BIOCHEMICAL AND AGRONOMICAL RESPONSES OF GRAPEVINES TO ALTERATION OF SOURCE-SINK RATIO BY CLUSTER THINNING AND SHOOT TRIMMING (1)

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ABSTRACT

The control of leaf to fruit ratio by the practice of entire clusters removal from the vine upon berry set has been used in traditional vineyards in order to regulate yield and to improve chemical composition of the berries. Although this practice has been settled in temperate zones, little is known about grapevines behavior in tropical growing areas. The aim of this work was to evaluate the biochemical and agronomical responses of two *Vitis vinifera* cultivars (Merlot and Cabernet Sauvignon) grown in Caldas, Minas Gerais, Brazil, a new winegrape region. Grapevines were submitted to cluster thinning (0%, 50% and 75% of cluster removal) and trimming treatments (trimmed and untrimmed) imposed at pea size stage. The source-sink alteration by fruit removal and shoot trimming had impact on yield, leaf sugar metabolism and grape composition. Although there was an improvement in color intensity of the berries with reduction of fruit load, the cluster thinning practice should be avoided in vineyards grown in the south of Minas Gerais State without impairment of wine grape quality. On the other hand, shoot trimming practice should be recommended only for Merlot in order to improve sugar grapes from vines with no cluster removal.

Key words: Vitis vinifera, cluster removal, canopy management, Ravaz index, fruit composition.

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RESUMO

RESPOSTAS BIOQUÍMICAS E AGRONÔMICAS DE VIDEIRAS À ALTERAÇÃO DA RAZÃO FONTE-DRENO PELO DESBASTE DE CACHOS E DESPONTE DE RAMOS

O controle da razão folhas:frutos pela prática da remoção de cachos inteiros após o estabelecimento das bagas tem sido utilizado nos vinhedos tradicionais, a fim de regular a produção e melhorar as características químicas das bagas. Embora essa prática esteja bem estabelecida em regiões temperadas, pouco se sabe a respeito do comportamento das videiras cultivadas em áreas tropicais. O objetivo deste trabalho foi avaliar as respostas agronômicas e bioquímicas de duas cultivares de *Vitis vinifera* (Merlot e Cabernet Sauvignon) cultivadas em Caldas, Minas Gerais, Brasil, nova região produtora de uvas finas. As videiras foram submetidas a tratamentos de desbaste de cachos (0%, 50% e 75% de cachos retirados) e desponte (com e sem desponte) realizados no estágio de ervilha. A alteração da fonte-dreno pela remoção dos frutos e desponte afetou a produção, o metabolismo de açúcares nas folhas e a composição das bagas. Embora tenha sido observado um aumento na intensidade de cor das bagas com a redução da carga de frutos, a prática do desbaste de cachos pode ser evitada nos vinhedos do sul de Minas Gerais, sem prejuízo à qualidade das uvas viníferas, enquanto a prática do desponte deve ser recomendada somente para Merlot, a fim de melhorar a concentração de açúcares nas uvas de videiras sem desbaste de cachos.

Palavras-chave: Vitis vinifera, remoção de cachos, manejo do dossel, Índice de Ravaz, composição dos frutos.

1. INTRODUCTION

Wine grape production depends on an optimal balance of leaves and shoots growth to produce carbohydrates for growth and ripening of clusters. The control of leaf to fruit ratio is the basis of many viticultural practices aiming the production of fruits with the required compositional characteristics. Cluster thinning, the removal of entire clusters from the vine following berry set, has been used as a corrective viticultural practice to regulate yield in commercial winegrape vineyards. Fruit removal is performed to increase the leaf area:fruit weight ratio in order to prevent over cropping and improve grape quality (Reynolds et al., 1996; Prajitna et al., 2007). High quality wines are usually produced from vineyards having low to moderate yields based on variety and cultural practices (JACKSON and LOMBARD, 1993). However, while several studies show effects of yield on grape composition, there are also indications of an associated lower wine quality (NAOR et al., 2002; Prajitna et al., 2007), whereas some data indicate little apparent response to yield variation (Ough and Nagaoka, 1984; Nuzzo and Matthews, 2006; REYNOLDS et al., 2007). In fact, there is considerable evidence that high yields can be consistently maintained provided that fruit environment is kept at close to optimum conditions (SMART et al., 1990).

