as a concentration (per 100 g berry FW).

At harvest, the shoot length, number of leaves, and total leaf area per shoot were measured from a representative shoot selected per experimental unit, based on Berli *et al.*[22]

### Statistical analysis

Analysis of variance and Fisher's multiple comparisons of means were used to discriminate between the averages by the minimum difference, with a significance level of $P \leq 0.05$. Statistical analyses were performed as a randomized block with the software InfoStat version 2009 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Córdoba, Argentina).

## RESULTS

### Vegetative growth, berry growth, and sugar content

Different techniques, doses, and timings were used in the 3-year field experiment. In year 1, $GA_5$, ProCa, and $GA_3$ were applied at 35, 20, and 0 DBV by dipping bunches three times. In year 2, single applications of $GA_5$ and $GA_3$, also by immersion, were tested at 60, 45, and 30 DBV. In year 3, applications of $GA_5$, $GA_4$, and a mixture of $GA_{4/7}$ by dipping or spraying the bunches were evaluated at 60 and 30 DBV. Vegetative growth of the shoots bearing the two fruit bunches was not significantly affected by any of the individual treatments applied to the fruit bunches, nor by the length of the shoots (Supporting Information, Fig. S1) or by leaf area per shoot (Supporting Information, Fig. S2). Berry growth at harvest, assessed as berry FW and berry DW, was in general not affected by any of the individual treatments, relative to the water control (Supporting Information, Figs S3 and S4), except for the 50 mg $L^{-1}$ dose of $GA_5$, applied at 30 DBV in the year 2 experiment, which reduced the berry FW (Supporting Information, Fig. S3(b)).

TSS on a per berry basis (absolute amounts) or in degrees Brix were in correspondence with berry FW and berry DW at harvest; that is, they were not affected by GAs or ProCa (Supporting Information, Figs S5 and S6).

### Bunch FW and number of berries per bunch

The GAs tested affected bunch FW and the number of berries per bunch at harvest significantly (Figs 2 and 3). In the year 1 experiment, the bunch FW and numbers of berries were increased by the 5 and 50 mg $L^{-1}$ doses of $GA_5$ and by 5 mg $L^{-1}$ of $GA_3$, by multiple applications (i.e. three times, at 35, 20, and 0 DBV; Figs 2(a) and 3(a)).

In the year 2 experiment with a single application, the effect of $GA_5$ was confirmed by 20 mg $L^{-1}$ $GA_5$ applied at 30 DBV, thus increasing the bunch FW and number of berries per bunch (Figs 2(b) and 3(b)). $GA_3$ did not promote bunch FW.

In the year 3 experiment, immersion in 50 mg $L^{-1}$ $GA_5$ or $GA_4$ at both moments of application (i.e. at 60 DBV and 30 DBV) increased the average bunch FW and the number of berries per bunch (Figs 2(c) and 3(c)). Likewise, spraying at 30 DBV with 10 mg $L^{-1}$ $GA_5$ or $GA_4$ increased the bunch FW. Application of 10 mg $L^{-1}$ $GA_{4/7}$ at 30 DBV by immersion and spraying, as well as 50 mg $L^{-1}$ $GA_{4/7}$ at 60 DBV by spraying, increased the bunch FW and the number of berries per bunch.

### Berry skin anthocyanins and total polyphenols content

Anthocyanins and TPI were positively and negatively affected by some GAs and ProCa treatments (Figs 4 and 5). In year 1, dipping the bunches in 5 mg $L^{-1}$ $GA_5$ as well as the higher doses of ProCa diminished the anthocyanins content and the TPI compared with the control (Figs 4(a) and 5(a)).

