

Quality improvement of ‘Primitivo’ grape by manual defoliation and abscisic acid application

Melhoria da qualidade da uva ‘Primitivo’ por desfolha manual e aplicação de ácido abscísico

Mejora de la calidad de la uva ‘Primitivo’ mediante defoliación manual y aplicación de ácido abscisico

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Isabela Leticia Pessenti

ORCID: <https://orcid.org/0000-0002-5176-3134>

State University of Ponta Grossa, Brazil

E-mail: isabelaleticiapessenti@gmail.com

Ricardo Antonio Ayub

ORCID: <https://orcid.org/0000-0003-3240-8417>

State University of Ponta Grossa, Brazil

E-mail: rayub@uepg.br

Renato Vasconcelos Botelho

ORCID: <https://orcid.org/0000-0001-9580-2572>

State University of Mid-Western of Paraná, Brazil

E-mail: rbotelho@unicentro.br

Sergio Ruffo Roberto

ORCID: <https://orcid.org/0000-0003-2357-187X>

State University of Londrina, Brazil

E-mail: sroberto@uel.br

Abstract

Climatic conditions have a strong influence in the cultivation of the vine. High precipitation and low temperatures, for example, can cause the grapes not reach adequate maturation indexes. In order to check the effect of defoliation and application of abscisic acid (*S*-ABA) to improve grape quality, an experiment was conducted in a commercial vineyard of ‘Primitivo’ grape, in South of Brazil, for two growing seasons. The treatments were as follows: 1) control; 2) manual defoliation in early maturation (*veraison*); 3) 15 days after the first

manual defoliation; 4) abscisic acid 200 mg L⁻¹; 5) abscisic acid 400 mg L⁻¹; 6) abscisic acid 600 mg L⁻¹. The variables soluble solids, titratable acidity, total polyphenols and anthocyanins, color intensity in berries, photosynthetically active radiation, chlorophyll index, percentage of defoliation and chlorosis were evaluated. Manual defoliation held 15 days after the first defoliation increased soluble solids and reduced titratable acidity, while the application of 400 mg L⁻¹ S-ABA increased the titratable acidity of the grape must. There was a higher photosynthetically active radiation near the bunch to defoliation treatments and S-ABA. The application of S-ABA provided chlorosis in leaves, followed by low chlorophyll content, causing the senescence. Hence, defoliation 15 days after first manual defoliation influenced soluble solids and titratable acidity, and application of abscisic acid increased levels of total polyphenols, anthocyanins and color intensity in 'Primitivo' berries skins.

Keywords: *Vitis vinifera* L.; Plant regulators; Skin color; Phenolic compounds.

Resumo

As condições climáticas têm uma forte influência no cultivo da videira. A alta precipitação e as baixas temperaturas, por exemplo, podem fazer com que as uvas não atinjam índices de maturação adequados. Com o objetivo de verificar o efeito da desfolha e da aplicação de ácido abscísico (S-ABA) na melhoria da qualidade da uva, foi realizado um experimento em um vinhedo comercial da uva 'Primitivo', no Sul do Brasil, durante duas safras. Os tratamentos foram os seguintes: 1) controle; 2) desfolha manual no início da maturação ('veraison'); 3) 15 dias após a primeira desfolha manual; 4) ácido abscísico 200 mg L⁻¹; 5) ácido abscísico 400 mg L⁻¹; 6) ácido abscísico 600 mg L⁻¹. Foram avaliadas as variáveis sólidos solúveis, acidez titulável, polifenóis totais e antocianinas, intensidade de cor nas bagas, radiação fotossinteticamente ativa, índice de clorofila, porcentagem de desfolha e clorose. A desfolha manual realizada 15 dias após a primeira desfolha aumentou os sólidos solúveis e reduziu a acidez titulável, enquanto a aplicação de 400 mg L⁻¹ de S-ABA aumentou a acidez titulável do mosto de uva. Houve uma maior radiação fotossinteticamente ativa próximo ao cacho para tratamentos de desfolha e S-ABA. A aplicação de S-ABA proporcionou clorose nas folhas, seguida de baixo teor de clorofila, causando a senescência. Portanto, a desfolha 15 dias após a primeira desfolha manual influenciou os sólidos solúveis e a acidez titulável, e a aplicação de ácido abscísico aumentou os níveis de polifenóis totais, antocianinas e a intensidade da cor nas cascas das bagas 'Primitivo'.

