Research Article

Pruning effect on content of quercetin and catechin in berry skins of cv. Blaufränkisch (*Vitis vinifera* L.)

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Abstract: The effect of pruning severity on quercetin and catechin content in berry skins of cv. Blaufränkisch (Vitis vinifera L.) was studied over 3 years. Different crop levels and canopy structures were obtained by retaining 8, 16, and 24 nodes per vine at pruning. Canopy density, which is proportionate to the shoot number per canopy volume, directly affects the intensity of photosynthetically active radiation (PAR). The quercetin content has been shown to be highly dependent on the light exposure of the berries in which it accumulates. An increase in node number linearly decreased skin catechin, and it is suggested that the decrease was caused by increased yield per vine.

Key words: Bud load, canopy structure, berry skin, quercetin, catechin

Introduction

Phenolic compounds have an important effect on grape and wine sensory quality, as they provide the color, taste, and aroma (Morrison and Noble 1990; Mazza et al. 1999). They have an important function in wine stability during aging due to their preservative effect (Gomez-Plaza et al. 2001). Phenolic compounds also have an important nutritive value when they are consumed through grapes and wine (Parker et al. 2007). Their importance in health protection has been confirmed, as they possess antioxidant and anticarcinogenic properties. Numerous reports indicate that a moderate consumption of red wine appears to be associated with a decrease in heart diseases (Frankel et al. 1993; Aviram and Fuhram 2002; Dell'Agli et al. 2004), and their antioxidant character give red wine its anticancer properties (Kanner et al. 1994; Soleas et al. 1997).

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Grape and wine phenolics include the nonflavonoids (hydroxycinnamates, hydroxy-benzoates, and stilbenes) and the flavonoids (flavan-3-ols, flavonols, and anthocyanins) (Waterhouse 2002). Flavan-3-ols (e.g. flavanols or catechin) are the most abundant class of flavonoids in grapes and wines. In the grape, they are found in both seeds and skins and are responsible for bitterness in wine, having some associated astringency. They include (+)-catechin, (-)-epicatechin, and (-)-epicatechin-3-*O*-gallate, which exist as both monomers and/or polymeric proanthocyanidins (Cortell and Kennedy 2006). Several studies have examined the effect of sun exposure and cluster thinning on flavan-3-ol concentration in skins. They respond to crop load and range from 100 to 240 mg g^{-1} (Zhao et al. 2006), and their concentrations are significantly lower in skins than seeds or stems. Cortell and Kennedy (2006) reported that cluster in shaded treatment had 0.74 ± 0.1 mg berry^{-1} of skin flavan-3-ols compared to 1.20 ± 0.1 mg berry^{-1} in the exposed treatment.

Flavonols are found in grape skin as glycosides, galactosides, and glucuronides of quercetin, myricetin, kaempferol, and isorhamnetin. Quercetin accumulates in grape skin and stems to protect against damage from ultraviolet (UV) light. Price et al. (1995) reported that quercetin concentrations in grape skins from clusters from different sun exposures ranged from 0.02 (shaded) to 0.12 mg g^{-1} (exposed) fresh weight.

The content of various flavonoids, such as flavonols, anthocyanins, and tannins, can be affected by external factors such as UV radiation, drought, pathogens, and temperature, as well as certain cultural practices in the vineyard. Examples of these practices are canopy management, irrigation, crop load, and timing of harvest (Bavaresco 2003; Downey et al. 2004). The direct link between the effect of crop load on specific polyphenols of grapes and wine has yet to be determined (Price et al. 1995; Zhao et al. 2006; Prajitna et al. 2007).

Winter pruning is the main tool for establishing an optimal ratio between vegetative and reproductive growth. The ratio between the vegetative organs and crop weight has crucial importance for grape and wine quality, and that ratio can be affected through different numbers of buds retained at pruning, the variation in shoot numbers, and defoliation, or by affecting the cluster number per vine (Kliewer and Weaver 1971; Bravdo et al. 1985). A balanced ratio between leaf area and crop load leads to an optimal distribution of assimilatives between leaves, shoots, and clusters, and to an optimal canopy microclimate.