Canopy management such as shoot trimming which consists of cutting off shoot tips is intended to avoid overhanging shoots which would otherwise shade the bunch zone in upright trellis systems. The principle of shoot trimming is to minimize vegetative dominance preventing physiological imbalances

between sources and sinks. Therefore it has also been used to improve fruit set and maximize carbohydrate partitioning to fruit during ripening (Koblet, 1987; Vasconcelos and Castagnoli, 2000).

In Brazil, a wider range of canopy and fruit management options needs to be explored to contribute to the improvement of grape quality, since the optimal yield for quality varies according to cultivar, climatic conditions, planting intensity, pruning and canopy management. There is no information in the literature regarding the impact of sink-source alteration on leaf and berries metabolism under environmental conditions in Brazil. Therefore, the identification of vineyard cultural practices that could influence sugar, acids and phenolics contents in wine grape is desirable. The purpose of the present experiment was to investigate vegetative vigor, yield and fruit composition of wine grapes Cabernet Sauvignon and Merlot cultivars submitted to different crop levels and reduction of canopy density by cluster removal and shoot trimming under climatic conditions of the high plains of the south of Minas Gerais State (Brazil).

2. MATERIAL AND METHODS

Experimental design and plant material

The trial was conducted in 2007 in a commercial vineyard located in Caldas, Minas Gerais State, in southeastern Brazil (21°55′S, 44°23′ W), at an altitude of 1,150 m. The cultivars studied were *Vitis vinifera* cv. Cabernet Sauvignon and cv. Merlot grafted on 1103 Paulsen, spaced 1.5 m between vines and 2.5

m between rows. The vineyard was planted in 2001 and both cultivars were trained on a vertical shoot position, north-south oriented, and spur pruned with two nodes spurs.

Three cluster-thinning and trimming treatments were imposed as completely randomized subplots within each cultivar main plot. The crop thinning treatments were applied as follows: control - no clusters removal (100% of cluster in the grapevine); 50% cluster thinning - one cluster removal per shoot (50% of cluster in the grapevine) and 75% cluster thinning - only one cluster for each two shoots left to develop (25% of cluster in the grapevine). The trimming treatments (trimmed and untrimmed) were done at approximately 30 cm above the last wire (canopy height was about 1.30 m). Both treatments were imposed at 90 days after pruning (at pea size stage) as a factorial design considering the cluster thinning and trimming as the main factors for each cultivar. There were 3 replicates per treatment with 5 to 6 vines each.

Vegetative vigor and Ravaz index

The vegetative vigor was measured as dormant pruning weight at winter and it was used to calculate the Ravaz index [kg fruit kg (dormant pruning)⁻¹]. The Ravaz index is an indication of vine balance: a value of 5 to 10 for *Vitis vinifera* cultivars indicates the vine is balanced, a value higher than 12 indicates overcropping, while a value below 3 indicates excessive vine size (SMART and ROBINSON, 1991).