Palavras-chave: *Vitis vinifera* L.; Reguladores vegetais; Cor da casca; Compostos fenólicos.

Resumen

Las condiciones climáticas tienen una fuerte influencia en el cultivo de la vid. Las altas precipitaciones y las bajas temperaturas, por ejemplo, pueden provocar que las uvas no alcancen índices de maduración adecuados. Con el fin de comprobar el efecto de la defoliación y la aplicación de ácido abscísico (S-ABA) para mejorar la calidad de la uva, se realizó un experimento en un viñedo comercial de uva 'Primitivo', en el sur de Brasil, durante dos temporadas de cultivo. Los tratamientos fueron los siguientes: 1) control; 2) defoliación manual en la maduración temprana ('envero'); 3) 15 días después de la primera defoliación manual; 4) ácido abscísico 200 mg L⁻¹; 5) ácido abscísico 400 mg L⁻¹; 6) ácido abscísico 600 mg L⁻¹. Se evaluaron las variables sólidos solubles, acidez titulable, polifenoles y antocianinas totales, intensidad de color en bayas, radiación fotosintéticamente activa, índice de clorofila, porcentaje de defoliación y clorosis. La defoliación manual realizada 15 días después de la primera defoliación aumentó los sólidos solubles y redujo la acidez titulable, mientras que la aplicación de 400 mg L⁻¹ S-ABA aumentó la acidez titulable del mosto de uva. Hubo una radiación fotosintéticamente activa más alta cerca del racimo a los tratamientos de defoliación y S-ABA. La aplicación de S-ABA proporcionó clorosis en las hojas, seguida de un bajo contenido de clorofila, provocando la senescencia. Por lo tanto, la defoliación 15 días después de la primera defoliación manual influyó en los sólidos solubles y la acidez titulable, y la aplicación de ácido abscísico aumentó los niveles de polifenoles totales, antocianinas y la intensidad del color en las pieles de las bayas "Primitivo".

Palabras clave: *Vitis vinifera* L.; Reguladores vegetales; Color de piel; Compuestos fenólicos.

1. Introduction

Berries color of red grapes is produced by a group of anthocyanins which provides important characteristics in high-quality red wines elaboration. The most intense the color, the most interesting it becomes from the functional and processing point of view, since dark-colored grapes have a higher content of phenolic compounds, in addition to the antioxidant, anticarcinogenic and antiviral capability (Abe et al., 2007).

The phenolic compounds concentration in wine is related to its concentration in the berries, and the defoliation can interfere within this characteristic, whereas the anthocyanins and flavonoids biosynthetic pathways are regulated by light and temperature sensitive enzymes. According to Baiano et al. (2015), pathways are regulated by enzymes light and

temperature dependent, and any change in the microclimatic conditions caused by defoliation, can cause significant impact over the accumulation of these compounds, in grape and wine.

The bunches that are exposed to the solar radiation have more accumulation of soluble solids, anthocyanins and of phenolic compounds with low content of titratable acidity, pH and concentration of malic acid as compared to grape bunches that are shaded (Baiano et al., 2015; Teixeira, 2004). Thus, defoliation could favors in ventilation and heat stroke in region of inflorescence and grapes bunches to provide better conditions for maturation.

Anthocyanins accumulation is regulated, at least in part, by abscisic acid, being exogenous applications of this plant regulator may increase the concentrations of anthocyanins and phenolics in grape (Buran et al., 2012). Several studies suggest that exogenous application of *S*-ABA provide increase in content of anthocyanins in grape skin, anticipating the harvest season (Barros et al., 2020; Cantín et al., 2007; Gardin et al., 2012; Wheeler et al., 2009).

The objective of this experiment was to evaluate the effect of defoliation and the application of abscisic acid on the quality of 'Primitivo' grape (*Vitis vinifera* L.).