The optimal canopy density, defined here as the number of leaf layers in the canopy, leads to favorable microclimatic conditions and allows light penetration through the canopy, which leads to an increase in dry matter and phenolic compounds in berry skins (Morrison and Noble 1990; Gao 1993; Bergqvist et al. 2001). It is generally agreed that low light reduces anthocyanins and other flavonoids, while increased light results in increased flavonoid content (Downey et al. 2004).

Under the conditions of shaded canopies, a significant decrease in berry flavonoid content has been reported (Smart et al. 1988). Furthermore, the increased canopy density leads to a rise in relative air humidity within the canopy, which then leads to a reduction in the intensity of transpiration and photosynthesis, a reduction in the growth of organs, and a decrease in flavonoid accumulation (Haselgrove et al. 2000). Increased leaf temperature increases the metabolic processes of the plant, and this leads to increased accumulation of metabolites (Ebadi et al. 1995). In the opposite situations, where there is a low number of shoots per canopy volume, high air temperatures can lead to a reduction in the intensity and even to a complete cessation of various metabolic processes (Jones 1992). Despite these statements, the optimal amount of cluster light exposure remains unclear (Bergqvist et al. 2001).

The objective of this study was to investigate the effect of different node numbers on vegetative and reproductive growth, and to explore the impact of light exposure and crop load on flavonoid accumulation in the berry skin over 3 seasons.

Materials and methods

Investigations were conducted on cv. Blaufränkisch (*Vitis vinifera* L.), planted in 1994 in an experimental vineyard at the experimental station "Radmilovac," which belongs to the Faculty of Agriculture, University of Belgrade. The location

belongs to the Sumadija-Velika Morava wine region, which is characterized as a maritime temperate or Cfb climate (Kottek et al. 2006). The vine spacing was 3 ´ 1 m. Vines were trained as double Guyot with a trunk height of 90 cm, and were pruned to a mix of canes and spurs. The vine row orientation was east-west. Yield was manipulated by winter pruning of vines to 8 (T_1), 16 (T_2), and 24 (T_3) nodes per vine, retained on both canes and spurs. The experiment was replicated in 3 blocks with all 3 treatments in each. Each treatment replicate consisted of 15 vines selected for their uniformity. Pruning treatments continued through the 3 seasons, and measurements were taken in each of the 2004, 2005, and 2006 cropping seasons. At commercial harvest, all bunches were counted from each vine and weighed to determine bunch weight. Berries were then removed and counted, and the mean berry weight per bunch was calculated.

Six samples of grapes from each treatment were collected when soluble solids in must reached 19%-20%. Each sample among the 6 collected consisted of about 10 clusters, totaling 1 kg of grapes on average. These clusters were randomly collected from each side of different vines. The samples were collected in black plastic bags and immediately stored at 4 °C. All berries from collected clusters were removed and placed in sealed plastic vessels and stored at –20 °C. Before analysis, frozen berries were thawed in a refrigerator at 4 °C. The berry skins from 50 randomly selected berries from each sample were then peeled with tweezers, freeze-dried, and ground with a laboratory mill. For sample extraction, 1 g of ground berry skin was placed in a 10 mL tube with 10 mL of methanol, which was adjusted to pH 2.0 with 1.0 M HCl, mixed, and incubated for least 3 h. The homogenate/methanol mixture was centrifuged at 9500 rpm for 20 min and a supernatant was decanted for absorbance. The concentration of catechin and quercetin was determined by high-performance liquid chromatography (HPLC) using an Agilent 1100 Series equipped with auto-injection, ChemStation, and 1100 Series DAD UV-Vis detector. The HPLC conditions were similar to those described by Romero-Pérez et al. (1999). The column used was a LiChrospher 100 RP-C18e (2550 × 4 mm, 5 μm). Elution was performed using mobile phase A (52.6 mL of concentrated HCl in 900 mL of distilled water) and mobile phase B (20% A + 80% acetonitrile). The gradient elution profile was as follows, with linear gradients for the time points: 0 min, 0% A, 100% B; 13 min, 82.0% A, 18.0% B; 15 min, 82.0% A, 18.0% B; 17 min, 77.0% A, 23.0% B; 21 min, 75.0% A, 25.0% B; 27 min, 68.5% A, 31.5% B; 35 min, 0% A, 100% B.