Leaf carbohydrate contents

The soluble (total soluble sugars, reducing sugars and sucrose) and insoluble (starch) carbohydrate concentrations were assessed on dried and powered leaf samples taken from four grapevines per treatment during the morning at ripening period. Soluble sugars were extracted from 100 mg samples with 80% (v/v) ethanol (80 °C, 20 min) and centrifuged (9,160 x g, 15 min). The pellet was twice extracted and the combined supernatants (10 mL) were used to analyses of total soluble sugars (TSS) and reducing sugars (RS) according to Somogy-Nelson method (Nelson, 1944). Starch retained in the pellet after sugar extraction was hydrolyzed with Termamyl[®] 120 L and Amyloglucosidase 300 L (Novozymes). Pellets were dried at room temperature and 2 mL of Termamyl (diluted 1:1000 in water) was added followed by incubation at 75 °C for 1 h. After incubation with Termamyl, 2 mL of Amyloglucosidase (28 unit mL⁻¹, in sodium acetate buffer, pH 4.8) was added and kept at 50 °C for 1 h. The released glucose was quantified by Somogy-Nelson method, after centrifugation (2,290

x g, 5 min). The starch content was obtained by multiplying the values for reducing sugars by a factor of 0.9, which allows for the reduced molecular weight of glucose in the polymer.

Yield and fruit composition

Total number of clusters and total fruit weight per vine were recorded at harvest. Mean cluster weight was calculated from yield and cluster per vine data. Chemical analyses (soluble solids, pH and titratable acidity) were performed on the juice of pressed berries (210 berries samples per treatment) collected, at harvest, from all vines and representative of all cluster positions within the canopy and of all positions within the cluster. Soluble solids (°Brix) were determined using a handheld temperaturecompensated refractometer (ATAGO Model Pal-1). The pH of undiluted juice of each sample was determined using a pHmeter (Micronal B474). An aliquot of 5 mL juice from each sample was placed into a 125 mL vial to which 45 mL of distilled water was added. Titratable acidity was determined by titration with 0.1 N NaOH to a phenolphthalein end point and expressed as meg L⁻¹.

The main sugars (glucose, fructose and sucrose) and acids (malic and tartaric) in the berries were obtained by HPLC method. Sugars were extracted from the fruit pulp, at a ratio of 1:4 (m/v) three successive times with 80% ethanol (v/v) at 80 °C. After centrifugation (10,000 x g, for 10 min at 25 °C), the supernatant was transferred to a 25 mL volumetric flask and the volume was topped up with 80% ethanol. An aliquot of 1 mL of each ethanolic extract was evaporated under vacuum in a "Speedvac" system (Savant, Co.). The volume was reconstituted with deionizied water. The contents of soluble sugars were analyzed by HPLC-PAD (Dionex, Sunnyvale, CA, USA) in a DX-500 chromatograph. This was coupled to a pulsed amperometric detector using a CarboPac PA1 (4.0 x 250 mm) column (Dionex) at 25 °C, 18 mM NaOH as mobile phase, and isocratic flow of 1 mL min⁻¹. The extractions were performed in triplicate for each sample.

The extraction of organic acids was carried out in triplicate in the juice collected from each sample. Samples were centrifuged at 10,000 x g for 10 min at 4 °C and the supernatant diluted in water. An aliquot of 10 μL of each diluted sample was injected into a HPLC-DAD (Hewlett Packard, model 1100) equipped with SupelcoGel C-610H column (Supelco 7.8 x 300 mm) at 15 °C and DAD detector at 245 nm. Water acidified with fosforic acid solution at 0.5% was used as mobile phase at a flow rate of 0.5 mL min $^{-1}$ in an isocratic condition.

Data analyses

The Statistica software package (StatSoft, Tulsa, OK) was used for data analysis. All results were subjected to two-way (thinning x trimming) analysis of variance (ANOVA) for each cultivar. The means were compared by Tukey test at the 5% level.