2. Material and Methods

This paper was based on literature and inspired by the authors Koyama (2014a; 2014b) Roberto et al (2013) na Yamamoto et al. (2015). These authors studied *Vitis labrusca* grape cultivars, and this article used *Vitis vinifera* L.

The experiment was conducted on cultivar Primitivo (*Vitis vinifera*) for two growing seasons 2015/2016 and 2016/2017, in a commercial vineyard located in Água Doce, Santa Catarina, Brazil (26°43'53 "S and 51°30'26" W; 1,300 m a.s.l.). The grapevines with 14 years old were grafted on the 'Paulsen 1103' rootstock and conducted in an espalier system, with spacing 1,5m between plants and 3,0 m between rows.

The experimental design was in randomized blocks, with 6 treatments, 4 replicates and 3 plants per plot, being evaluated the central plant. The treatments were as following: 1) control (no treatment); 2) manual defoliation in early maturation (DIM); 3) manual defoliation 15 days after the first defoliation (D15); 4) *S*-ABA 200 mg L⁻¹ (ABA200); 5) *S*-ABA 400 mg L⁻¹ (ABA400); 6) *S*-ABA 600 mg L⁻¹ (ABA600).

The applications of commercial aqueous solutions of *S*-ABA (Valent BioSciences Corporation, Libertyville, IL, USA) were performed at the beginning of the maturation of clusters (*veraison*) with the use of a knapsack sprayer until runoff, being applied directly on

the leaves until the height of bunches, from the base of the branch. The manual defoliation was performed at the beginning of the ripening and 15 days after the date of the first defoliation until the bunches from the base, being withdrawn by 6 leaves per branch. The evaluations of the plants in the field were performed during the harvest, in 19/02/2016 and 22/02/2017, first and second cycle, respectively.

At harvest, it was performed the measurement photosynthetically active radiation (PAR) with the appliance Pyranometer ProCheck ® Version 7 (Decagon Devices, Pullman, Washington, USA), in the 10:00 h to 14:00 h. The measurements were made at the time of the bunch, with the result expressed in $\mu \text{ mol m}^{-2} \text{ s}^{-1}$. In the 2016/2017, the index of defoliation and chlorosis, and the index of defoliation were assessed to check the presence of leaves on the branches. The index of chlorosis, was performed to check the presence of chlorosis in leaves. The data of defoliation and chlorosis index were transformed into a percentage of the total of leaves of the branch (%). Chlorophyll assessment was carried out on the day of the harvest with chlorophyllometer CFL model 1030 (ClorofiLOG, Porto Alegre, RS, Brazil), which expressed the results in an index called ICF itself: Chlorophyll index Falker. For each repetition, there were two readings taken when leaf was fully expanded.

At harvest, 60 samples of berries from each experimental plot were collected. The berries were taken from different portions of the bunches, after this samples were subjected to weighing and separation of hulls. The pulps were macerated and was used to analyze to determine soluble solids (°Brix), using manual refractometer model 103 (Biobrix, Sao Paulo, Brazil). The titratable acidity (% of tartaric acid) was analyzed through mini titler model HI 84532 with NaOH 0.1 M (Hanna, Woonsocket, Rhode Island, EUA) (IAL - Instituto Adolfo Lutz, 2008).

For total polyphenols content, method of Singleton and Rossi (1965) was followed, using Folin Ciocalteu reagent and calibration curve. The reading of the samples was carried out using spectrophotometer UV 760 nm model in 1650 PC (Shimadzu, Kyoto, Japan). The readings obtained with extracts were interpolated on the standard curve, and the results were expressed as mg equivalent of gallic acid L^{-1} .

The method used for total anthocyanins was described by Lee and Francis (1972). The content of 1 g of skin in advance was removed using tweezers and macerated in porcelain crucibles with 10 mL of extracting solution (50% ethanol 95% + 50% hydrochloric acid 1.5 M). When the samples were completely macerated, the net content was stored in vial protected from light (covered with foil), then washed the rest of the macerated skin to the crucible. When, the test tubes received the entire solution it is kept left under refrigeration at

4° C for 20 hours. At the end of this period, the extract was filtered, washing it with 25 mL of extracting solution, leaving the total extract in bottle covered with aluminum foil for 2 hours. Then, 2 mL of the extract was removed, by adding 10 mL of extracting solution and subsequent unrest solution was shaken on vortex. Absorbance was determined using a spectrophotometer model 1650 UV PC (Shimadzu, Kyoto, Japan) at 535 nm for the determination of anthocyanins, the values expressed in mg of anthocyanins per 100 g of plant material. To quantify the content of anthocyanins equation was used $(FD * VA) * -198.2$, where VA = value of absorbance and FD = dilution factor.

