

Rootstock-scion interaction: 3. Effect on the composition of Cabernet Sauvignon wine

Alberto Miele¹ & Luiz Antenor Rizzon²

Abstract - The interaction among rootstock, scion and environment may induce different responses to the grapevine physiology and consequently to the grape and wine composition. The vineyards of Serra Gaúcha, Brazil, are established in soils that may have different physicochemical attributes. Furthermore, the grapevines are grafted on a wide diversity of rootstocks. Therefore, this study aimed to determine their effect on the wine composition of the Cabernet Sauvignon (CS) grafted on Rupestris du Lot, 101-14 Mgt, 3309 C, 420A Mgt, 5BB K, 161-49 C, SO4, Solferino, 1103 P, 99 R, 110 R, Gravesac, Fercal, Dogridge and Isabel, featuring some genetic diversity altogether. The experimental design was in randomized blocks, with 15 treatments, three replicates and 10 vines per plot. The grapes were harvested at maturity for three years, and then wines were made in glass recipients of 20 L. When alcoholic and malolactic fermentations were over, the wine analyses were performed on twenty-five variables related to alcohol, acidity, dry extract, polyphenols and volatile compounds. The main results show that all variables were affected year by year, some of them by the rootstock and a few by the interaction between rootstock and year. The rootstock effect was observed mainly on variables related to alcohol, acidity and ashes. Results show that the CS/101-14 Mgt wine had higher alcohol content than CS/Dogridge and CS/Isabel wines, which was probably due to the 101-14 Mgt rootstock favoring an early grape ripening. However, higher pH values were observed in the CS/Rupestris du Lot, CS/5BB K and CS/Gravesac than CS/420A Mgt, CS/110 R and CS/Isabel wines. These results show that there is a diversity of rootstocks that can be used by the growers due to the Cabernet Sauvignon wine composition similarities.

Index terms: *Vitis vinifera* L., grapevine, phenolic compounds, volatile substances.

Interação entre porta-enxerto e copa: 3. Efeito na composição do vinho Cabernet Sauvignon

Resumo - A interação entre porta-enxerto, copa e meio ambiente pode induzir respostas diferenciadas na fisiologia da videira e, conseqüentemente, na composição da uva e do vinho. Os vinhedos da Serra Gaúcha são estabelecidos em diferentes tipos de solo, cada um com diferentes propriedades físico-químicas. Além disso, as videiras são enxertadas em uma ampla diversidade de porta-enxertos. Portanto, este estudo teve como objetivo determinar seu efeito na composição do vinho Cabernet Sauvignon (CS) proveniente de videiras enxertadas em Rupestris du Lot, 101-14 Mgt, C 3309, 420A Mgt, K 5BB, C 161-49, SO4, Solferino, P 1103, R 99, R 110, Gravesac, Fercal, Dogridge e Isabel, que, juntos, possuem certa diversidade genética. O delineamento experimental foi em blocos casualizados, com 15 tratamentos, três repetições, 10 plantas por parcela. As uvas foram colhidas durante três anos, na maturidade, e os vinhos foram feitos em recipientes de vidro de 20 L. As análises dos vinhos foram realizadas quando as fermentações alcoólica e maloláctica foram concluídas. Avaliaram-se 25 variáveis relacionadas a álcool, acidez, extrato seco, polifenóis e compostos voláteis. Os principais resultados mostram que todas as variáveis foram afetadas significativamente pelo ano, algumas pelo porta-enxerto e poucas pela interação porta-enxerto e ano. O efeito do porta-enxerto foi observado principalmente em relação ao álcool, à acidez e às cinzas. Os resultados mostram que o teor de álcool foi maior no vinho CS/101-14 Mgt que nos vinhos CS/Dogridge e CS/Isabel, devido, provavelmente, ao 101-14 Mgt favorecer uma maturação mais precoce da uva. Entretanto, valores de pH mais elevados foram constatados nos vinhos CS/Rupestris du Lot, CS/K 5BB e CS/Gravesac que nos CS/420A Mgt, CS/R 110 e CS/Isabel. Estes resultados mostram que há uma diversidade de porta-enxertos que pode ser usada pelos produtores, devido à similaridade da composição do vinho Cabernet Sauvignon.

Termos para indexação: *Vitis vinifera* L., videira, compostos fenólicos, substâncias voláteis.

Corresponding author:
alberto.miele@embrapa.br
Received: Janeiro 24, 2017.
Accepted: July 13, 2017.

Copyright: All the contents of this journal, except where otherwise noted, is licensed under a Creative Commons Attribution License.



¹Agronomist, Dr., Embrapa Uva e Vinho, Bento Gonçalves-RS, Brasil. E-mails: alberto.miele@embrapa.br ORCID 0000-0001-9465-570X

²Enologist, Dr., Embrapa Uva e Vinho, Retired. E-mail: luiz.rizzon@terra.com.br

Introduction

The vineyards are established with grafted grapevines in most wine regions worldwide. This is mainly due to the possibility of vine roots being fed by the insect phylloxera (*Daktulosphaira vitifoliae* Fitch, Homoptera: Phylloxeridae), which can cause damage to the vines and, therefore, affecting the vineyard yield, grape quality and, consequently, the property economy. Therefore, a question arises: what rootstock should be used? Indeed, the choice of a rootstock by growers depends on some characteristics due to the interaction between rootstock, scion and environment. The purpose of the production is another important point to be considered.

The characteristics, typicality and quality of wines are a complex issue, which depends on several factors. Indeed, they depend primarily on the grapevine genetics, environment, cultural practices used in the vineyards and enological procedures during the winemaking process. Among the main group of substances influencing the wine composition and characteristics, stand out organic acids, phenolic compounds, aromatic substances, minerals and amino acids. A substance can act alone or interacts with others.