3. RESULTS AND DISCUSSION

The results showed that the source-sink alteration by fruit removal and shoot trimming in Merlot and Cabernet Sauvignon had impact on yield, leaf sugar metabolism, and grape composition. The crop level among cluster thinning treatments ranged around 10 clusters vine⁻¹ (75% cluster thinning), 15 clusters vine⁻¹ (50% cluster thinning) and 27 clusters vine⁻¹ (control) in Merlot (Figure 1a), whereas in Cabernet Sauvignon the averages of cluster thinning treatments were 12, 16 and 26 clusters vine⁻¹, respectively (1d). In Merlot the total yield for non thinned vines averaged approximately 5 kg vine⁻¹, 3 kg vine⁻¹ for 50% cluster thinning and 1.8 kg vine⁻¹ for 75% cluster thinning (Figure 1b). In Cabernet Sauvignon these averages were 3.4, 1.8 and 1.3 kg vine ¹, respectively (Figure 1e). In both cultivars there was an increase in yield of shoot trimmed vines with 50% and 75% cluster thinning which seems to be related to an increase in cluster weight (Figures 1b, c, e, f). In general, cluster thinning resulted in a decrease of cluster weight in both cultivars in untrimmed grapevines with significant differences at Tukey test between control and 75% cluster thinning. In trimmed vines, however, there was no significant difference among cluster weight regardless cluster thinning treatment (Figure 1c, f).

The shoot trimmed contributed to increase the yield in the thinned vines, probably due to reduction of competition for photoassimilates between the tip growing shoots and fruits, contributing to increase the cluster weight. Fruit set can increase when the shoot trimming is done at flowering (SMART and ROBINSON, 1991). However, in our study as the tip removal was done one month after the fruit set, the number of berries may not have accounted for the cluster weight increase observed in the trimmed vines. Probably, the trimmed shoot could have contributed to increase the fruit sink strength favoring carbohydrate partitioning from leaves to berries in those vines at low crop loads (Figure 1c, f).

In contrast, in both cultivars, the cluster thinning treatment decreased the cluster weight in untrimmed vines. The results found in the literature are controversial. Some studies show that the cluster weight is increased by cluster thinning (Reynolds et al., 1994), others indicate cluster weight decrease (Miller et al., 1996), and still others came to the conclusion that the cluster weight was not influenced by this practice (Keller et al., 2005; Nuzzo and Matthews, 2006).

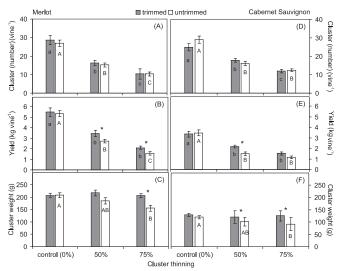


Figure 1. Effects of cluster thinning and shoot trimming on cluster number per vine, yield per vine and cluster weight in 'Merlot' (a, b, c) and in 'Cabernet Sauvignon' grapevines (d, e, f). Data are means \pm se (n = X). Cluster thinning means with different letters in columns (trimmed and untrimmed) are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

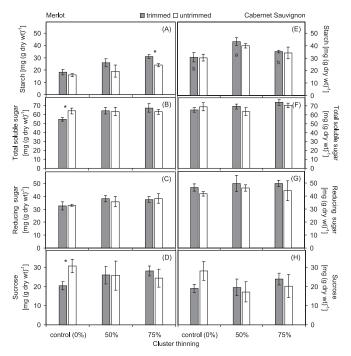


Figure 2. Effects of cluster thinning and shoot trimming on starch, total soluble sugar, reducing sugar and sucrose in leaves of 'Merlot' (a, b, c, d) and 'Cabernet Sauvignon' (e, f, g, h) grapevines. Data are means ± se (n = X). Cluster thinning means with different letters in colums (trimmed and untrimmed) are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

Cluster removal caused a significant increase only in leaf starch at 50% cluster removal in trimmed Cabernet Sauvignon grapevines, whereas the effect of shoot trimming was only observed in Merlot (Figure 2a, e). In this cultivar, the trimmed vines with 75% cluster thinning showed a significant increase in leaf starch compared to untrimmed vines, whereas untrimmed control vines (0% cluster thinning) showed significant increase in leaf total soluble sugar and sucrose.