The single leaf area, main shoot leaf area, and lateral shoot leaf area were estimated according to the method of Lopes and Pinto (2000). During the 15-31 May period of each year, 50 leaves were randomly collected from various vines in all experimental treatments. The leaves were immediately placed in plastic bags and kept and transported in a field refrigerator. Leaf area (LA) and the length of 2 inferior leaf veins (l) were measured using an HP 3600 scanner and Adobe Photoshop 7.0 in laboratory conditions. These data were used to calculate the regression between l and LA. The formula obtained ($LA = -74.7687 + 17.6594 \times l$, $r^2 = 0.93$) was used for nondestructive calculation of leaf surface on the basis of leaf vein length data collected in the vineyard. Also during the 15-31 May periods, 30 shoots were collected randomly from each treatment. They were transported to the laboratory in the same manner as the leaves. The total leaf area (MLA1), separately for main shoots and lateral shoots, was calculated for all collected shoots individually. Leaf number (NL1) and the largest (L1) and smallest leaf area (S1) were then determined for each main shoot. Multiple regression analysis was used to obtain the relationship between the dependent variable MLA1 and 3 independent variables (NL1, L1, and S1). The formula obtained ($MLA1 = -2504.21 + 172.684 \times NL1 + 9.10372 \times L1 + 5.20723 \times S1$) was used for nondestructive calculation of leaf surface area for main shoots. For the lateral shoots, the analogous formula was used with independent variables for lateral shoots (leaf number and the largest and smallest leaf area).

The mean weight of vine canes collected at winter pruning was determined by measuring the weight of canes per each vine in all experimental treatments using a handheld digital scale. The photosynthetically active radiation (PAR) was measured 3 times during July on 6 vines per treatment using a line quantum sensor SunScan System - SS1 (Delta-T Devices Ltd., UK). Measurements were taken around noon (between 1100 and 1300 hours) on sunny days in the

fruiting zone of the canopy (1.0 m above the ground surface) on the south side as incident PARi. Transmitted PARt was the measurement on the north side of the canopy, and means were calculated as PAR.

All data were analyzed using analysis of variance (ANOVA). Treatment effects were compared using mean separation by LSD and polynomial contrasts. Regression analysis was conducted to determine the relationship between different factors and phenolic compounds. All analyses were performed using STATGRAPHICS Plus Version 5.1 (Statistical Graphics Corp. 2001.). All reported correlation coefficients were significant at the P = 0.05 level.

Results

The vegetative growth, expressed as pruning weight and leaf area per vine, was related to the bud number left at pruning. An increase in bud number caused an increase in pruning weights per vine. The lowest pruning weight was recorded in T_1 (367 g vine^{-1}) and then in T_2 (695 g vine^{-1}), and the largest was in T_3 (1103 g vine^{-1}). Similarly, vine leaf area was increased with the increasing bud number per vine. The total leaf area per vine was significantly less (P < 0.05) in T_1 than in T_2 or T_3 (Table 1). The canopy density, which is proportionate to the shoot number per canopy volume, directly affects the intensity of PAR (Smart et al. 1988). The largest PAR was recorded in T_1, then in T_2, and the lowest was recorded in T_3.

Yield increased linearly with the increasing of bud number per vine. The average crop weight per vine was significantly less (P < 0.05) in T_1 than in T_2 or T_3 (Table 1). The content of catechin was lower in T_3 than in T_2, and highest in T_1. Quercetin content was highest in T_1 and lowest in T_3 (Table 1).

Regression analysis indicates that the phenolic content was moderately influenced by the pruning severity, which induced both different vine leaf areas and yields. The coefficient of correlation between vine leaf area and quercetin was moderate ($r^2 = -0.69$), as well as between PAR and quercetin ($r^2 = 0.73$) (Figures 1a and 1b). Yield and catechin content were moderately correlated (r = 0.60) (Figure 2).