Grafting vines is one of the cultural practices that can influence yield components (MIELE; RIZZON, 2017), and consequently have effect on grape composition (MIELE et al., 2009a; LEÃO et al., 2011; JOGALIAH et al., 2013; CHOU; LI, 2014; GONG et al., 2014; SOUZA et al., 2015). If rootstocks can affect the grapevine yield components and the grape composition, they can also influence the wine composition. Researches related to this topic were conducted in the main winemaking countries of the world. There are not many, however they cover a wide range on wine composition. These works are related to its general composition (HEDBERG et al., 1986; MAIN et al., 2002; STEVENS et al., 2016), phenolic compounds (HALE; BRIEN, 1978; CIRAMI et al., 1984; WALKER et al., 1998; HARBERTSON; KELLER, 2012; JOGALIAH et al., 2015; SILVA et al., 2015), volatile compounds (OUGH et al., 1968), potassium (WALKER; BLACKMORE, 2012), chloride (WALKER et al., 2010), fermentation process (STOCKERT et al., 2013) and the wine general quality (HALE; BRIEN, 1978; OLLAT et al., 2003; RENOUF et al., 2010; WOOLDRIDGE et al., 2010).

As the results of these works may differ from one another and that the place where the research is done may have important role in the composition of the wine, an experiment was conducted to determine the effect of 15 rootstocks on the Cabernet Sauvignon wine composition made in Serra Gaúcha, Brazil.

Material and Methods

The experiment was carried out for three years in a

Cambissolo soil of Embrapa Uva e Vinho — coordinates: 29°09'44" S and 51°31'50" W; altitude: 640 m — where the climatological normal temperature is 17.3°C and 1,683 mm for rain. The vineyard was established in a Cambissolo soil (FLORES et al., 2012), which is equivalent to an Inceptisol, according to the Soil Taxonomy. The presence or absence of fungi, insects and nematodes was not evaluated. Data related to the vineyard, such as soil preparation, planting, trellising, spaces between rows and plants, pruning and training grapevines, canopy management and control of diseases, pests and weeds were described in previous paper (MIELE; RIZZON, 2017).

The treatments consisted of Cabernet Sauvignon (*Vitis vinifera* L.) grapevines grafted on 15 rootstocks, i.e., Rupestris du Lot, 101-14 Millardet et de Grasset, 3309 Couderc, 420A Millardet et de Grasset, 5BB Kober, 161-49 Couderc, SO4, Solferino (local name of an unknown rootstock), 1103 Paulsen, 99 Richter, 110 Richter, Gravesac, Fercal, Dogridge and Isabel (*V. labrusca* L.). In fact, Isabel is not a rootstock, but the most widely cultivated grapevine (mainly own rooted) in Serra Gaúcha, with the production going to wineries to make wine and grape juice.

The experimental design was randomized blocks, with 15 treatments (CS/rootstocks), three replicates, 10 vines per plot. The area of each block was 675 m² and the entire experiment 2,025 m².

The grape ripening was evaluated by measuring the total soluble solids (°Brix) content of the grapes of 45 plots, which was done by a hand refractometer. When the total soluble solids of the grape juice were stabilized, the grapes were harvested. Then, they were placed in plastic boxes, weighted and taken to the winery close to the vineyard for processing. The grapes were crushed, destemmed and the liquid and solid phases were transferred to 20-L glass recipients. Sucrose was not added to the grape musts for the sugar correction. Then, 50 mg L⁻¹ of SO₂ were added to each recipient. In addition, 0.20 g L⁻¹ of active dry yeast (*Saccharomyces cerevisiae*) was added and the glass recipients were fitted with rubber stoppers and water-filled airlocks. After eight days of maceration and alcoholic fermentation, the wines were pressed off the skins and transferred to 9-L glass recipients also fitted with rubber stoppers and water-filled airlocks. These recipients were kept at 24°C±1°C until sugar concentration was less than 4.0 g L⁻¹. The malolactic fermentation was naturally processed, which was regularly evaluated by paper chromatography, and then total SO₂ was adjusted to 50 mg L⁻¹. When this fermentation was over, the wines were transferred to 750-mL glass bottles, sealed with cork, and stored at 15°C in a temperature-controlled room.

In the same year, the following variables were determined: density, alcohol, titratable acidity, volatile acidity, fixed acidity, pH, dry extract, reducing sugars, reduced dry extract, alcohol in weight/reduced dry extract,

ashes, alkalinity of ashes, absorbance – 420 nm (I 420) and 520 nm (I 520) –, color intensity (I 420 + I 520), hue (I 420/I 520), anthocyanins, tannins, total polyphenols index, volatile compounds – ethyl acetate, methanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol + 3-methyl-1-butanol and sum of higher alcohols.

The classical variables were determined by physicochemical methods (RIBÉREAU-GAYON et al., 1982); anthocyanins, by pH difference; tannins, by acid hydrolysis; absorbance at 420 nm and 520 nm, by UV/VIS spectrophotometry using a 1-mm path length cell (RIBÉREAU-GAYON; STONESTREET, 1965, 1966).

The volatile compounds were determined by a Perkin Elmer GS AutoSystem XL gas chromatograph with flame ionization detection, equipped with a 60 m length capillary column, polyethylene glycol WAX stationary phase (N9316406). The wine samples (3 µL) were directly injected into the chromatograph and the internal standard was a 10% solution of 4-methyl-2-pentanol at 1 g L⁻¹ (BERTRAND, 1975).

The data were submitted to Anova and Tukey's multiple range test, and correlations among the variables were determined.

Results and Discussion

The effect of 15 rootstocks on the physicochemical composition of Cabernet Sauvignon wine is shown in Tables 1, 2 and 3. All variables were affected by the year, some by the rootstock and few for the year and rootstock interaction. The rootstock effect was observed mainly on variables related to alcohol, acidity and ashes, and few on volatile substances.

The density (Table 1) was higher ($p < 0.01$) in the CS/Dogridge wine and lower in CS/101-14 Mgt and CS/420A Mgt wines which is in accordance with results of the alcohol content where the CS/101-14 Mgt wine had the highest content ($p < 0.01$) and CS/Dogridge and CS/Isabel the lowest. The titratable acidity and fixed acidity were higher ($p < 0.05$) in the CS/Isabel wine and lower in CS/3309 C. However, the pH had a different behavior because higher values ($p < 0.001$) were found in the CS/Rupestris du Lot, CS/5BB K and CS/Gravesac wines and lower in CS/420A Mgt, CS/110 R and CS/Isabel. These differences were probably because the pH indicates the real concentration of H⁺ ions that are ionized or dissociated in the solution while titratable acidity estimates the quantity of titratable acids.