Several studies have shown that leaf photosynthesis can be influenced by the presence of developing fruit. A positive effect of crop load on photosynthesis has been reported in Vitis vinifera L. (Kaps and Cahoon, 1989; Edson et al., 1995; Naor et al., 1997) and also in other species of Citrus (IGLESIAS et al., 2002), Malus (PALMER et al., 1997; WÜNSCHE et al., 2000) and Prunus (LAYNE and FLORE, 1993; Ben Mimoun et al., 1996; Di Vaio et al., 2001). Although the photosynthesis was not measured in our study, the lowest starch concentration in leaves of non thinned vines suggests a higher carbon partitioning to cluster maybe induced by a greater number of bunches. The reduction in the number of clusters probably decreased the carbohydrate sink strength leading to accumulation of starch in the leaves of thinned vines. Similar results on leaf carbohydrate status were also observed in mango leaves (URBAN et al., 2004).

The tip shoot removal has also contributed to increase the level of the insoluble sugar in leaves of thinned vines, probably due to a decrease in carbon partitioning to shoot growth and clusters and/or to increased photosynthetic rate of leaves induced by shoot tip removal (Toda, 1991). Therefore, our results are compatible with the assumption that a decrease in the fruit sink activity leads to carbohydrate accumulation in the leaf that could promote a feedback inhibition of photosynthesis as reported in previous studies (Krapp et al., 1993; Martínez-Carrasco et al., 1993; Paul and Driscoll, 1997; Iglesias et al., 2002, Urban et al., 2004). However, the nature of this response is not necessarily conclusive, nor is it fully understood.

The thinning treatments had no impact on pH and soluble solids in berries of both cultivars (Figure 3a, b, d, e). The total acidity was higher in berries of non thinned in both trimmed and untrimmed vines of Merlot (Figure 3c). The effects of shoot trimming was only observed in fruit soluble solids of Cabernet Sauvignon, which showed a slightly, but statistically significant, increase of soluble solids in berries of untrimmed, 50% and 75% cluster thinned vines (Figure 3d).

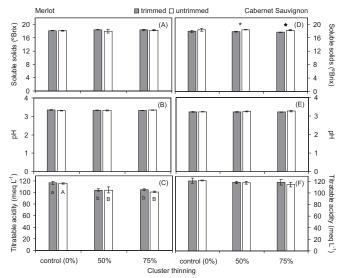


Figure 3. Effects of cluster thinning and shoot trimming on soluble solids, pH and titratable acidity in berries of 'Merlot' (a, b, c) and 'Cabernet Sauvignon' (d, e, f) grapevines. Data are means \pm se (n = X). Cluster thinning means with different letters in colums (trimmed and untrimmed) are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

The berries of untrimmed vines showed the highest level of the main sugars glucose and fructose in most of cluster thinning treatments in both cultivars (Figure 4). However, in Merlot, the shoot trimming increased the level of glucose only in berries of unthinned vines. In the untrimmed vines, the effect of cluster removal on the level of soluble sugars was different between cultivars. There was a significant interaction between treatments in Merlot. In this cultivar, the cluster thinning caused an increase in glucose and fructose in berries of untrimmed vines, whereas in Cabernet Sauvignon there was no statistical difference among cluster thinning treatments. (Figure 4a, b, d, e). In both cultivars, the sucrose content was slightly affected by shoot trimming where the berries from trimmed vines showed the highest concentration (Figure 4c, f).

In this experiment, the fruit sink strength seems to be more evident when the shoot was trimmed as showed in figure 4, where the levels of the main sugars (glucose and fructose) were higher in berries from non thinned vines in both cultivars. Considering the premise that the source of sugar produced in grapevines is from leaf photosynthesis, probably there was a favorable carbon partitioning to cluster with the increased fruit load. On the other hand, it is also interesting to mention that in the untrimmed vines the berry sugar accumulation was not associated to strength of fruit sink since the effect of fruit load on levels of soluble sugars in berries from untrimmed vines was different between cultivars.

