Mechanical pruning and soil organic amendments in vineyards of Syrah: effects on grape composition

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ABSTRACT

Aim: The interaction of mechanized pruning systems and soil organic amendment can increase vine productivity. However, since the increase in productivity may affect grape composition, this study aimed to understand the effects of the interaction between these two practices.

Methods and materials: Two field trials were implemented in Shiraz vineyards in two different wine regions. Mechanical hedge pruning was compared with hand spur pruning and four different organic amendments were tested: biochar, municipal solid waste compost, cattle manure and sewage sludge.

Results: Mechanical pruning reduced the total soluble solids (TSS) and pH of the grapes, but had no effects on the other variables. Organic amendments also reduced TSS, especially sewage sludge, and tended to reduce total anthocyanins and total phenols. The effect of the interaction between both factors on grape composition was never significant. A negative relationship between yield and TSS was observed, while titratable acidity had no relationship with yield. Total anthocyanins were also negatively related to yield. A positive relation between TSS and total anthocyanins was also observed.

Conclusions: The results show that the combination of mechanical pruning and soil organic amendment is a powerful tool for increasing productivity, but it has some effects on grape composition.

Significance and impact of study: The reduction in some fruit composition variables showed that, while mechanical pruning does not significantly affect grape composition, the choice of type and amount of organic amendment will depend on the destination of the grapes.

KEYWORDS
mechanical pruning, biochar, cattle manure, municipal solid waste compost, sewage sludge, grape composition.
INTRODUCTION

The adoption of mechanized pruning systems leads to significant increases in production (Clingeleffer, 1984; Lopes et al., 2000; Keller and Mills, 2007). This yield increase is not normally associated with a reduction in grape quality (Gatti et al., 2011; Palma et al., 2010) - except for when production exceeds the productive capacity of vines (Bovio and Lisa, 1996) - and may even, in many cases, result in higher grape quality (Clingeleffer, 2009; Terry and Kurtural, 2011). Adaptation to mechanical pruning is achieved when the rise in production, due to the higher bud load, is compensated by canopy efficiency (Poni et al., 2004).

Dokoozlian (2013) mentions that when crop load is properly managed, box-pruned grapevines will produce similar or improved fruit composition and wine quality compared to hand-pruned grapevines. With its largely unaffected yield and grape composition, this is a solid and reliable approach in a wine market that demands greater efficiency and competitiveness.

Low soil fertility is one of the limiting factors of vineyard productivity in Portugal and it can compromise the production increase promoted by mechanical pruning (Botelho et al., 2020). Low fertility is correlated with low soil organic matter content, which can be improved by applying organic amendments from different sources containing high levels of organic matter and substantial amounts of nutrients (Ribeiro et al., 2009; Fanguereiro et al., 2012; Illera-Vives et al., 2015; Botelho et al., 2020).

According to Morlat and Symoneaux (2008), chemical modifications to the soil due to the application of organic amendments can play a significant role in the nutrient status of the vine and, consequently, affect grape and wine composition. Nitrogen (N), which results from the mineralisation of soil organic matter, is a nutrient that affects vine growth and grape composition. High levels of N tend to delay grape maturation (Spayd et al., 1994; Hilbert et al., 2003), as well as to decrease total soluble solids, maintain titratable acidity, increase pH and decrease polyphenol concentration (Spayd et al., 1994; Morlat and Symoneaux, 2008), even in low vigour vineyards (Gatti et al., 2020).

From the reviewed literature, mechanical pruning seems to be a suitable strategy for reducing production costs and increasing productivity, while the organic amendment of vineyard soil increases productivity (Morlat, 2008) and contributes to tackling the problems associated with predicted climatic changes (Fraga et al., 2012). However, to the best of our knowledge, the interactions between organic amendments and mechanical pruning have not been previously studied. The present study therefore aims to understand the effects of the interaction between those two vineyard practices on future grape composition.