In year 2, with only a single application by submersion, 50 mg $L^{-1}$ $GA_5$ at 30 DBV markedly increased the anthocyanins



  

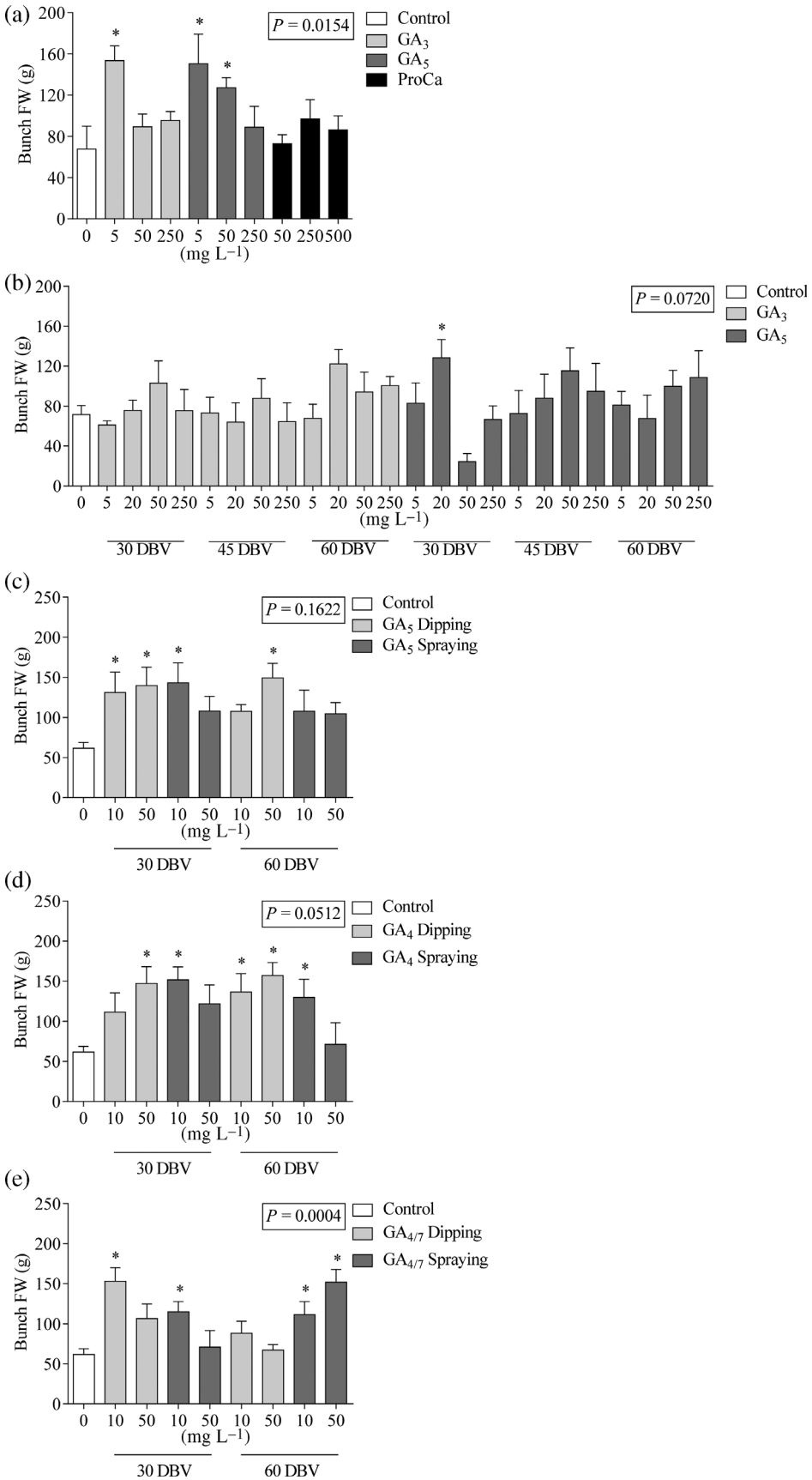


**Figure 2.** Bunch fresh weight (FW) at harvest in (a) year 1, (b) year 2, and (c–e) year 3. Values are means plus/minus standard error of the mean; an asterisk placed atop the treatment bars indicates a significant difference against the control (Fisher's least significant difference; $P \leq 0.05$). DVB: days before veraison; GA, gibberellin; PGR: plant growth regulator; ProCa: prohexadione-calcium.