The analyses regarding the color of extracts were made according to the method of Iland et al. (2004). The extract was diluted at a rate 1:10 and analyzed in spectrophotometer model 1650 UV PC (Shimadzu, Kyoto, Japan) at wavelengths 420, 520 and 620 nm. The intensity of color was obtained by formula $Abs_{420} + Abs_{520} + Abs_{620}$.

3. Results and Discussion

In relation to the photosynthetically active radiation observed, on the surface of the bunches, treatments DIM (manual defoliation in early maturation) and D15 (manual defoliation fifteen days after the first) obtained the highest values in both growing seasons evaluated (Table 1). In the second cycle, the ABA treatments also provided higher photosynthetically active radiation (PAR) on the surface of the bunches in relation to the control treatment, but significantly lower than the treatments with defoliation. It is observed that in the first cycle the PAR values were lower than the second cycle, this is due to the presence of clouds.

Table 1 - Photosynthetically active radiation (PAR), soluble solids content (SS) (°Brix), titratable acidity (TA) (% tartaric acid) in 2015/2016 and 2016/2017.

| Treatments | PAR ($\mu\text{mol}/\text{m}^2 \text{ s}^{-1}$) | | SS (° Brix) | | TA (% AT) | |
|------------|---|---------|-------------|---------|-----------|---------|
| | 2015/16 | 2016/17 | 2015/16 | 2016/17 | 2015/16 | 2016/17 |
| TEST | 187.5c | 197.2d | 14.7a | 14.5b | 0.90ab | 0.43b |
| DIM | 249.7b | 1091.7a | 14.8a | 15.1ab | 0.75bc | 0.46b |
| D15 | 341.4a | 1192.2a | 14.5a | 16.0a | 0.70c | 0.39c |
| ABA200 | 129.0d | 742.5b | 14.3ab | 15.4ab | 0.47d | 0.44b |
| ABA400 | 164.0cd | 530.0c | 14.4ab | 13.5c | 0.94a | 0.54a |
| ABA600 | 142.0cd | 760.2b | 13.4b | 14.5b | 0.47d | 0.45b |
| CV (%) | 12.27 | 10.87 | 3.2 | 2.86 | 10.34 | 3.02 |

Test: control; DIM: manual defoliation in early maturation; D15: manual defoliation fifteen days after the first defoliation; ABA200: S-ABA 200 mg L⁻¹; ABA400: S-ABA 400 mg L⁻¹; ABA600: S-ABA 600 mg L⁻¹. Medium followed by the same letter do not differ by Tukey test ($p \leq 0.05$). Vertical bars represent the standard deviation (n = 4). Source: The authors.

Solar radiation, photosynthetically active radiation (PAR) and luminosity are important climatic parameters for vine development. These factors are related to the process of photosynthesis, the accumulation of sugars content of the grapes and, consequently, their quality, as they directly influence the secondary metabolism of the grapevine, increasing phenolic compounds (Malinovski, 2013). Many authors affirm that luminosity causes increase in the concentration of total monomeric anthocyanins in the grapes, however, this compound is reduced when the bunches are submitted to elevated temperatures (Malinovski, 2013). At this phase of grapes maturation, the bunches are more exposed to the sun contain more total flavonoids contents (up to ten times) than the shaded bunches. This is due to the increase of the 3-glycoside concentration of quercetin, campferol and myrcetin (Spayd et al., 2002).

For the photosynthetically active radiation (PAR) on the surface of the clusters, treatments DIM and D15 provided increases of 132 and 181%; 553 and 605% for the two growing seasons, respectively, due to the absence of leaves. The ABA treatments also led to the defoliation of plants in the second cycle with photosynthetically active radiation (PAR) increments in 376 and 385%, respectively. The levels of photosynthetically active radiation

(PAR, 400 - 700 nm) incident on the canopy, especially at the height of the bunches, is very important in determining the composition of the grape. Some studies have demonstrated that an increase of the insolation in the bunches provides greater accumulation of sugars and soluble solids contents (Comiran et al., 2012).