MATERIALS AND METHODS

The trial was carried out over four years (2012 to 2015) on Vitis vinifera L. cv. Shiraz in two vineyards: Quinta do Côro (QC) located in the Tejo wine region and Quinta do Gradil (QG) located in Lisboa wine region. In QC, the vines were grafted onto 99R in 1999 and spaced at 1.0 m x 2.5 m for a density of 4000 plants/ha. In QG, the vines were grafted onto 1103P, were planted in 2005 and spaced at 1.0 m x 2.6 m, for a density of 3846 plants/ha. Row orientation was N-S in both cases. Soil and climate are described in Botelho et al. (2020).

The pruning system and organic amendment were compared in both trial fields in a strip-plot design, with three replications forming a strip-split-plot design. Each block comprised eight adjacent rows to which pruning treatment was randomly carried out, creating two groups of four adjacent rows each with a different pruning treatment. The 60 m rows were divided into five 12 m sections, to which organic amendments were randomly applied. The organic amendments were spread all over the seven inter-rows, creating a stripe perpendicular to the row orientation. Each one of the 30 experimental units consisted of 48 vines.

Two pruning techniques were applied: 1) manual spur pruning (MAN), in which six to seven two-node spurs per vine were retained and 2) mechanical pruning (MEC), in which the pruning effect of four cutting bars was simulated (two parallel to the ground and two perpendicular to the ground) working at a distance of 15 cm from the cordon. For both techniques, the cordon was 70 cm above the soil surface. Canopy management is described in Botelho et al. (2020).

Five organic amendment treatments were applied: 1) no organic amendment or fertiliser (Ctrl), 2) 8500 kg/haper year of biochar (Bioe), 3) 16100 kg/ha per year of municipal solid waste compost (MSWC), 4) 24000 kg/ha per year of...
cattle manure (Manure), and 5) 34000 kg/ha per year of sewage sludge (Sludge). The quantity of each organic amendment is expressed in fresh weight and its definition is based on the application of 5000 kg of dry organic matter per ha and per year. The composition of the organic amendments is given in Botelho et al. (2020). The organic amendments were scattered by hand.

Before bud burst, the organic amendments were spread over the soil and incorporated with a light disk harrow. The nutrients supplied by each organic amendment per year are as follows:

- Bioc: 72.0 kg/ha of total N, 7.2 kg/ha of total P and 43.8 kg/ha of total K;
- MSWC: 226.0 kg/ha of total N, 110.4 kg/ha of total P and 124.9 kg/ha of total K;
- Manure: 179.4 kg/ha of total N, 101.9 kg/ha of total P and 432.2 kg/ha of total K;
- Sludge: 503.7 kg/ha of total N, 460.5 kg/ha of total P and 108.6 kg/ha of total K.

In order to determine yield, clusters from each of six previously selected vines in each replication vine were weighed at harvest.

Grape total soluble solids (TSS), titratable acidity (TA), pH, total anthocyanins and total phenols were analysed in 100 berry samples, which had been randomly collected at harvest from each block and treatment (Harvest dates: QC: 11/09/2013, 8/09/2014 and 15/09/2015; QG: 26/09/2013, 15/09/2014 and 29/09/2015). TSS, TA and pH were analysed according to OIV recommended methods (OIV, 2019). For the anthocyanin and phenol analysis, grape extraction was carried out according to the method proposed by Carbonneau and Champagnol (1993): the skins and seeds were macerated for 24 h at 20 °C, using ethanol (96 %) and tartaric acid solution at pH 3.2 as solvents. Then the extract was centrifuged at 1096 g for 10-15 min before being used. Spectrophotometric analyses were performed for total anthocyanins (Ribéreau-Gayon and Stonestreet, 1965) and total phenols (Ribéreau-Gayon, 1970).

Data for all the variables were tested to verify if the assumptions of analysis of variance (ANOVA) were met using Shapiro-Wilk’s test. The data was then subjected to a three-way ANOVA (site x pruning x organic amendment), using the general linear procedure for the strip-split-plot design and F-test. The significance level was set at α = 0.05 and means were separated using Tukey’s honestly significant difference test. The statistical analysis was performed using Statistix software package (version 9.0; Analytical Software, Tallahassee, FL). Regression analysis was used to study the relationships between continuous variables.

**RESULTS**

Yield was increased by mechanical pruning in 2014 and by organic amendments in the last two years of the study, with the exception of Bioc. The effect of the interaction between pruning system and organic amendments on yield was significant in all three years with the organic amendments having significant effects in MEC only (Botelho et al., 2020).