  

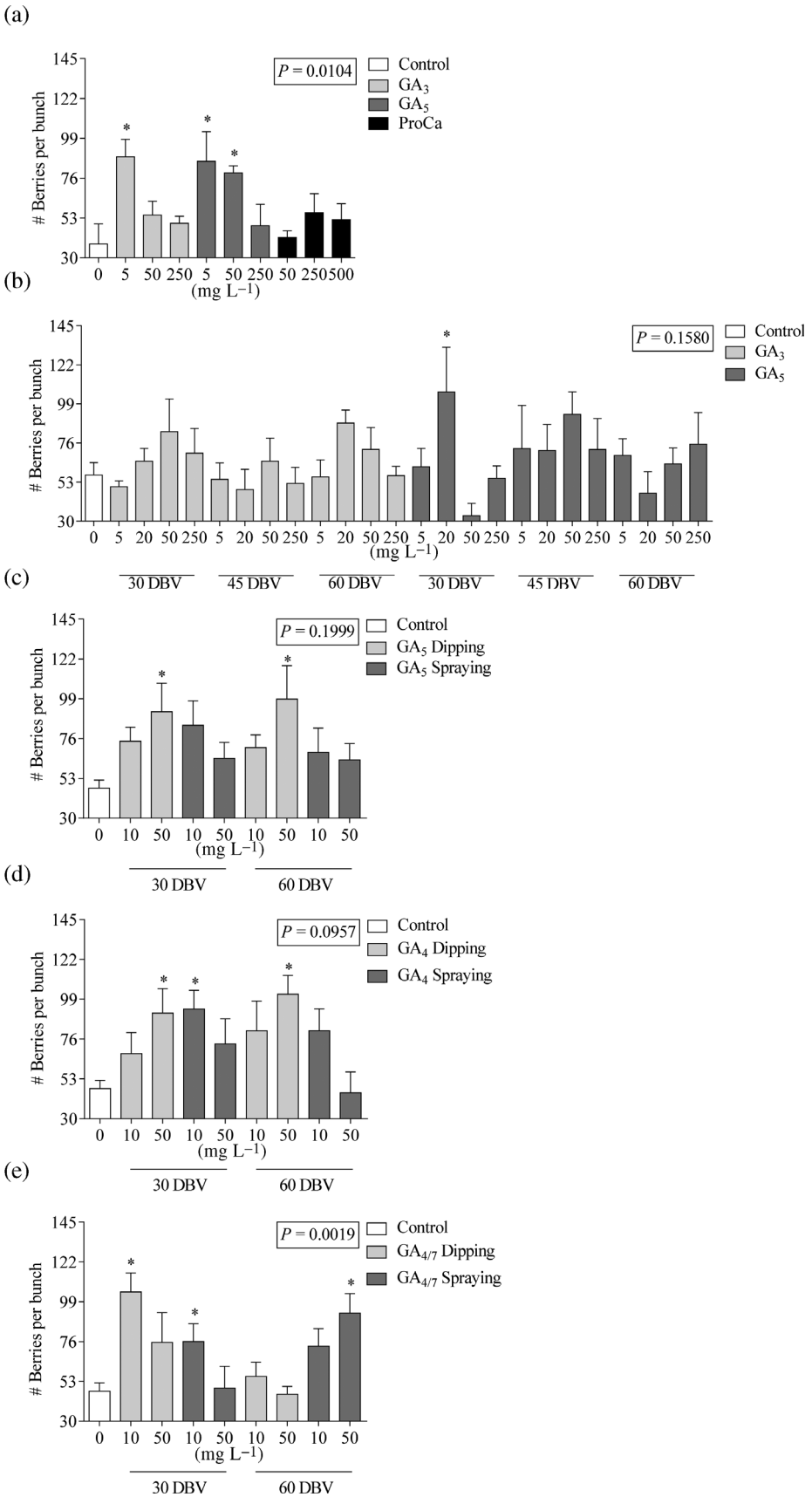


**Figure 3.** Number of berries per bunch at harvest in year 1 (a), year 2 (b) and year 3 (c, d, e). Values are means plus/minus standard error of the mean; significant differences against the control are indicated with an asterisk placed atop the treatment bars (Fisher's least significant difference; $P \leq 0.05$). DVB: days before veraison; GA, gibberellin; PGR: plant growth regulator; ProCa: prohexadione-calcium.