Photosynthetically active radiation (PAR) provides the energy for photosynthesis and primary production of green plants. In the grapevine it has several effects, mainly because of its relation with the quality of the light, especially the ultraviolet (UV) radiation that stimulates the production of some important compounds used in the determination of the characteristics of aerial biomass production of leaves and bunches of the grape (Grifoni et al., 2008).

The literature related to the elimination of leaves during the course of the vegetative cycle of the vine is relatively extensive, with sometimes conflicting results. This is because its effect may vary depending on different factors, especially the defoliation intensity, the season in which it is carried out, the climatic conditions that occur during the vegetative cycle of the vine, the structure and texture of the soil, the cultivar that is being evaluated and the set of cultural practices that are used in the vineyard (Miele et al., 2013).

According to Mandelli et al. (2008), defoliation can provide desirable effects, such as a decrease in leaf area index and, as a consequence, an increase in vegetative canopy 'porosity' and better exposure of grape clusters to the sun. However, this practice increases the possibility of burning the grape. Care should be taken in regions where the temperature is very high at the time of fruit ripening. Another point to consider is the decrease in the incidence of fungal diseases, especially gray mold rot, caused by the fungus *Botrytis cinerea*. Besides that, defoliation must be carried out with caution, since it will eliminate specialized organs for photosynthesis and consequent translocation of sugar to the fruit, mainly the synthesis and accumulation of starch in the perennial parts of the plant.

In the 2015/2016 cycle, the ABA600 treatment reduced the soluble solids content, but did not differ from the ABA400 and ABA 200 treatments. In the second cycle, the D15 treatment obtained the highest value for soluble solids not differing from DIM and ABA200 treatments (Table 1). For the titratable acidity, in the two growing seasons evaluated, the ABA400 obtained the highest values, differing significantly from the other treatments, while the treatment D15 obtained reductions in relation to the control (Table 1).

Intrigliolo et al. (2014) observed an increase in soluble solids (SS) concentration, while the titratable acidity (TA) and pH remained unchanged in the defoliation treatments when compared to the control in Mandó grape cultivar in Spain. However, Mandelli et al.

(2008), observed that the chemical characteristics were not affected, independently of the type of defoliation used in grape cv. Merlot, in Bento Gonçalves, RS.

Koyama et al. (2014) found that the exogenous application of *S*-ABA increased the SS content as the ABA dosage increased in 'Isabel' grape, while there was a decrease in TA. However, other authors describe that the application of *S*-ABA had little or no effect on the chemical characteristics of the berries (Jeong et al., 2004; Peppi; Fidelibus; Dokoozlian, 2006).

The levels of chlorophyll a, b and total chlorophyll were measured only in the second cycle (2016/2017) (Table 2). All ABA treatments significantly reduced chlorophyll content a, b and total chlorophyll of leaves.

Table 2 - Percentage of defoliation (%), percentage of leaves with chlorosis (%), Chlorophyll a, b and total in 2016/2017 of grape cv. Primitivo.

| Treatments | Percentage of defoliation (%) | Percentage of leaves with chlorosis (%) | Chlorophyll a | Chlorophyll b | Total chlorophyll |
|------------|-------------------------------|---|---------------|---------------|-------------------|
| TEST | 4.9 b | 1.3 b | 35.7 a | 10.4 ab | 46.1 a |
| DIM | 34.7 a | 0 b | 32.4 ab | 11.1 a | 43.6 ab |
| D15 | 42.2 a | 0 b | 31.8 ab | 10.2 ab | 42 ab |
| ABA200 | 20.2 ab | 24.6 a | 28 bc | 7 c | 35.1 bc |
| ABA400 | 26.5 ab | 26.9 a | 22.3 cd | 5.4 c | 27.7 c |
| ABA600 | 27 ab | 2.3 a | 21.2 d | 5.5 c | 26.7 c |
| CV (%) | 38,6 | 38,54 | 10 | 37,46 | 10,25 |

Test: control; DIM: manual defoliation in early maturation; D15: manual defoliation fifteen days after the first defoliation; ABA200: *S*-ABA 200 mg L⁻¹; ABA400: *S*-ABA 400 mg L⁻¹; ABA600: *S*-ABA 600 mg L⁻¹. Medium followed by the same letter do not differ by Tukey test ($p \leq 0.05$). Vertical bars represent the standard deviation (n = 4). Source: The authors.