Table 1 shows the results of yield and TSS (°Brix) and TA of grape musts from the 2013, 2014 and 2015 vintages of the Quinta do Côro (QC) and Quinta do Gradil (QG) experimental sites. Data from 2012 are not shown because no significant effects were found and the results are reported in Correia (2014). The pruning system affected TSS in all three years of the experiment, with the MEC treatments producing musts with lower TSS. Even though the tendency was always the same, there were only differences in QC in 2014 and 2015. The juice pH was lower with MEC every year, but in 2015 the differences only existed in QC (Table 2). On the other hand, TA was higher with MEC in 2013 only.

In terms of organic amendments, Sludge showed the lowest TSS values, while Ctrl and Bioc tended to obtain the highest values. Meanwhile the differences in juice pH and TA were mainly not significant (with the exception of pH in 2014, which was only different in QG), with a random relationship between treatments and none standing out from the rest.

Total anthocyanins and total phenols were not affected by the pruning system (Table 2). On the other hand, in 2015, the organic amendments had an effect on both variables, with Ctrl having higher levels of total anthocyanins and total phenols than MSWC and Sludge; meanwhile Sludge had lower levels when compared to all the other treatments.

The interaction between treatments (pruning system x organic amendment) was not significant in any of the three years.

The relationship between yield and grape TSS is depicted in Figure 1. There was a tendency for a decrease in TSS with increase in yield.
There was a consistent difference between QC and QG, and the covariance analysis showed that the lines of the two sites were parallel, with QG having less TSS when compared to QC. The relationship between the two variables was high, especially in QC.

The relationship between yield and TA is depicted in Figure 2. There was no relationship between TA and yield. QC grapes tended to have less TA than those from QG. However, for a wide range of yield levels no differences were observed in TA.

The relationship between total anthocyanins and yield (Figure 3) was inverse, with total anthocyanins decreasing as yield increased; however, the correlation between both variables was sometimes weak. In 2014, an increase in total anthocyanins with increased yield was observed for the grapes from hand-pruned vines in QC, but again the correlation between both variables was weak ($r^2 = 0.21$). No consistent differences were observed in this correlation between pruning system and site.

The relationship between TSS and total anthocyanins (Figure 4) shows a good correlation between both variables, with an increase in total anthocyanins with the increase in TSS.

DISCUSSION

Some physical-chemical characteristics of grapes (Tables 1 and 2) were affected by the three factors in the study. Overall, TSS was negatively affected by MEC in all three years, as observed by other authors (Reynolds, 1988; Clingeleffer and Krake, 1992; Bovio and Lisa, 1996; Pérez-Bermúdez et al., 2015). However, there was a correlation between the TSS decrease and the yield increase induced by MEC, particularly as the organic amendments (namely Manure, MSWC and Sludge) provided more principal macronutrients, particularly N, and increased...
When the yield was similar in both MEC and MAN treatments there were no considerable differences in TSS between both treatments (Figure 1).

The higher yield associated with lower leaf area to fruit ratio delays ripening (Clingeleffer, 1988; Spayd et al., 1994; Wessner and Kurtural, 2013; Zheng et al., 2017). Thus, it can be assumed that if the harvest of the MEC grapes had been postponed the results would have been different (Zheng et al., 2017). In the case of QC, some delay in the harvest of MEC grapes would not be problematic, because September is usually a dry month in the Tejo wine region. However, in QG it could be a problem since the harvest would be postponed to October when rainfall usually occurs in Lisboa wine region and Botrytis cinerea infections would most likely occur.

Although the TSS in grapes was reduced by the increase in yield, the total sugar production (data not shown) increased in the treatments with higher yield, as also observed by Pérez-Bermúdez et al. (2015). In addition to the reduction in TSS caused by the increase in productivity, there was a significant tendency for Sludge grapes to have lower TSS when compared to the other organic amendments, including those with similar yields. Hilbert et al. (2003) and Delgado et al. (2004) had also observed a delay in ripening due to high N supply, without an increase in productivity or a decrease in leaf to fruit ratio. Delgado et al. (2004) and Korboulewsky et al. (2004) attribute the decrease in TSS in berries to the increase in vine vigour caused by high N supply, which changes the balance in carbon partitioning, thus favouring vegetative growth over reproductive growth.
FIGURE 1. Influence of pruning system, organic amendments and site on the relation between yield and total soluble solids of Shiraz grapes, in Tejo and Lisboa Wine Regions.

Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: see legend.

Regression equations:
\[
y = -0.75x + 27.77, \quad r^2 = 0.77 \text{ (MEC QC)};
\]
\[
y = -0.72x + 28.10, \quad r^2 = 0.71 \text{ (MAN QC)};
\]
\[
y = -0.67x + 23.76, \quad r^2 = 0.40 \text{ (MEC QG)};
\]
\[
y = -0.52x + 23.28, \quad r^2 = 0.52 \text{ (MAN QG)}.
\]

FIGURE 2. Influence of pruning system, organic amendments and site on the relation between yield and total acidity of Shiraz grapes from Tejo and Lisboa Wine Regions, Portugal.

Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: see legend.

Regression equations:
\[
y = 0.019x + 5.37, \quad r^2 = 0.0198 \text{ (MEC QC)};
\]
\[
y = 0.074x + 4.75, \quad r^2 = 0.1250 \text{ (MAN QC)};
\]
\[
y = 0.005x + 6.29, \quad r^2 = 0.0001 \text{ (MEC QG)};
\]
\[
y = 0.172x + 5.32, \quad r^2 = 0.1896 \text{ (MAN QG)}.
\]
FIGURE 3. Influence of pruning system, organic amendments and site on the relation between yield and total anthocyanins of Shiraz grapes from Tejo and Lisboa Wine Regions, Portugal in 2013 (a), 2014 (b) and 2015 (c). Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions: see legend. Regression equations:

a) $y = -204.9x + 2807.5, r^2 = 0.98$ (MEC QC);
$y = -269.5x + 2615.8, r^2 = 0.53$ (MAN QC);
$y = -139.1x + 1900.9, r^2 = 0.86$ (MEC QG);
$y = -132.4x + 1820.8, r^2 = 0.11$ (MAN QG).

b) $y = -63.8x + 1703.7, r^2 = 0.86$ (MEC QC);
$y = 240.7x + 109.9, r^2 = 0.21$ (MAN QC);
$y = -102.4x + 1356.4, r^2 = 0.17$ (MEC QG);
$y = -181.1x + 1348.2, r^2 = 0.12$ (MAN QG).

c) $y = -84.8x + 1612.3, r^2 = 0.59$ (MEC QC);
$y = -254.2x + 2886.6, r^2 = 0.66$ (MAN QC);
$y = -124.6x + 2002.2, r^2 = 0.63$ (MEC QG);
$y = -206.5x + 2350.8, r^2 = 0.85$ (MAN QG).

FIGURE 4. Relationship between total soluble solids and total anthocyanins of Shiraz grapes from Tejo and Lisboa Wine Regions, Portugal. Data represent single treatment (pruning system × organic amendment × site) averages and data were pooled over repetitions. Regression equation: $y = 16.94x^2 - 637.8x + 6968.5, r^2 = 0.60.$
In this study, vine vigour (Botelho et al., 2020) tended to be higher in Sludge, when compared to Ctrl and Bioc, and is probably the cause of the lower TSS value.

In MEC, there was a clear tendency for grape pH to decrease; this was also observed by Morris and Cawthon (1981), Clingeleffer and Krake (1992) and Holt et al. (2008). The lower pH in MEC is probably related to a delay in ripening and/or to a lower potassium concentration in grapes (data not shown) (Conde et al., 2007).

In general, grape juice TA was not affected by pruning, although TA values were higher in 2013 with MEC treatments. Similar results have been obtained by other authors (Clingeleffer, 1988; Holt et al., 2008; Gatti et al., 2011). Given that cluster exposure to sunlight was higher in MEC treatments (Botelho et al., 2020), grapes from those treatments would be expected to have lower TA, as reported by Reynolds et al. (1986). The temperature of exposed clusters is higher (Smart and Sinclair, 1976) and TA is inversely correlated to the temperature to which the grapes are subjected (Spayd et al., 2002), because the reduction in TA during ripening is related to the respiration rate of berries, which in turn is a function of temperature (Jackson and Lombard, 1993).