Table 1. Rootstock effect on the physicochemical composition of the Cabernet Sauvignon wine in the wine-growing region of Serra Gaúcha-RS, Brazil, over three years.

| | Density (g mL ⁻¹) | Alcohol (% v v ⁻¹) | Titratable acidity (meq L ⁻¹) | Volatile acidity (meq L ⁻¹) | Fixed acidity (meq L ⁻¹) | pH | Dry extract (g L ⁻¹) | Reducing sugars (g L ⁻¹) | Reduced dry extract (g L ⁻¹) |
|---------------------------------|----------------------------------|-----------------------------------|---|---|--|----------------------|-------------------------------------|--|--|
| Rootstock | | | | | | | | | |
| Rupestris du Lot | 0.9952 ^a ab | 11.58 ab | 71 ab | 12 a | 58 ab | 3.88 a | 24.0 a | 2.67 a | 22.4 a |
| 101-14 Mgt | 0.9947 b | 12.04 a | 72 ab | 14 a | 58 ab | 3.77 abcd | 24.2 a | 2.94 a | 22.2 a |
| 3309 C | 0.9950 ab | 11.54 ab | 69 b | 13 a | 56 b | 3.84 abc | 23.2 a | 2.55 a | 21.7 a |
| 420A Mgt | 0.9948 b | 11.52 ab | 72 ab | 13 a | 58 ab | 3.72 bcd | 22.6 a | 2.68 a | 20.9 a |
| 5BB K | 0.9957 ab | 11.44 ab | 72 ab | 14 a | 57 ab | 3.88 a | 24.8 a | 3.18 a | 22.6 a |
| 161-49 C | 0.9950 ab | 11.78 ab | 72 ab | 13 a | 58 ab | 3.75 abcd | 23.9 a | 2.80 a | 22.1 a |
| SO4 | 0.9955 ab | 11.50 ab | 70 ab | 13 a | 57 ab | 3.84 abc | 24.3 a | 2.80 a | 22.5 a |
| Solferino | 0.9955 ab | 11.35 ab | 72 ab | 14 a | 58 ab | 3.82 abcd | 23.9 a | 2.79 a | 22.1 a |
| 1103 P | 0.9951 ab | 11.30 ab | 73 ab | 13 a | 59 ab | 3.78 abcd | 23.1 a | 2.52 a | 21.6 a |
| 99 R | 0.9949 ab | 11.35 ab | 72 ab | 12 a | 59 ab | 3.75 abcd | 22.9 a | 2.46 a | 21.5 a |
| 110 R | 0.9950 ab | 11.48 ab | 72 ab | 13 a | 59 ab | 3.70 cd | 23.3 a | 2.75 a | 21.5 a |
| Gravesac | 0.9951 ab | 11.76 ab | 70 ab | 13 a | 56 ab | 3.87 a | 24.7 a | 2.59 a | 23.1 a |
| Fercal | 0.9951 ab | 11.17 ab | 73 ab | 12 a | 61 ab | 3.74 abcd | 22.1 a | 2.42 a | 20.7 a |
| Dogridge | 0.9959 a | 10.98 b | 71 ab | 13 a | 57 ab | 3.84 abc | 23.3 a | 2.52 a | 21.8 a |
| Isabel | 0.9956 ab | 10.81 b | 74 a | 12 a | 61 a | 3.68 d | 21.6 a | 2.43 a | 20.2 a |
| Year | | | | | | | | | |
| 1999 | 0.9957 a | 10.64 b | 79 a | 10 c | 70 a | 3.61 c | 22.2 b | 2.23 b | 21.0 b |
| 2000 | 0.9955 a | 10.48 b | 66 c | 11 b | 55 b | 3.76 b | 20.5 c | 2.08 b | 19.4 c |
| 2001 | 0.9945 b | 13.21 a | 71 b | 19 a | 51 c | 4.02 a | 27.7 a | 3.72 a | 25.0 a |
| Significance^b | | | | | | | | | |
| R | 0.0016** | 0.0084** | 0.0277* | 0.3846 ^{ns} | 0.0196* | <0.0001*** | 0.1626 ^{ns} | 0.7889 ^{ns} | 0.0621 ^{ns} |
| Y | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** |
| R x Y | 0.8628 ^{ns} | 0.4093 ^{ns} | 0.6627 ^{ns} | 0.4223 ^{ns} | 0.4700 ^{ns} | 0.2493 ^{ns} | 0.4383 ^{ns} | 0.7961 ^{ns} | 0.2334 ^{ns} |

^aMeans within columns, for rootstock and year separately, followed by different small letters differ significantly by Tukey's multiple range test; ^bSignificance (p value) of rootstock (R), year (Y), rootstock and year interaction (R x Y); * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = not significant.

The ashes (Table 2) were also significantly ($p < 0.001$) affected by rootstock, where CS/Gravesac and CS/Dogridge wines showed the highest contents and CS/420A Mgt, CS/161-49 C and CS/110 R the lowest. The alkalinity of ashes showed almost the same behavior, because CS/Dogridge had the highest value and CS/161-49 C and CS/110 R the lowest ($p < 0.001$). Among the volatile substances (Table 3), the rootstock had only effect on ethyl acetate, whose concentration in wine was higher ($p < 0.01$) in CS/101-14 Mgt and lower in CS/99 R, CS/Fercal, CS/Dogridge and CS/Isabel. However, there was no effect ($p > 0.05$) of rootstock in the variables volatile acidity, dry extract, reducing sugars, reduced dry extract (Table 1), alcohol/reduced dry extract, all polyphenols (Table 2) and most volatile substances (Table 3).

Considering the three-year average data, there was no significant correlation ($p > 0.05$) between grapevine yield, although the yield/vine varied from 8.99 kg plant⁻¹

(CS/Isabel) to 16.21 kg plant⁻¹ (CS/110 R) (MIELE; RIZZON, 2017), and the variables evaluated in wine in this work. However, there was a significant effect among some variables related to wine composition. Therefore, there was a significant and negative correlation between the wine density and its alcohol content ($r = -0.64$, $p < 0.05$). In fact, the values of these two variables in CS/Dogridge wine were 0.9959 g mL⁻¹ and 10.98% v v⁻¹, respectively, and that of CS/101-14 Mgt were 0.9947 g mL⁻¹ and 12.04% v v⁻¹, i.e., the alcohol content was 9.65% higher in the last combination. It is known that this rootstock presents low to medium vigor (HARDIE; CIRAMI, 1988), allowing for this earlier grape ripening.