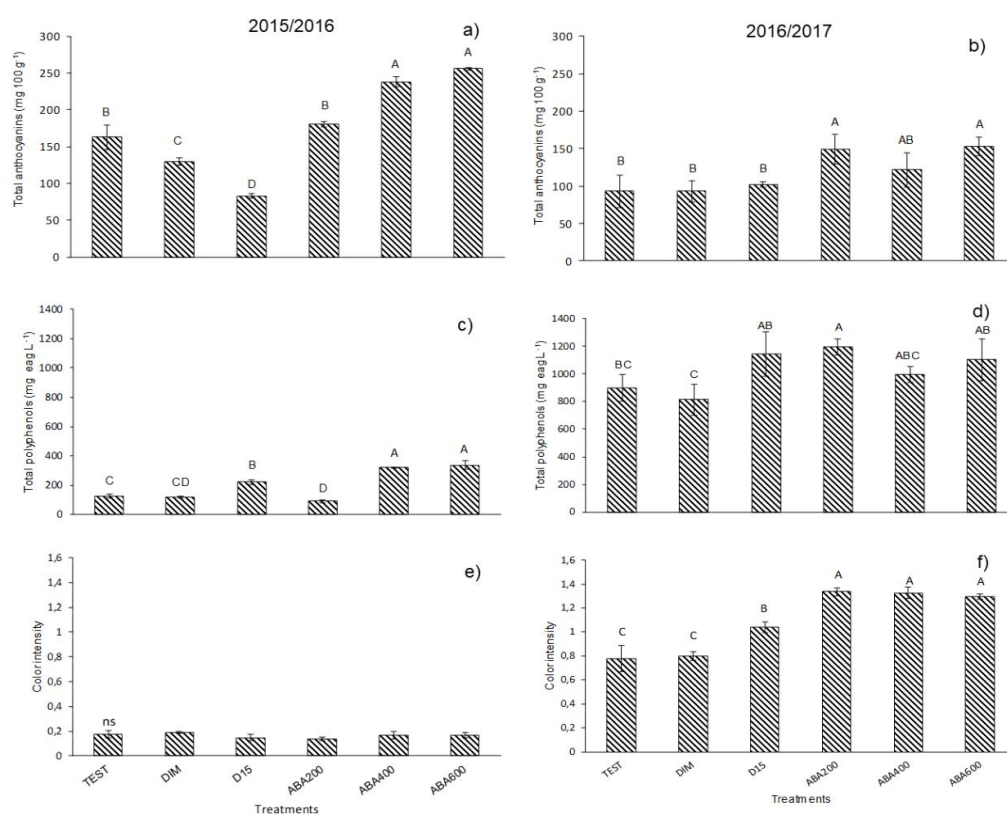
For defoliation percentage (%), treatments DIM and D15 obtained the highest values (34.7 and 42.2%) due to the treatments performed. The treatments with ABA presented intermediate values, and also were statistically different from the control that presented the lowest defoliation percentage (5%) (Table 2). For the percentage of leaves with chlorosis, all treatments with ABA presented this symptom with the highest values (24.7, 27.0 and 25.3%, respectively), differing statistically from the other treatments (Table 2).

Neto et al. (2017) designated chlorophylls as a, b, c and d where, chlorophyll a is the most abundant and important, corresponding to approximately 75% of the green pigments found in plants. In this work, a concentration (below 40) of chlorophyll a, b and total in the treatments with *S*-ABA applications was observed, due to the chlorophyll degradation caused by the ABA, this could also be observed by the chlorosis verified in the leaves.