  

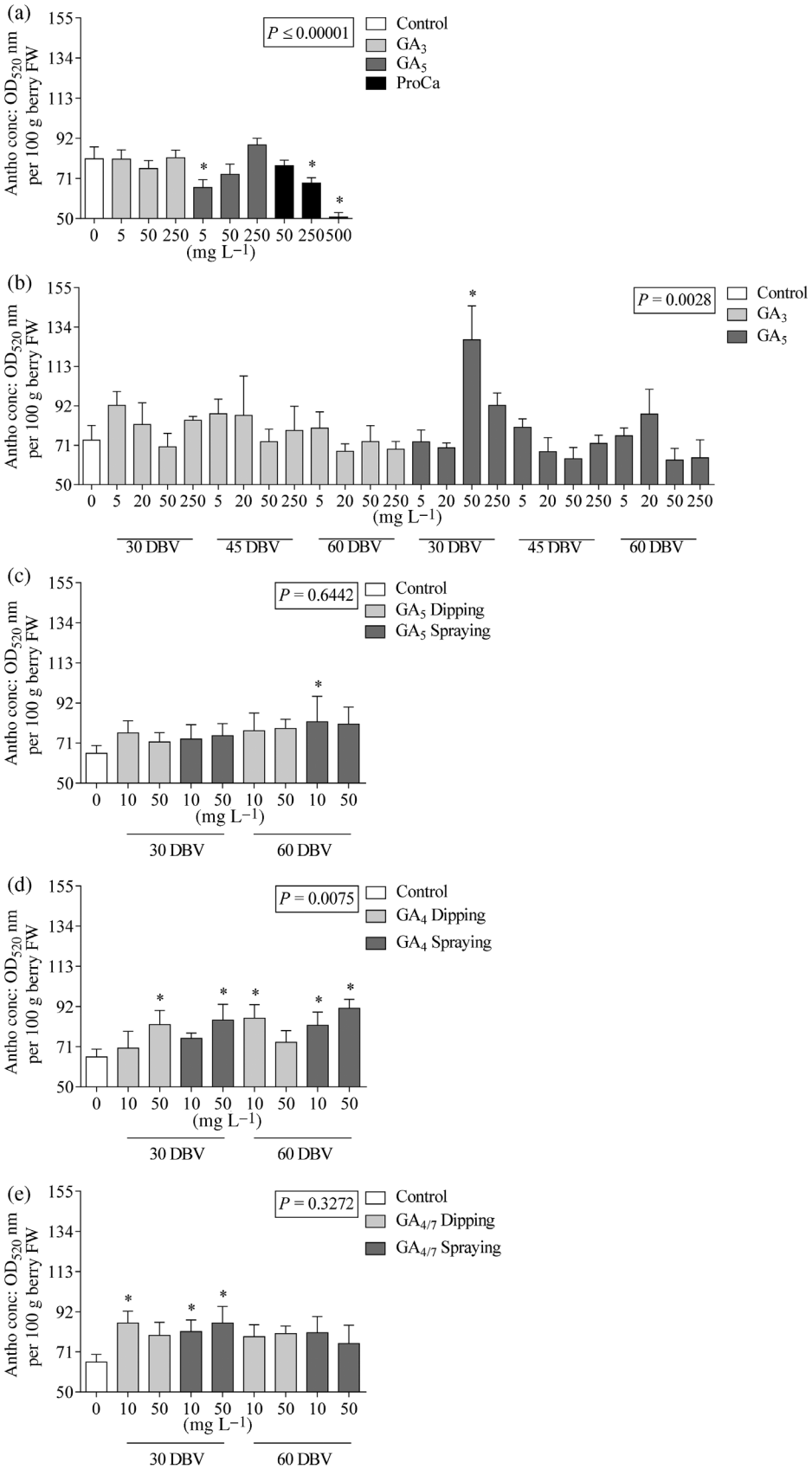


**Figure 4.** Total anthocyanin (Antho conc) in berry skins at harvest in (a) year 1, (b) year 2, and (c–e) year 3. Values are means plus/minus standard error of the mean; an asterisk placed atop the treatment bars indicates a significant difference against control (Fisher's least significant difference; $P \leq 0.05$). DVB: days before veraison; FW: fresh weight; GA, gibberellin; PGR: plant growth regulator; ProCa: prohexadione-calcium.