There are very few works concerning the effect of rootstock in the alcohol content of wines. One of them shows that the grapevine IAC 116-31 Rainha grafted on IAC 766 Campinas had higher alcohol than the same cultivar grafted on 106-8 Riparia do Traviú, but there

Table 2. Rootstock effect on the physicochemical composition of the Cabernet Sauvignon wine in the wine-growing region of Serra Gaúcha-RS, Brazil, over three years.

| | Alcohol in weight/ Reduced dry extract | Ashes (g L ⁻¹) | Alkalinity of ashes | I 420 | I 520 | Color intensity (I 420 + I 520) | Hue (I 420/I 520) | Tannins (g L ⁻¹) | Anthocyanins (mg L ⁻¹) | Total polyphenols index (I 280) |
|---------------------------------|--|-------------------------------|------------------------|------------------------|------------------------|--|-------------------------|---------------------------------|---------------------------------------|--|
| Rootstock | | | | | | | | | | |
| Rupestris du Lot | 4.13 ^a | 3.43 abc | 35.8 abc | 0.361 a | 0.466 a | 0.889 a | 0.77 a | 1.81 a | 334.8 a | 40.1 a |
| 101-14 Mgt | 4.34 a | 3.26 abc | 32.5 abc | 0.371 a | 0.511 a | 0.917 a | 0.72 a | 1.93 a | 348.2 a | 41.7 a |
| 3309 C | 4.25 a | 3.37 abc | 33.4 abc | 0.361 a | 0.476 a | 0.898 a | 0.75 a | 1.84 a | 347.5 a | 39.3 a |
| 420A Mgt | 4.40 a | 2.92 c | 32.6 abc | 0.326 a | 0.439 a | 0.822 a | 0.74 a | 1.71 a | 307.0 a | 37.1 a |
| 5BB K | 4.05 a | 3.45 ab | 36.7 abc | 0.322 a | 0.390 a | 0.767 a | 0.80 a | 1.83 a | 299.1 a | 36.9 a |
| 161-49 C | 4.32 a | 2.93 bc | 30.9 c | 0.321 a | 0.413 a | 0.801 a | 0.76 a | 1.65 a | 258.4 a | 36.1 a |
| SO4 | 4.08 a | 3.41 abc | 36.8 ab | 0.353 a | 0.443 a | 0.830 a | 0.78 a | 1.74 a | 297.1 a | 40.0 a |
| Solferino | 4.12 a | 3.33 abc | 35.7 abc | 0.320 a | 0.404 a | 0.778 a | 0.78 a | 1.68 a | 276.3 a | 36.9 a |
| 1103 P | 4.17 a | 3.36 abc | 35.3 abc | 0.347 a | 0.464 a | 0.868 a | 0.74 a | 1.86 a | 308.5 a | 38.6 a |
| 99 R | 4.22 a | 3.21 abc | 33.2 abc | 0.354 a | 0.482 a | 0.899 a | 0.74 a | 1.77 a | 320.9 a | 38.1 a |
| 110 R | 4.28 a | 3.03 bc | 31.8 bc | 0.348 a | 0.479 a | 0.882 a | 0.72 a | 1.95 a | 312.1 a | 39.2 a |
| Gravesac | 4.07 a | 3.56 a | 37.0 ab | 0.371 a | 0.463 a | 0.895 a | 0.79 a | 1.78 a | 329.1 a | 39.9 a |
| Fercal | 4.32 a | 3.15 abc | 33.7 abc | 0.333 a | 0.451 a | 0.837 a | 0.74 a | 1.67 a | 291.1 a | 36.3 a |
| Dogridge | 4.04 a | 3.65 a | 37.8 a | 0.323 a | 0.413 a | 0.790 a | 0.78 a | 1.54 a | 291.6 a | 36.7 a |
| Isabel | 4.30 a | 3.17 abc | 33.5 abc | 0.334 a | 0.461 a | 0.856 a | 0.71 a | 1.63 a | 307.3 a | 38.3 a |
| Year | | | | | | | | | | |
| 1999 | 4.06 b | 2.90 c | 32.6 b | 0.296 b | 0.428 b | 0.724 b | 0.70 c | 1.32 b | 231.8 c | 30.7 b |
| 2000 | 4.33 a | 3.19 b | 33.0 b | 0.238 c | 0.319 c | 0.557 c | 0.76 b | 1.04 c | 321.0 b | 29.1 b |
| 2001 | 4.24 a | 3.77 a | 38.0 a | 0.496 a | 0.605 a | 1.101 a | 0.82 a | 2.94 a | 373.1 a | 55.4 a |
| Significance^b | | | | | | | | | | |
| R | 0.0594 ^{ns} | <0.0001 ^{***} | 0.0004 ^{***} | 0.1324 ^{ns} | 0.0503 ^{ns} | 0.4155 ^{ns} | 0.0674 ^{ns} | 0.2876 ^{ns} | 0.1344 ^{ns} | 0.1364 ^{ns} |
| Y | <0.0001 ^{***} | <0.0001 ^{***} | <0.0001 ^{***} | <0.0001 ^{***} | <0.0001 ^{***} | | | <0.0001 ^{***} | <0.0001 ^{***} | <0.0001 ^{***} |
| R x Y | 0.8125 ^{ns} | 0.0963 ^{ns} | 0.1450 ^{ns} | 0.9326 ^{ns} | 0.9978 ^{ns} | 0.7993 ^{ns} | 0.9728 ^{ns} | 0.8123 ^{ns} | 0.9571 ^{ns} | 0.9376 ^{ns} |

^aMeans within columns, for rootstock and year separately, followed by different small letters differ significantly by Tukey's multiple range test; ^bSignificance (p value) of rootstock (R), year (Y), rootstock and year interaction (R x Y); * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, ns = not significant.