Discussion

Differences in node number per vine resulted in a different shoot number per vine, and thus they affected both the vine leaf area and vine pruning weight. Our results support previous studies examining the influence of shoot and bud number per vine, respectively, on total leaf area (Zamboni et al. 1996; Miller et al. 1997). The increase in node number per vine, from 8 to 16 to 24, increased the vine leaf area. The vines with a high node number developed a larger total leaf area compared to those with a low node number, apparently due to an increased number of leaves, as shoot number per vine increased.

Table 1. Influence of bud load on the vegetative and reproductive growth and phenolic composition of cv. Blaufränkisch. Data represent average values for the studied period (2004-2006).

	Pruning weights per vine (g)	Leaf area per vine (m^2)	PAR (µmol m^{-2} s^{-1})	Yield per vine (kg)	Catechin (mg g^{-1} FW)	Quercetin (mg g^{-1} FW)
T_1	367[c]	2.7[c]	105.7[a]	2.03[c]	0.0314[a]	0.0672[a]
T_2	695[b]	5.0[b]	69.4[b]	3.44[b]	0.0226[b]	0.0504[b]
T_3	1103[a]	7.4[a]	47.8[c]	4.80[a]	0.0163[c]	0.0313[c]
LSD$_{(0.05)}$	81.1974	1.6642	19.0089	0.9702	0.0057	0.0071

T_1: 8 buds per vine, T_2: 16 buds per vine, T_3: 24 buds per vine.
Means separated by LSD multiple range test (P ≤ 0.05). Data followed by same letter in each column are not significantly different.
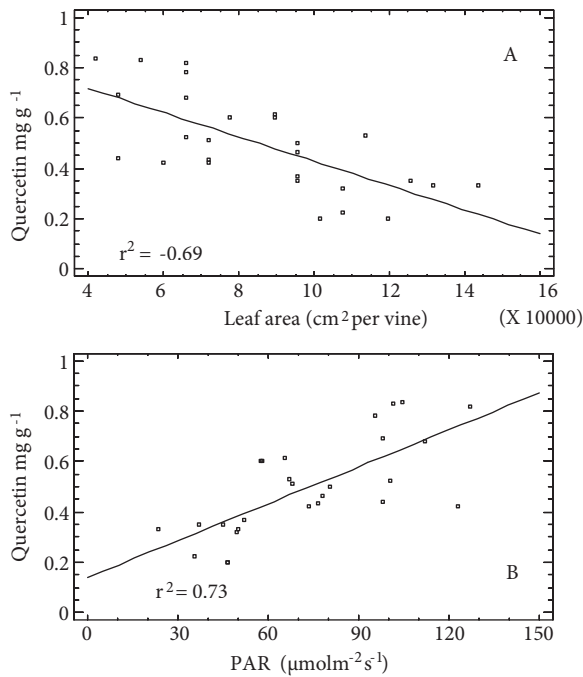
Figure 1. a) Relationship between vine leaf area and quercetin; b) PAR and quercetin in berry skins of cv. Blaufränkisch. The correlation coefficients were calculated using all individual PAR and quercetin measurements during the 2004-2006 period.
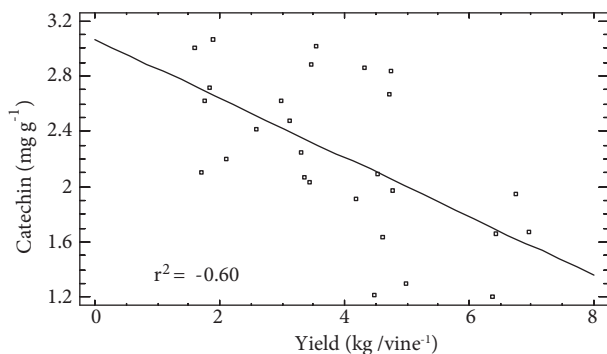

Figure 2. Relationship between yield and content of catechin in berry skins of cv. Blaufränkisch, 2004-2006.