  

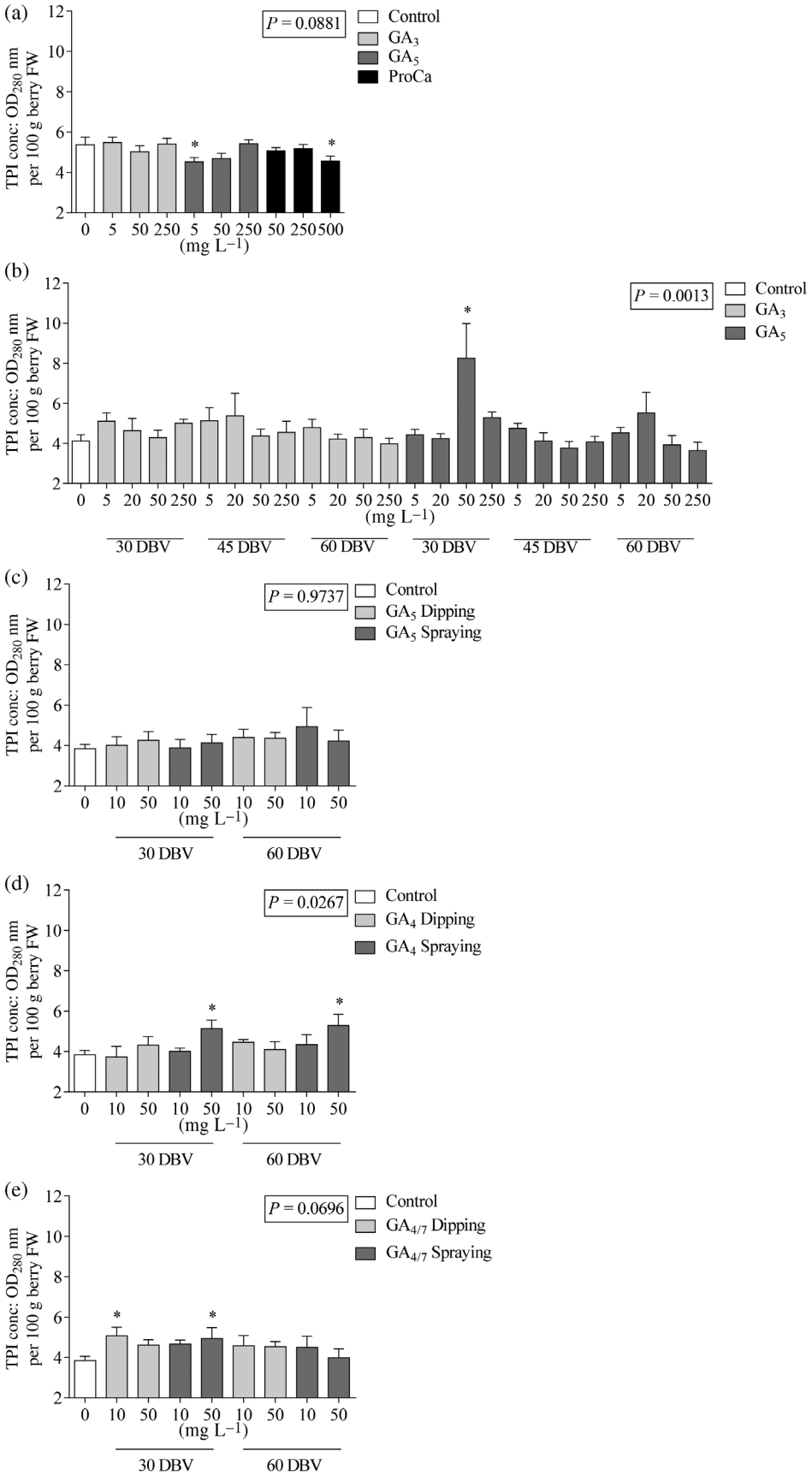


**Figure 5.** Total polyphenols index (TPI conc) in berry skins at harvest in (a) year 1, (b) year 2, and (c–e) year 3. Values are means plus/minus standard error of the mean; an asterisk placed atop the treatment bars indicates a significant difference against control (Fisher's least significant difference; $P \leq 0.05$). DVB: days before veraison; FW: fresh weight; GA, gibberellin; PGR: plant growth regulator; ProCa: prohexadione-calcium.



  

and the TPI in berry skins at harvest (Figs 4(b) and 5(b)). $GA_3$ applications, in both years, did not affect anthocyanin or TPI.

In year 3, spraying 10 mg $L^{-1}$ $GA_5$ at 60 DBV increased anthocyanins, relative to control (Figs 4(c) and 5(c)). Applications of $GA_4$ in both concentrations, moments, and application methods generally increased anthocyanins (Fig. 4(d)). However, with respect to TPI, only spraying 50 mg $L^{-1}$ $GA_4$ at 30 DBV and 60 DBV increased it significantly (Fig. 5(d)). $GA_{4/7}$ at both assayed doses (10 and 50 mg $L^{-1}$) and application methods increased anthocyanins and TPI, relative to the control, but only when applied at 30 DBV (Figs 4(e) and 5(e)).

## DISCUSSION

All the treatments applied to the developing grape berry bunches scarcely influenced the vegetative growth of shoots, most probably due to the targeted mode of application and the timing, when the plants exhibited advanced vegetative growth. The earliest treatment started at 60 DBV and corresponded to the pea-sized berry developmental stage.

Berry FW was not affected by the treatments, with the exception of 50 mg $L^{-1}$ $GA_5$ applied at 30 DBV in year 2, which reduced it. It was expected that $GA_5$ might work in the grape berry as a competitive substrate inhibitor of the action of $GA_3$-oxidase,[14] thereby yielding reduced levels of endogenous growth effectors; that is, $GA_1$ and $GA_4$. Also note that biosynthesis of active GAs may be restricted by feedback regulation; that is, the expression of $GA_{20}$-oxidase and $GA_3$-oxidase (final steps in GA biosynthesis) is controlled by endogenous GA levels.[1] Ravest *et al.