Table 3. Rootstock effect of the volatile substances on the Cabernet Sauvignon wine in the wine-growing region of Serra Gaúcha, Brazil, over three years.

| | Ethyl acetate (mg L ⁻¹) | Methanol (mg L ⁻¹) | 1-Propanol (mg L ⁻¹) | 2-Methyl-1-propanol (mg L ⁻¹) | 2-Methyl+3- Methyl-1-butanol (mg L ⁻¹) | Sum of higher alcohols (mg L ⁻¹) |
|---------------------------------|--|-----------------------------------|-------------------------------------|--|--|--|
| Rootstock | | | | | | |
| Rupestris du Lot | 95.2 ^a ab | 165.4 a | 27.2 a | 86.1 a | 363.8 a | 477.3 a |
| 101-14 Mgt | 120.5 a | 154.7 a | 25.8 a | 90.7 a | 337.7 a | 454.3 a |
| 3309 C | 103.0 ab | 153.0 a | 31.6 a | 87.6 a | 332.4 a | 451.7 a |
| 420A Mgt | 105.8 ab | 136.0 a | 35.1 a | 88.2 a | 308.1 a | 431.4 a |
| 5BB K | 110.0 ab | 151.1 a | 32.7 a | 95.1 a | 312.9 a | 440.9 a |
| 161-49 C | 104.9 ab | 146.5 a | 26.0 a | 87.2 a | 325.6 a | 438.8 a |
| SO4 | 93.6 ab | 157.2 a | 34.1 a | 90.5 a | 319.0 a | 443.6 a |
| Solferino | 106.2 ab | 141.3 a | 33.3 a | 92.4 a | 304.3 a | 427.8 a |
| 1103 P | 95.2 ab | 147.2 a | 28.6 a | 89.5 a | 339.4 a | 457.5 a |
| 99 R | 85.4 b | 137.6 a | 30.5 a | 92.4 a | 355.4 a | 478.3 a |
| 110 R | 101.5 ab | 137.4 a | 25.7 a | 90.0 a | 334.4 a | 458.0 a |
| Gravesac | 99.4 ab | 143.4 a | 32.3 a | 92.5 a | 333.2 a | 458.2 a |
| Fercal | 82.9 b | 137.9 a | 32.5 a | 97.5 a | 359.8 a | 489.8 a |
| Dogridge | 85.1 b | 146.3 a | 36.3 a | 99.6 a | 332.8 a | 468.6 a |
| Isabel | 81.1 b | 132.8 a | 23.8 a | 92.4 a | 356.6 a | 472.8 a |
| Year | | | | | | |
| 1999 | 58.4 c | 121.7 c | 34.2 a | 83.7 c | 332.9 b | 412.0 b |
| 2000 | 108.3 b | 136.8 b | 36.2 a | 110.1 b | 263.3 c | 349.0 b |
| 2001 | 127.4 a | 167.3 a | 20.7 b | 170.1 a | 407.0 a | 609.0 a |
| Significance^b | | | | | | |
| R | 0.0043** | 0.1621 ^{ns} | 0.4522 ^{ns} | 0.6492 ^{ns} | 0.2560 ^{ns} | 0.2309 ^{ns} |
| Y | <0.0001*** | 0.0007*** | <0.0001*** | <0.0001*** | <0.0001*** | <0.0001*** |
| R x Y | 0.0521 ^{ns} | 0.0002*** | 0.8861 ^{ns} | 0.0320* | 0.6847 ^{ns} | 0.1426 ^{ns} |

^aMeans within columns, for rootstock and year separately, followed by different small letters differ significantly by Tukey's multiple range test; ^bSignificance (*p* value) of rootstock (R), year (Y), rootstock and year interaction (R x Y); * = *p* < 0.05, ** = *p* < 0.01, *** = *p* < 0.001, ns = not significant.

was no effect (*p* > 0.05) with IAC 21-14 Madalena and BRS Lorena cultivars (SILVA et al., 2015). The total soluble solids contents of grapes give an idea of its alcohol potential. Indeed, total soluble solids were significantly affected by the rootstock (KODUR et al., 2013; BERDEJA et al., 2014), but most research shows that there was no effect, or little one, on the total soluble solids of the grape must (KELLER et al., 2012; CHOU; LI, 2014).

As expected, there was a significant and negative correlation between titratable acidity and pH (*r* = -0.74; *p* < 0.05). The titratable acidity varied little, between 69 and 74 meq L⁻¹, being in accordance with Brazilian legislation whose limits are between 55 and 130 meq L⁻¹. In the other hand, its pH was high which is inherent to this cultivar. The titratable acidity and pH are the most important variables related to wine acidity, due to organic acids present in the grape berries, especially tartaric and malic acids. The titratable acidity of wine is in general lower than that of grape must because of the precipitation of tartaric acid during cooling (if it is done) and reduction of malic acid to lactic acid during malolactic fermentation. The acidity affects wine sensory perception because it improves its

brightness and equilibrium, and contributes positively to the conservation of wine.

The Cabernet Sauvignon has a medium to long vegetative cycle. It needs adequate solar radiation during grape ripening, which promotes the leaf photosynthetic activity and consequently the biosynthesis of sucrose. This sugar is then transported through the phloem tissue to the grape berries where it is broken and accumulated in the form of glucose and fructose. Concomitantly, a decrease of organic acids in grape berries is processed. Therefore, the relationship between sugar and organic acids in grape must is an important factor for the quality of the grape and wine. In this way, according to the Serra Gaúcha conditions a total soluble solids (°Brix)/titratable acidity ratio over 30 in grape juice is supposed to be adequate for this purpose, at least for the Cabernet Sauvignon wine.

The mean volatile acidity content was moderate, varying from 12 to 14 meq L⁻¹. In the two first years, volatile acidity showed lower levels, but in the third one it was high (19 meq L⁻¹) (Table 1) because grapes were overripe. These results are in accordance with the

Brazilian legislation, whose limit is 20 meq L⁻¹. However, it is advisable to maintain wines with low volatile acidity parameters because it may have negative interference on the wine sensory characteristics and quality.

The dry extract varied from 21.6 to 24.8 g L⁻¹ (Table 1), and according to Boulton et al. (1998) it is an important tool to regulate wine bodies. Its main components are glycerol and organic acids and, secondarily, reducing sugars. Indeed, all evaluated wines were dry according to the Brazilian legislation because sugar concentrations were less than 4 g L⁻¹. This result shows that most sugar was transformed into alcohol by the yeasts (Table 1). The alcohol/reduced dry extract ratio was a little higher than 4.00 (Table 2), where 5.20 is the maximum permitted.

The ashes are made up of minerals present in the wine after calcination in the muffle, the contents of which varied from 2.92 to 3.65 g L⁻¹ (Table 2). This means that all wines had parameters according to the Brazilian legislation that establishes a minimum of 1.5 g L⁻¹ (BRASIL, 1998). The alkalinity of ashes shows how much organic acids are present in the free form in wines, where lower values show more free forms. In the present work, it varied from 30.9 to 37.8 (Table 2), which means that a large amount of organic acids in the Cabernet Sauvignon wines was in free form.