Slight differences were observed in grape pH between the different organic amendments in 2014, but they are not relevant from a practical point of view. Therefore, organic amendments did not affect pH or TA of grapes, as observed by other authors (Gaiotti et al., 2017; Morlat and Symoneaux, 2008).

Total anthocyanins was not affected by the pruning system in any of the three years. Other studies also found no differences when mechanical pruning was applied (Main and Morris, 2008; Poni et al., 2004; Wessner and Kurtheral, 2013). Some studies observed an increase in total anthocyanins, (Brillante et al., 2018; Holt et al., 2008; Zheng et al., 2017), but this is more likely due to a smaller berry size than to greater anthocyanin synthesis per unit area of berry skin (Zheng et al., 2017). There is a positive relationship between TSS and total anthocyanins (Figure 4); therefore, as MEC grapes have lower TSS, they would also be expected to have lower total anthocyanins. As a matter of fact, there was a tendency for grapes from MEC to have lower total anthocyanins when the differences in TSS were higher; however, when the difference in TSS was lower (2013), no differences were found in total anthocyanins.

An increase in total anthocyanins with TSS was reported by Pirie and Mullins (1977), but they found a better correlation with skin sugar content, which was not assessed in this study. Other authors have also observed higher total anthocyanins with higher TSS (Castellarin et al., 2007; Palliotti and Cartechini, 2000; Xi et al., 2018). Darné (1993) suggested the existence of two metabolic pathways for anthocyanin synthesis: one from the tannins accumulated in seeds and skins before veraison, and the other from sugars in senescent leaves.

With respect to the effect of organic amendments on total anthocyanins, there were only differences between treatments in 2015, with lower values in MSWC and Sludge compared to Ctrl. The higher yield observed in these treatments, and the consequent delay in ripening, may be related to the decrease in anthocyanins (Figure 3). A negative relationship between yield and total anthocyanins has also been reported in other studies (Palliotti and Cartechini, 2000; Xi et al., 2018). However, according to Hilbert et al. (2003), a high N supply interferes with the metabolic pathway of anthocyanins by delaying quantitative and qualitative biosynthesis, and it enhances their degradation in the final steps of berry maturation. Therefore, both these phenomena may have led to the lower total anthocyanins in MSWC and, particularly, in Sludge. In the sludge treatment, the high phosphorus supply may also have been the cause of the lower total anthocyanins: when in excess, P can inhibit the induction of phenylalanine ammonia-lyase and chalcone synthase activity, leading to reduced anthocyanin concentrations in the berries (Kakegawa et al., 1995).

Total phenols were not affected by the pruning system, as has been observed by other authors (Main and Morris, 2008; Poni et al., 2004; Wessner and Kurtheral, 2013). In our four-year study, the organic amendments did not affect total phenols. Other authors, such as Gaiotti et al. (2017) and Morlat and Symoneaux (2008), observed a decrease in grape total phenols; however, their results were obtained after longer-term applications of organic amendments (for 5 and 17 years respectively).

CONCLUSIONS

The results show that the increase in yield promoted by mechanical pruning and soil organic amendment decreased grape TSS, while TA, total anthocyanins and total phenols were not affected. On the other hand, pH was decreased by mechanical
pruning only. A negative relationship between yield and total anthocyanins was observed, especially due to the increase in productivity that some organic amendments promoted. Total anthocyanins showed a positive relationship with TSS.

During a time when the market demands wines with lower alcohol concentration, the application of both mechanical pruning and soil organic amendment seems to be a suitable strategy for achieving this goal without affecting other berry composition variables. Moreover, the reduction in pH favours wine stability. Such pH reduction is particularly important in warm Mediterranean conditions, especially in a climate change scenario.

Overall, the application of both mechanical pruning and soil organic amendment is a viable option for reducing management costs, increasing yield and producing grapes suitable for the modern market. However, the amounts used in this experiment are not viable since they supply too much N and P. Since the application of sludge and municipal solid waste compost can lead to a reduction in total anthocyanins and total phenols, it is important to take into account the destination market of the grapes when making decisions about their use in the vineyard and the quantities to be applied.

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