*[23] analyzed GA metabolites during berry growth and development of grapevine contrasting phenotypes. They found that large and medium-sized berries contained similar quantities of bioactive GAs and suggested that the final berry size is not directly correlated to GA concentration. The lack of effect of the applied GAs on individual berry weight may be related to the increased number of sinks (berries) per bunch. That is, the sources (leaves) were able to feed more berries, but berry growth was limited since an increase in the amount of photoassimilates was delivered to more sinks. Alternatively, the effect of GAs application may be lower than expected since a seeded wine grape cultivar was used (where a relatively high level of GAs was already present in the tissues).

Our applications of ProCa at 35, 20, and 0 DBV did not yield significant reductions in berry size or increases in skin-to-berry ratio, likely due to lateness of the treatments and/or the varietal response. Lo Giudice *et al.*[24] found that Cabernet Sauvignon and Chardonnay grape bunches treated with ProCa at 1–2 weeks post-bloom reduced berry weight with no impact on fruit set, whereas the ProCa applications at pre-bloom, or during the bloom period, decreased fruit set. Such a reduction was then related to improved sensory characteristics and wine quality (color intensity, total anthocyanin, and total phenols), all variables that depend on the total polyphenol concentration. We thus expected our ProCa treatments to increase the proportion of skin-derived flavor and aroma precursors in the 'must' and resultant wine, thereby indirectly increasing wine quality. We also expected a direct reduction of flavonoid biosynthesis by ProCa. However, our applications of ProCa did not yield significant reductions in berry size, but the anthocyanins and TPI were reduced. Similar results were observed by Rademacher[17] for anthocyanins in some other crops, and also by Puhl *et al.*,[18] who found decreased flavonol biosynthesis in ProCa-treated grape leaves, flowers, and green berries. The basis of the ProCa reduction in flavanols appears to be due to reduced activity of flavanone 3-hydroxylase, a key enzyme in the synthesis of dihydroquercetin and other flavonoids.[17]