No variables related to polyphenols were significantly affected by the rootstock, but were by the year ($p < 0.001$) (Table 2). In general, the data found in the present work were observed in previously conducted researches on Cabernet Sauvignon and other grape varieties. Regarding Cabernet Sauvignon, the levels of anthocyanins, tannins, color intensity and hue were similar to those of a work carried out during six years with this variety (RIZZON; MIELE, 2002). In addition, the results had the same range of contents as the work developed with Merlot (RIZZON; MIELE, 2003; MIELE et al., 2009b), Cabernet Franc (RIZZON; MIELE, 2001; MANFROI et al., 2006) and Tannat (RIZZON; MIELE, 2004). However, the Cabernet Sauvignon wine showed different results from Isabel (syn. Isabella) and Bordô (syn. Ives), two *V. labrusca* varieties. Bordô wine had lower tannins and higher anthocyanins, color intensity and hue (TECCHIO et al., 2007) while Isabel wine had lower parameters of these four variables (RIZZON; MIELE, 2006) than those of Cabernet Sauvignon wine.

Polyphenols are very complex and important compounds for red wines, anthocyanins and tannins being the main substances of this group that may have influence on wine characteristics and quality. Anthocyanins are pigments mainly responsible for the wine red color, which varies according to grape variety, cultural practices and enological procedures. There are five groups of anthocyanins in grapes, named cyanidin, delphinidin, peonidin, petunidin and malvidin, which are present in different proportions in grape berries of each variety.

They are found as 3-glucosides in *V. vinifera* L. where malvidin-3-glucoside is the most common, and in other species, such as *V. labrusca* L., 3,5-diglucosides are found. This genetic mediated difference is an important way to differentiate red grape varieties from these two species.

The tannins comprise a vast and complex set of substances, where phenolic acids, flavonoids and tannins polymers are the most important in grapes. They may be astringent and bitter, which demand special attention of the winemaker due to their possible effect on wine characteristics and quality. Besides the factors affecting tannins in grapes and during wine processing, aged wines can have new molecules coming from oak wood. In general, the wines have a high amount and wide range of properties that allow one to distinguish different product types (BOULTON et al., 1998). So, the phenolic composition of a wine is due to the grape variety, the winemaking procedures which may have effect on the physics phenomenon (diffusion of compounds from the grape solid phase to the must and the tannins extraction from the wood during aging) and chemical and biochemical reactions (oxidation, degradation and condensation) (CHEYNIER et al., 1998).

Except for ethyl acetate, all other volatile substances were not affected ($p > 0.05$) by the rootstock. However, they were by the year and there was an interaction between the rootstock and the year for methanol and 2-methyl-1-propanol (Table 3). There are very few studies related to the effect of rootstocks on the volatile substances present in wines. Indeed, in an early work, Ough et al. (1968) demonstrated that there was a significant effect of the rootstock on the amyl alcohols, 2,3-butanediols and total volatile esters but there was no significant effect on acetaldehyde and isobutyl alcohol.

The mean concentration of ethyl acetate (98.0 mg L⁻¹) was below the threshold of this compound, which is estimated to be around 180 mg L⁻¹. High concentrations, however, transmit an acetic character to wines with a negative effect on their overall quality. Low contents of this compound are due to the grape sanitary state and to the alcoholic fermentation conditions. The mean methanol content found was 141.9 mg L⁻¹, which is below the maximum specified by the Brazilian legislation (300 mg L⁻¹), and contents higher than this could be harmful to the human health. In general, wines of *V. labrusca* L. varieties have higher methanol concentrations than *V. vinifera* L. Indeed, Rizzon and Miele (2006) working with Isabel wines found mean values of 290.6 mg L⁻¹ and in Bordô wines Tecchio et al. (2007) found 290.9 mg L⁻¹. Methanol is formed by the hydrolysis of pectin present in grape berries and their concentration is mainly due to the maceration of the grape solid parts, the skin in particular.

Higher alcohol levels are due to secondary compounds that originate from alcoholic fermentation. The concentrations depend on the nitrogen content within

the grape must, yeasts and factors which influence the process such as temperature, oxygen and sulfur dioxide (BERTRAND, 1975). High concentrations of these substances are negative for the quality of the wine as they are responsible for undesirable aromas outcome. In fact, high contents of amyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) can be responsible for the vegetal or herbaceous character of wines. The average content of 1-propanol was 30.4 mg L⁻¹, which depends on the sanitary state of the grape, and it is formed by fermentations caused by bacteria (DIRNINGER; SCHAEFFER, 1990).

The effect of rootstock could be due, directly or indirectly, to its genetic diversity and inter-relationship with the scion and the environment. Indeed, grafting vines may have effect in different ways, such as in vine physiology, yield components, vigor, fruitfulness, berry size, berry color and phenology which in turn can influence the wine composition.

Conclusions

All the evaluated variables are affected by the year, eight of 25 by the rootstock and only two by the interaction between the rootstock and the year. The rootstock effect is shown mainly in variables related to alcohol, acidity and ashes.

The highest alcohol content in the Cabernet Sauvignon wine is found with the CS/101-14 Mgt combination and the lowest with CS/Dogridge and CS/Isabel. The highest pH is found with CS/Rupestris du Lot, CS/5BB K and CS/Gravesac wine combinations and the lowest with CS/420A Mgt, CS/110 R and CS/Isabel.

The results of this research show that, depending on the production goal, the Cabernet Sauvignon grapevine can be grafted on all evaluated rootstocks, except for cv. Isabel, at least for the soil and climate conditions in which the experiment was carried out.

Acknowledgements

Authors gratefully thank colleagues of Embrapa Uva e Vinho, who have worked for nine years managing grapevines in the vineyard and performing wine analyses for three years. Thanks also go to Inra-Centre de Bordeaux-Aquitaine for its kindness in providing the vegetative material of two rootstocks, Fercal and Gravesac.