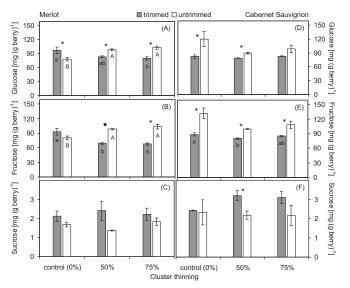


Figure 4. Effects of cluster thinning and shoot trimming on glucose, fructose and sucrose in berries of 'Merlot' (a, b, c) and 'Cabernet Sauvignon' (d, e, f) grapevines. Data are means \pm se (n = X). Cluster thinning means with different letters in colums (trimmed and untrimmed) are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

However, in general, the effect of shoot trimming on sucrose accumulation was similar in both cultivars where the berries from vines with no shoot tip removal showed lower sugar levels as compared to trimmed vines. The physiological mechanisms controlling these interactions remain an interesting puzzle.

In Merlot, there was no effect of treatments on citric and tartaric acid (Figure 5 b, c). The malic acid was only affected by removal of fruits in trimmed vines, which non thinned vines showed the highest values (Figure 5a). In Cabernet Sauvignon, tartaric, citric and malic acids were not affected by cluster thinning treatments in untrimmed vines. Trimmed vines showed lower organic acid levels in 50% cluster thinning treatment (Figure 5d, e, f). In both cultivars there was a trend for heavier crop to have high acidity expressed mainly by malic acid. However, only in Merlot the cluster thinning significantly decreased the malic acid levels and titratable acidity (Figure 3c) as showed by other authors (Keller et al., 2005).

In general, in both cultivars, the anthocyanins and total phenols extracted from skin berries were more affected by cluster thinning than shoot trimming (Figure 6a, b, d, e). These compounds were significantly higher in berries from the vines with the lowest crop yield. In trimmed vines, there was a trend to increase the levels of anthocyanins and total phenols with yield reduction. On the other hand, the

untrimmed vines of Merlot cultivar showed an inverse pattern with lower levels at 50% cluster thinning treatment. Merlot berries with the highest yield (no cluster removal treatment) showed higher anthocyanin and total phenols in the skins in untrimmed vines, whereas in 50% and 75% cluster thinning the trimming treatment increased anthocyanins and total phenols levels. In Cabernet Sauvignon there was no significant effect of trimming on anthocyanins and phenols. There was a similar pattern in both cluster thinning and shoot trimming treatments, where the cluster reduction increased the level of these phenolic compounds.

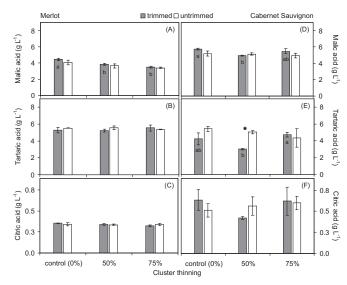


Figure 5. Effects of cluster thinning and shoot trimming on malic acid, tartaric acid and citric acid in berries of 'Merlot' (a, b, c) and 'Cabernet Sauvignon' (d, e, f) grapevines. Data are means \pm se (n = X). Cluster thinning means with different letters in colums are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

In Merlot, total phenolics extracted from seeds were only affected by shoot trimming, where the trimmed vines showed the highest values (Figure 6c). In Cabernet Sauvignon the significant reduction in phenolic compounds in seeds was only caused by cluster thinning (Figure 6f).

Yield pruning weight⁻¹ ratio (Ravaz index) ranged from 1.42 to 3.78 in Cabernet Sauvignon, whereas in Merlot, the ratio ranged from 3.84 to 10.3 among treatments (Figure 7a, b). In both cultivars, trimmed vines showed the highest ratios as compared to untrimmed vines.