Esperança et al. (2014) determined indices of chlorophyll in the leaf limb of apple trees submitted to increasing doses of abscisic acid (ABA). The treatments with two applications, ABA 750 + 750 mg L⁻¹ and ABA 1,500 + 1,500 mg L⁻¹ promoted a greater reduction of the chlorophyll index, followed by the treatment of ABA 1,500 mg L⁻¹ with a single application. ABA promotes reduction of chlorophyll indices and consequently accelerates the process of foliar senescence, and can thus be used as a defoliation agent in the apple tree crop.

For the total anthocyanins and total polyphenols levels in the 2015/2016 cycle (Figures 1a and 1c), ABA400 and ABA600 treatments were higher and differed statistically from the others. In the second cycle, the treatments ABA200 and ABA600 presented anthocyanin levels significantly higher than those of the control treatments, DIM and D15, but did not differ from ABA400 (Figure 1b). For total polyphenol content, the ABA200 treatment was superior to the control (Figure 1d). In the second cycle the values of total polyphenols were much higher in relation to the first cycle.

Figure 1 - Total anthocyanins ($\text{mg } 100 \text{ g}^{-1}$).



(a), total polyphenols ($\text{mg gallic acid equivalent L}^{-1}$) (c), colour intensity (e) in 2015/2016, total anthocyanins ($\text{mg } 100 \text{ g}^{-1}$) (b), total polyphenols (mg eaq. L^{-1}) (d) and colour intensity (f) in 2016/2017 of grape cv. Primitivo. Test: control; DIM: manual defoliation in early maturation; D15: manual defoliation fifteen days after the first defoliation; ABA200: S-ABA 200 mg L^{-1} ; ABA400: S-ABA 400 mg L^{-1} ; ABA600: S-ABA 600 mg L^{-1} . Medium followed by the same letter do not differ by Tukey test ($p \leq 0.05$). Vertical bars represent the standard deviation ($n = 4$). Source: The authors.

For color intensity, there were no significant differences in the first cycle (Figure 1e). In the second cycle, all doses of ABA provided higher color intensities than the other treatments. The D15 treatment also increased the color intensity of berry skin berries, but in a lower magnitude than the treatments with ABA (Figure 1f). As in total polyphenols, the values of color intensity for the second cycle were higher as compared to the first cycle.

The content of anthocyanins increased 146 and 158% (1st cycle); and 160 and 163% (2nd cycle), with treatments ABA400 and ABA600; and ABA200 and ABA600, respectively, and the total polyphenol content increased 254 and 267% (1st cycle); and 132% (2nd cycle), with doses 400 mg L^{-1} ; and 600 mg L^{-1} ; and 200 mg L^{-1} , respectively. The results corroborate with those obtained by Koyama et al. (2014), which, regardless of the concentrations and frequency of application, obtained an increase in the content of anthocyanins in 'Isabel' grape juice, as well as those verified by Jeong et al. (2004), in which the application of S-ABA

stimulated the accumulation of anthocyanins in 'Cabernet Sauvignon' grape. Peppi et al. (2007) also observed that S-ABA increased the total amount of anthocyanins in 'Redglobe' grapes.

Gardin et al. (2012) observed an increase in the anthocyanins and total polyphenols content with the application of ABA and etephon. Both plant regulators act on the biosynthesis of these compounds, mainly the ABA that is directly involved in the synthesis of anthocyanins. The author further states that levels of pigmentation in skin of ABA-treated grapes were accompanied by increased activity of CHF1 (chalcone-flavone isomerase). The activity of CFHI is closely related to anthocyanin biosynthesis.

4. Conclusion

The defoliation 15 days after the first defoliation can be adopted to improve the gustatory characteristics of cv. 'Primitivo', such as soluble solids content and acidity. However, S-ABA can be applied to increase anthocyanin content, total polyphenols and color intensity.

It is necessary to check more defoliation times to increase the levels of soluble solids and total acidity. Apply different doses of abscisic acid to increase the total anthocyanin content. Apply defoliation and abscisic acid in different cultivars and locations.

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Percentage of contribution of each author in the manuscript

Isabela Leticia Pessenti – 50%

Ricardo Antonio Ayub – 20%

Renato Vasconcelos Botelho – 20%

Sergio Ruffo Roberto – 10%