Pérez and Gómez[25] found strong correlations amongst increases in berry size, invertase activity, and hexose content of the seedless grape cultivar Sultana treated with $GA_3$. However, ProCa applications did not reduce fruit soluble solids in grapes.[24] From the foregoing, it may be concluded that endogenous levels of the grape berry GAs are sufficient to sustain 'normal' sugar loading. A similar conclusion was reached by Moreno *et al.*[3] with seeded cv. Malbec; that is, $GA_3$ applications resulted in no additional effect on the grape berry's sugar levels, possibly due to a relatively high level of GAs already present in the tissues.

The pool of active GAs is maintained by controlling their biosynthesis and their deactivation,[8] mainly through 2$\beta$-hydroxylation, and also by conjugation with sugars, by methylation, and by epoxidation of the 16,17-double bond.[1] Our ProCa treatments had no effect on bunch FW, suggesting that, in Malbec, sufficient concentration of bioactive GAs can be operating and that they are pre-existing, or in any case they are deconjugated from the pool stored in vacuoles. On the other hand, we observed that $GA_{4/7}$ applied 30 DBV by immersing the bunches was more effective in increasing the bunch FW, whereas the aspersion technique resulted in superior results when done 60 DBV (pea size). This may be explained by a better incorporation of $GA_7$ with the aspersion technique, since $GA_7$ is catabolized more slowly[9] because of the double bond between the C1 and C2 of the A ring (lower affinity for the 2$\beta$-hydroxylase), and so higher concentrations persist longer.

In grapevine, the number of berries in a bunch is determined by the number of flowers per inflorescence, but also by fruit set (flowers that turn into berries) and berry abscission. Previous work has shown that the effect of applied $GA_3$ on berry set and bunch weight is responsive to timing of the GA application, with applications at full bloom reducing the number of flowers that set.[5] However, in the experiments presented here, the effects of late application (during pea size and later stages) of $GA_3$ (year 1, three applications), $GA_5$, $GA_4$, and $GA_{4/7}$ on increasing numbers of berries per bunch are likely related to the improvement of berry sink strength[3] and the reduction of berry drops. Some cultivars, including Malbec, exhibit a tendency to show reproductive disorders,[26] like poor fruit set due to excessive abortion of flowers and ovaries (which may occur for up to 4 weeks after anthesis), which is termed shatter, shedding, or 'coulure'. On the other hand, 'millerandage' is characterized by the presence of normal-size seeded berries in conjunction with small-size (<3 mm) and mid-size (3–6 mm) seedless fruits in the same bunch; it is also called 'hens and chicks' because large and small berries exist within a bunch. Small- and mid-size berries are called shot berries and are seedless or exhibit seed traces and can remain in the bunch up until harvest with irregular size growth and a different ripening level.[27] In seeded grapes, fertilization is followed by a second period of cell division and cell expansion in correlation with high concentrations of GAs produced by the embryos.[28] Therefore, it is plausible to hypothesize that application of GAs avoids the mentioned reproductive disorders by allowing the cell division and expansion to continue, which may then be reflected in a greater number of berries and in a superior bunch FW at harvest time.

Previous reports have shown that different developmental processes may involve specific active GAs. Hirano *et al.*[29] observed that, even though $GA_1$ is the predominant active GA in rice,

anthers accumulated $GA_4$. Furthermore, in plants of *V. vinifera* Pinot Noir (a seeded grape), a higher accumulation of $GA_1$ than $GA_4$ was observed in inflorescences at anthesis, whereas only $GA_4$ was detected at later stages.[8] Possibly, our applications of $GA_4$ and $GA_{4/7}$ after fruit set increased the internal concentration of $GA_4$, improving the strength of berries as a sink and reducing the drop of fruits (and thus increasing the number of berries per bunch at harvest), but more studies will be necessary to validate the hypothesis.