Analysis of variance showed significant differences in PAR among pruning treatments and a reduction in light exposure (PAR) with the increasing node number due to increased canopy density. There is a well known link between vine leaf area and light quality in the canopy. Murisier and Ziegler (1992) reported that an increased node number increased total leaf area, but the exposed area remained unchanged due to the overlapping of leaves. Dokoozlian and Kliewer (1995) also found that total photosynthetic photon flux density (PPFD) decreased daily from 5.4 to 0.24 mol m^{-2}, due to the increasing of leaf area densities from 2.2 to 12.1 $m^2 m^{-1}$.

Yield was strongly affected by the node number per vine. Other studies also revealed that retaining more nodes at pruning increased the yield due to an increase in total shoot and hence cluster number. Increasing the node number from 30 to 50 per vine in Pinot gris, Pinot noir, and Sauvignon (Zamboni et al. 1996) or in Pinot noir (Heazlewood et al. 2006) increased the yield per vine.

Significant differences among the pruning treatments were found for quercetin and catechin in the berry skin. As expected, sun exposure was associated with an increase in the quercetin content in berry skins (Figure 1b). Earlier studies have shown that an increased node number per vine will increase shoot number and the number of leaf layers, and decrease the percentage of the exposed leaf area. An increase in the content of quercetin in favorable light conditions compared to shaded clusters was determined by Price et al. (1995) and Downey et al. 2006. Adams (2006) also reported that in the red wine cultivars, the amount of flavonols was highly dependent on light exposure of the tissues in which they accumulated.

Similarly to the quercetin content, the node number per vine was negatively correlated with the skin catechin. Despite reports of the effect of light exposure on the quercetin concentration, no similar effects on the catechin levels in grapes were reported. Recent studies have shown the benefits of low yield on phenolics (Mazza et al. 1999; Guidoni et al. 2002) and on resveratrol in red wine (Prajitna et al. 2007). The difference in catechin content in the skin extracts between pruning treatments was probably due to the difference in the yield per vine. This agrees with the results of Zhao et al. (2006) for cv. Cabernet Sauvignon vines with a varying crop level. They also reported that the total concentration of catechins in the skin was higher in treatments with lower yield per vine compared with the treatment with no cluster removal.

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References

Adams OD (2006) Phenolics and ripening in grape berries. Am J Enol Vitic 57: 249-256.

Aviram M, Fuhram B (2002) Wine flavonoids protect against LDL oxidation and atherosclerosis. Ann NY Acad Sci 957: 146-161.

Bavaresco L (2003) Role of viticultural factors on stilbene concentrations of grape and wines. Drugs Exp Clin Res 29: 181-187.

Bergqvist J, Dokoozlian N, Ebisuda N (2001) Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin valley of California. Am J Enol Vitic 52: 1-7.

Bravdo B, Hepner Y, Loinger C, Cohen S, Tabacman H (1985) Effect of crop level and crop load on growth, yield, must and wine composition and quality of Cabernet Sauvignon. Am J Enol Vitic 36: 125-131.

Cortell J, Kennedy J (2006) Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system. J Agric Food Chem 54: 8510-8520.

Dell'Agli M, Busciala A, Bosisio E (2004) Vascular effects of wine polyphenols. Cardiovasc 63: 593-602.

Dokoozlian NK, Kliewer WM (1995) The light environment within grapevine canopies. I. Description and seasonal changes during fruit development. Am J Enol Vitic 46: 209-218.

Downey MO, Dokoozlian NK, Krstic MP (2006) Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. Am J Enol Vitic 57: 257-268

Downey MO, Harvey JS, Robinson SP (2004) The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust J Grape and Wine Res 10: 55-73.

Ebadi A, May P, Sedgley M, Coombe BG (1995) Effect of low temperature near flowering time on ovule development and pollen tube growth in the grapevine (*Vitis vinifera* L.) cvs Chardonnay and Shiraz. Aust J Grape and Wine Res 1: 11-18.

Frankel EN, Waterhouse AL, Kinsella JE (1993) Inhibition of human LDL oxidation by resveratrol. Lancet 341: 1103-1104.

Gao Y (1993) Studies in Fruit Color Development in Red Seedless Table Grape Reliance (*Vitis* hybrid). PhD Dissertation. The Ohio State University.