References

- BERDEJA, M.; HILBERT, G.; LAFONTAINE, M.; STOLL, M.; GOMES, E.; RENAUD, C.; DELROT, S. Effects of drought stress and rootstock genotype on grape berry quality. *Acta Horticulturae*, The Hague, n.1038, p.375-377, 2014.
- BERTRAND, A. **Recherches sur l'analyse des vins par chromatographie en phase gazeuse**. 1975. 291f. Thèse (Doctorat d'État ès Sciences)-Institut d'Œnologie, Université de Bordeaux II, Talence, 1975.
- BOULTON, R.B.; SINGLETON, V.S.; BISSON, L.F.; KUNKEE, R.E. **Principles and practices of winemaking**. Gaithersburg: Aspen, 1998.
- BRASIL. Portaria no. 283, de 18 de junho de 1998. Aprova normas e procedimentos para o registro de estabelecimento, bebidas e vinagres, inclusive vinhos e derivados da uva e do vinho e expedição dos respectivos certificados. **Diário Oficial da República Federativa do Brasil**. Brasília, DF, 22 jun. 1998. Seção 1, n.106.
- CHEYNIER, V.; MOUTOUNET, M.; SARNI-MANCHADO, P. Les composés phénoliques. In: FLANZY, C. (Ed.). **Œnologie: fondements scientifiques et technologiques**. Paris: Tec&Doc, 1998. p.123-162.
- CHOU, M-I.; LI, K-T. Rootstock and seasonal variations affect anthocyanin accumulation and quality traits of 'Kyoho' grape berries in subtropical double cropping system. *Vitis*, Siebeldingen, v.53, n.4, p.193-199, 2014.
- CIRAMI, R.M.; McCARTHY, M.G.; GLENN, T. Comparison of the effects of rootstock on crop, juice and wine composition in a nematode-infested Barossa Valley vineyard. *Australian Journal of Experimental Agriculture*, Melbourne, v.24, n.125, p.283-289, 1984.
- DIRNINGER, N.; SCHAEFFER, A. Évolution des alcools supérieurs et du méthanol dans les eaux-de-vie de fruits produits dans le Nord-Est de la France. *Cahier Scientifique*, Paris, n.126, p.7-15, 1990.
- FLORES, C.A.; PÖTTER, R.O.; SARMENTO, E.C.; WEBER, E.J.; HASENACK, H. **Os solos do Vale dos Vinhedos**. Pelotas: Embrapa Clima Temperado, 2012.
- GONG, H.J.; BLACKMORE, D.H.; CLINGELEFFER, P.R.; SYKES, S.R.; WALKER, R.R. Variation for potassium and sodium accumulation in a family from a cross between rootstocks K 51-40 and 140 Ruggeri. *Vitis*, Siebeldingen, v.53, n.2, p.65-72, 2014.

- HALE, C.R.; BRIEN, C.J. Influence of Salt Creek rootstock on composition and quality of Shiraz grapes and wine. **Vitis**, Siebeldingen, v.17, n.2, p.139-146, 1978.
- HARBERTSON, J.F.; KELLER, M. Rootstock effects on deficit-irrigated winegrapes in a dry climate: grape and wine composition. **American Journal of Enology and Viticulture**, Davis, v.63, n.1, p.40-48, 2012.
- HARDIE, W.J.; CIRAMI, R.M. Grapevine rootstocks. In: COOMBE, B.G.; DRY, P.R. (Ed.). **Viticulture: resources**. Adelaide: Winetitle, 1988. v.1, p.154-176.
- HEDBERG, P.R.; McLEOD, R.; CULLIS, B.; FREEMAN, B.M. Effect of rootstock on the production, grape and wine quality of Shiraz vines in the Murrumbidgee irrigation area. **Australian Journal of Experimental Agriculture**, Collingwood, v.26, n.4, p.511-516, 1986.
- JOGAIAH, S.; KITTURE, A.R.; SHARMA, A.K.; SHARMA, J.; UPADHYAY, A.K.; SOMKUWAR, R.G. Regulation of fruit and wine quality parameters of 'Cabernet Sauvignon' grapevines (*Vitis vinifera* L.) by rootstocks in semiarid regions of India. **Vitis**, Siebeldingen, v.54, n.2, p.65-72, 2015.
- JOGAIAH, S.; OULKAR, D.P.; BANERJEE, K.; SHARMA, J.; PATIL, A.G.; MASKE, S.R.; SOMKUWAR, R.G. Biochemically induced variations during some phenological stages in Thompson Seedless grapevines grafted on different rootstocks. **South African Journal of Enology and Viticulture**, Stellenbosch, v.34, n.1, p.36-45, 2013.
- KELLER, M.; MILLS, L.J.; HARBERTSON, J.F. Rootstock effects on deficit-irrigated winegrapes in a dry climate: vigor, yield formation, and fruit ripening. **American Journal of Enology and Viticulture**, Davis, v.63, n.1, p.29-39, 2012.
- KODUR, S.; TISDALL, J.M.; CLINGELEFFER, P.R.; WALKER, R.R. Regulation of berry quality parameters in 'Shiraz' grapevines through rootstocks (*Vitis*). **Vitis**, Siebeldingen, v.53, n.3, p.125-128, 2013.
- LEÃO, P.C de S.; BRANDÃO, E.O.; GONÇALVES, N.P. da S. Produção e qualidade de uvas de mesa 'Sugraone' sobre diferentes porta-enxertos no submédio do Vale do São Francisco. **Ciência Rural**, Santa Maria, v.41, n.9, p.1526-1531, 2011.
- MAIN, G.; MORRIS, J.; STRIEGLER, K. Rootstock effects on Chardonnay productivity, fruit, and wine composition. **American Journal of Enology and Viticulture**, Davis, v.53, n.1, p.37-40, 2002.
- MANFROI, L.; MIELE, A.; RIZZON, L.A.; BARRADAS, C.I.N. Composição físico-química do vinho Cabernet Franc proveniente de videiras conduzidas no sistema lira aberta. **Ciência e Tecnologia de Alimentos**, Campinas, v.26, n.2, p.290-296, 2006.
- MIELE, A.; RIZZON, L.A. Rootstock-scion interaction: 1. Effect on the yield components of the Cabernet Sauvignon grapevine. **Revista Brasileira de Fruticultura**, Jaboticabal, v.39, n.1, p.1-9, 2017.
- MIELE, A.; RIZZON, L.A.; GIOVANNINI, E. Efeito do porta-enxerto no teor de nutrientes em tecidos da videira 'Cabernet Sauvignon'. **Revista Brasileira de Fruticultura**, Jaboticabal, v.31, n.4, p.1141-1149, 2009a.
- MIELE, A.; RIZZON, L.A.; MANDELLI, F. Manejo do dossel vegetativo e seu efeito na composição do vinho Merlot. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.44, n.4, p.463-470, 2009b.
- OLLAT, N.; TANDONNET, J.P.; LAFONTAINE, M.; SCHULTZ, H.R. Short and long term effects of three rootstocks on Cabernet Sauvignon vine behaviour and wine quality. **Acta Horticulturae**, Leuven, v.617, p.95-100, 2003.
- OUGH, C.S.; COOK, J.A.; LIDER, L.A. Rootstock-scion interactions concerning wine making. II. Wine compositional and sensory changes attributed to rootstock and fertilizer level differences. **American Journal of Enology and Viticulture**, Davis, v.19, n.4, p.254-265, 1968.
- RENOUF, V.; TREGOAT, O.; ROBY, J-P.; VAN LEEUWEN, C. Soils, rootstocks and grapevine varieties in prestigious Bordeaux vineyards and their impact on yield and quality. **Journal International des Sciences de la Vigne et du Vin**, Talence, v.44, n.3, p.127-134, 2010.
- RIBÉREAU-GAYON, J.; PEYNAUD, E.; SUDRAUD, P.; RIBÉREAU-GAYON, P. **Traité d'œnologie: sciences et techniques du vin: analyse et contrôle des vins**. 2nd ed. Paris: Dunod, 1982. v.1.
- RIBÉREAU-GAYON, P.; STONESTREET, E. Dosage des tanins du vin rouge et détermination de leur structure. **Chimie Analytique**, Paris, v.48, n.4, p.188-196, 1966.
- RIBÉREAU-GAYON, P.; STONESTREET, E. Le dosage des anthocianes dans le vin rouge. **Bulletin de la Société Chimique de France**, Paris, v.9, p.2649-2652, 1965.
- RIZZON, L.A.; MIELE, A. Avaliação da cv. Cabernet Franc para elaboração de vinho tinto. **Ciência e Tecnologia de Alimentos**, Campinas, v.21, n.2, p.249-255, 2001.