We also assessed the effects of our treatments on the next year's flowering and fruiting of the same vines. Based on bunch counts after fruit set, there was no effect (data not shown). A similar finding was found (Jackson DI, Donaldson M, and Pharis, RP unpublished results) for cv. Chardonnay, where the $GA_5$ structural variant C-16,17-dihydro $GA_5$ had been applied to the nodes of the laydown cane in the previous year.[30]

Commercial applications of $GA_3$ to grapevines used in the production of table grapes (mainly seedless) have been well described.[5] However, few examples where $GA_3$ has been trialed on wine grape cultivars are available. In those, the primary objective was to reduce bunch compactness, since tight bunches are more susceptible to bunch rot.[6] $GA_3$ applied in table grapes varieties reduced the skin-to-berry ratio and, consequently, decreased berry color.[7] Nevertheless, in our experiments, skin-to-berry ratio was not affected by $GA_3$ treatment and, accordingly, the anthocyanins content was not modified with respect to the control. We infer that mainly the cultivar (a seeded cultivar, with embryo and relatively high level of GAs), timing of treatment, and mode of application differences account for our present results on cv. Malbec berry color.

The effects of $GA_5$, however, had not been examined on either table or wine grape cultivars, except for an example for a ring D-modified $GA_5$ variant on cv. Chardonnay.[30] In the present study, the $GA_5$ treatments enhanced both TPI and anthocyanin concentrations, suggesting, although speculatively, that $GA_5$ application may act as an enhancer of polyphenol synthesis. Similar to $GA_5$ application, we observed that applications of $GA_4$ and $GA_{4/7}$ also increased the anthocyanins and TPI in the berry skin, which is probably related to the activation of transcription for flavonoid enzymes genes. Weiss[31] observed in petunia that $GA_1$ and $GA_4$ control flavonoid gene transcription, inducing the production of a regulatory protein. More studies will be necessary to evaluate the direct and indirect effects of $GA_5$, $GA_4$, and $GA_{4/7}$ on grape berry skin flavonoid content.

Our results demonstrate that exogenous applications of $GA_5$, across a relatively wide dose range (5 to 50 mg $L^{-1}$), increase the numbers of final berries at harvest, thereby increasing the average FW per bunch produced by a selected wine grape variety. $GA_5$ is notably effective in accomplishing this increased bunch harvest weight, without affecting vegetative growth and berries sugar content. Consequently, exogenous applications of $GA_5$ may increase the yields of wine grapes. Grape berries harvested from grapevines receiving exogenous applications of $GA_5$ also exhibit higher TPI and higher anthocyanin contents, thereby increasing the winemaking quality of the berries. We have observed similar benefits with applications of $GA_4$ and the $GA_{4/7}$ mixtures (with similar doses). In contrast, only the very lowest dose (5 mg $L^{-1}$) of $GA_3$ significantly promoted berry number and grape yield per plant. Finally, it is important to highlight that the agronomic benefits reported in the present study – that is, a higher yield and a higher content of phenolics in berry skin – were not only observed by applying $GA_5$, $GA_4$, and $GA_{4/7}$ by dipping, but also by spraying, which is a method of application being very common in commercial vineyards.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest/competing interests.

## CONSENT TO PARTICIPATE

FB, RA, and RB agree with the final contents of the manuscript and its submission to the journal; Professor Richard P. Pharis, who passed away in June 2018, actively participated in the general orientation of the manuscript. Thus, the manuscript is dedicated to his memory.

## AUTHOR CONTRIBUTIONS

**FB:** conceptualization, methodology, investigation, formal analysis, data curation, supervision, project administration, funding acquisition, writing original draft and editing. **RA:** conceptualization, methodology, investigation, formal analysis, data curation, writing original draft and editing. **RP:** conceptualization, methodology, supervision, project administration, funding acquisition. **RB:** conceptualization, methodology, supervision, project administration, funding acquisition, writing original draft and editing.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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