Gómez-Plaza E, Gil-Muñoz R, López-Roca JM, Martínez-Cutillas A, Fernández-Fernández JI (2001) Phenolic compounds and color stability of red wines: effect of skin maceration time. Am J Enol Vitic 52: 266-270.

Guidoni S, Allara P, Schubert A (2002) Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. Am J Enol Vitic 53: 224-226.

Haselgrove L, Botting D, van Heeswijck R, Hoj PB, Dry PR, Ford C, Land PGI (2000) Canopy microclimate and berry composition: the effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv. Shiraz grape berries. Aust J Grape and Wine Res 6: 141-149.

Heazlewood JE, Wilson S, Clark RJ, Gracie AJ (2006) Pruning effects on Pinot Noir vines in Tasmania (Australia). Vitis 45: 165-171.

Jones HG (1992) Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology. Cambridge University Press, Cambridge.

Kanner J, Frankel EN, Granit R, German JB, Kinsella JE (1994) Natural antioxidants in grapes and wines. J Agric Food Chem 42: 64-69.

Kliewer WM, Weaver RJ (1971) Effect of crop level and leaf area on growth, composition, and coloration of Tokay grapes. Am J Enol Vitic 22: 172-177.

Kottek MJ, Grieser C, Beck B, Rudolf B, Rubel F (2006) World Map of the Köppen-Geiger climate classification updated. Meteorol 15: 259-263.

Lopes CMA, Pinto PA (2000) Estimation de la surface foliare principale et secondaire d'un rameau de vigne. Progres Agricole et Viticole 117: 160-166.

Mazza G, Fukumoto L, Delaquis P, Girard B, Ewert B (1999) Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. J Agric Food Chem 47: 4009-4017.

Miller DP, Howell GS, Flore JA (1997) Influence of shoot number and crop load on potted Chambourcin grapevines. II: Whole-vine vs. single-leaf photosynthesis. Vitis 36: 109-114.

Morrison JC, Noble AC (1990) The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. Am J Enol Vitic 41: 193-200.

Murisier F, Ziegler R (1992) Effects of bud load and planting density on yield, grape quality, and growth of Chasselas. In: IV International Symposium on Grapevine Physiology, Italy.

Parker T, Wang XH, Pazmiño J, Engeseth N (2007) Antioxidant capacity and phenolic content of grapes, sun-dried raisins, and golden raisins and their effect on ex vivo serum antioxidant capacity. J Agric Food Chem 55: 8472-8477.

Prajitna A, Dami IE, Steiner TE, Ferree DC, Scheerens JC, Schwartz SJ (2007) Influence of cluster thinning on phenolic composition, resveratrol, and antioxidant capacity in Chambourcin wine. Am J Enol Vitic 58: 346-350.

Price SF, Breen PJ, Valladao M, Watson BT (1995) Cluster sun exposure and quercetin in Pinot noir grapes and wine. Am J Enol Vitic 46: 187-194.

Romero-Pérez AI, Ibern-Gómez M, Lamuela-Raventós RM, de la Torre-Boronat MC (1999) Piceid, the major resveratrol derivative in grape juices. J Agric Food Chem 47: 1533-1536.

Smart RE, Smith SM, Winchester RV (1988) Light quality and quantity effects on fruit ripening for Cabernet Sauvignon. Am J Enol Vitic 39: 250-258.

Soleas GJ, Diamandis EP, Goldberg DM (1997) Resveratrol: a molecule whose time has come? And gone? Clin Biochem 30: 91-113.

Waterhouse A (2002) Handbook of Antioxidants, 2nd edition (Eds. L Packer, E Cadenas). Marcel Dekker, New York.

Zamboni M, Bavaresco L, Komjanc R (1996) Influence of bud number on growth, yield, grape and wine quality of Pinot gris, Pinot noir and Sauvignon (*Vitis vinifera* L.). In: IHC Workshop on Strategies to Optimize Wine Grape Quality, Conegliano, pp. 411-417.

Zhao XJ, Wang X, Liu B Li J, Sun YX, Shu HR (2006) Effect of cluster thinning on catechins in berries of *Vitis vinifera* cv. Cabernet Sauvignon. Vitis 45: 103-104.