- RIZZON, L.A.; MIELE, A. Avaliação da cv. Cabernet Sauvignon para elaboração de vinho tinto. **Ciência e Tecnologia de Alimentos**, Campinas, v.22, n.2, p.192-198, 2002.
- RIZZON, L.A.; MIELE, A. Avaliação da cv. Merlot para elaboração de vinho tinto. **Ciência e Tecnologia de Alimentos**, Campinas, v.23, p.16-161, 2003.
- RIZZON, L.A.; MIELE, A. Avaliação da cv. Tannat para elaboração de vinho tinto. **Ciência e Tecnologia de Alimentos**, Campinas, v.24, n.2, p.223-229, 2004.
- RIZZON, L.A.; MIELE, A. Efeito da safra vitícola na composição da uva, do mosto e do vinho Isabel da Serra Gaúcha, RS, Brasil. **Ciência Rural**, Santa Maria, v.36, n.3, p.959-964, 2006.
- SILVA, M.J.R. da; TECCHIO, M.A.; MOURA, M.F.; BRUNELLI, L.T.; IMAIZUMI, V.M.; VENTURINI FILHO, W.G. Composição físico-química do mosto e do vinho branco de cultivares de videiras em resposta a porta-enxertos. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.50, n.11, p.1105-1113, 2015.
- SOUZA, C.R. de; MOTA, R.V. da; FRANÇA, D.V.C.; PIMENTEL, R.M. de A.; REGINA, M. de A. Cabernet Sauvignon grapevine grafted onto rootstocks during the autumn-winter season in southeastern Brazilian. **Scientia Agricola**, Piracicaba, v.72, n.2, p.138-146, 2015.
- STEVENS, R.M.; PECH, J.M.; TAYLOR, J.; CLINGELEFFER, P.; WALKER, R.R.; NICHOLAS, P.R. Effects of irrigation and rootstock on *Vitis vinifera* (L.) cv. Shiraz berry composition and shrivel, and wine composition and wine score. **Australian Journal of Grape and Wine Research**, Glen Osmond, v.22, n.1, p.124-136, 2016.
- STOCKERT, C.M.; BISSON, L.F.; ADAMS, D.O.; SMART, D.R. Nitrogen status and fermentation dynamics for Merlot on two rootstocks. **American Journal of Enology and Viticulture**, Davis, v.64, n.2, p.195-202, 2013.
- TECCHIO, F.M.; MIELE, A.; RIZZON, L.A. Composição físico-química do vinho Bordô de Flores da Cunha, RS, elaborado com uvas maturadas em condições de baixa precipitação. **Ciência Rural**, Santa Maria, v.37, n.5, p.1480-1483, 2007.
- WALKER, R.R.; BLACKMORE, D.H. Potassium concentration and pH inter-relationships in grapevine and wine of Chardonnay and Shiraz from a range of rootstocks in different environments. **Australian Journal of Grape and Wine Research**, Glen Osmond, v.18, n.2, p.183-193, 2012.
- WALKER, R.R.; BLACKMORE, D.H.; CLINGELEFFER, P.R. Impact of rootstock on yield and ion concentrations in petioles, juice and wine of Shiraz and Chardonnay in different viticultural environments with different irrigation water salinity. **Australian Journal of Grape and Wine Research**, Glen Osmond, v.16, n.1, p.243-257, 2010.
- WALKER, R.R.; CLINGELEFFER, P.R.; KERRIDGE, G.H.; RÜHL, E.H.; NICHOLAS, P.R.; BLACKMORE, D.H. Effects of rootstock Ramsey (*Vitis champini*) on ion and organic acid composition of grapes and wine, and on wine spectral characteristics. **Australian Journal of Grape and Wine Research**, Glen Osmond, v.4, n.3, p.100-110, 1998.
- WOOLDRIDGE, J.; LOOW, P.J.E.; CONRADIE, W.J. Effects of rootstocks on grapevine performance, petiole and must composition, and overall wine score of *Vitis vinifera* cv. Chardonnay and Pinot Noir. **South African Journal of Enology and Viticulture**, Stellenbosch, v.31, n.1, p.45-48, 2010.