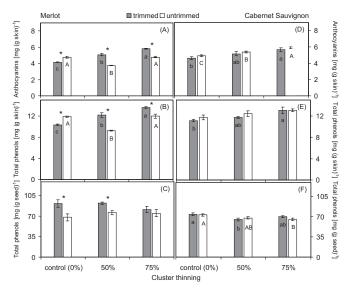


Figure 6. Effects of cluster thinning and shoot trimming on anthocyanin, skin total phenols and seed total phenols in berries of 'Merlot' (a, b, c) and 'Cabernet Sauvignon' (d, e, f) grapevines. Data are means ± se (n = X). Cluster thinning means with different letters in colums (trimmed and untrimmed) are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

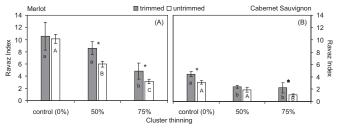


Figure 7. Effects of cluster thinning and shoot trimming on Ravaz Index of 'Merlot' (a) and 'Cabernet Sauvignon' (b) grapevines. Data are means \pm se (n = X). Cluster thinning means with different letters in colums (trimmed and untrimmed) are statistically different (p<0.05). Trimmed and untrimmed treatments in each cluster thinning with * are statistically different (p<0.05).

Several studies have shown that a fruit:pruning-weight ratio (Ravaz index or crop load) of 5 to 10 is an indicator of balanced vines capable of producing high-quality fruit (Braydo et al. 1985, Reynolds 1989, Smart et al. 1990). According to this ratio, Merlot varied between the ideal and undercropped and Cabernet Sauvignon had a tendency to be undercropped in most of pruning treatments. Despite the wide range observed there was no clear relationship between this ratio and fruit composition in both cultivars. The range of fruit composition in the current experiment was small in comparison with the variation in crop level or crop load.

Furthermore, the crop level does not have the same effect on sugar and phenolic composition of the berries. There was a benefit of low yield on total phenolic and anthocyanins contents in both cultivars (Figure 6) as also showed by other authors (MAZZA et al., 1999). However, the highest phenolic compounds levels were observed in vines where the range of crop load was out of the range considered vine balanced (Ravaz index below 5). This result could be better associated to environmental conditions at the cluster zone than to vine balance. Possibly the cluster thinning provides an increasing of light and temperature at the cluster zone. Previous studies have shown that climatic factors, such as light and temperature, exert relevant influence on polyphenol synthesis but minor effects on sugar amount (Wicks and KLIEWER, 1983; SPAYD et al., 2002). Moreover, some studies revealed that phenolic compounds in berries have been more affected by environmental conditions of the seasons than by agronomical practices (Keller et al., 2005).

Our data suggest that in the vineyards under study the fruit environment was kept close to optimum conditions. Although there was an improvement of color intensity shown by an increase in anthocyanins content in the skin of berries of cluster thinned vineyards, the amount of sugar was also improved in berries from non thinned vines. Considering that the sugar accumulation is a limiting factor in berries harvested under high humidity conditions and also that the cluster thinning is potentially an expensive process in terms of labor, time-consuming and reduction of crop yield, the application of this cultural practice should be avoided in vineyards conducted in the south of Minas Gerais State. On the other hand, the shoot trimming should be recommended as a cultural practice only for Merlot because there was a positive effect of tip shoot removal on sugar grape accumulation of unthinned vines.

4. CONCLUSIONS

- 1. The alteration of source-sink relationship in grapevine by cluster thinning and shoot trimming had impact on leaf carbohydrate partitioning, yield and grape quality of field grown Merlot and Cabernet Sauvignon.
- 2. In both cultivars, the fruit load reduction increased leaf starch content, decreased cluster weight and improved fruit color and total phenols in skin berries.
- 3. In Merlot, the cluster thinning decreased the concentration of glucose and fructose in berries of trimmed vines, whereas in Cabernet Sauvignon there was no statistical difference.

4. In Merlot, the shoot trimming increased sugar grape from control vines and phenolics compounds in grapes from thinned vines, whereas in Cabernet Sauvignon there was no positive effect of tip shoot removal on grape composition.

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