INTERACTION EFFECTS OF NITROGEN AND POTASSIUM FERTILIZATION ON ANTHOCYANIN COMPOSITION AND CHROMATIC FEATURES OF TEMPRANILLO GRAPES

EFFETS D'INTERACTION ENTRE FERTILISATION AZOTÉE ET FERTILISATION POTASSIQUE SUR LA COMPOSITION ANTHOCYANIQUE ET LES CARACTÉRISTIQUES CHROMATIQUES DU RAISIN CV. TEMPRANILLO

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Abstract: A factorial experiment (3 nitrogen and 3 potassium fertilization doses) was carried out in a Tempranillo vineyard located in the 'Ribera del Duero' Apellation d'Origine area (Spain) in 2000. N and K supplies caused decreases in delphinidin and petunidin monoglucoside content in grape skins. High nitrogen doses increase L* coordinate and chroma of the skins. High potassium with unbalanced nitrogen supplies enhances hue and yellow component of colour. Possible effects of N and K fertilization on the copigmentation of anthocyanins are discussed.

Résumé : La fertilisation est une des techniques culturales du vignoble ayant le plus de répercussion sur la quantité et la qualité de la récolte. La disponibilité en éléments nutritifs affecte l'accumulation des polyphénols dans le raisin durant la maturation et, par conséquent, la couleur et l'astringence du vin. L'objectif de ce travail est d'évaluer les effets additifs et d'interaction de différentes doses de fertilisation azotée et potassique appliquées au vignoble sur la composition en anthocyanes et les caractéristiques chromatiques des raisins cv. Tempranillo. Une expérience factorielle a été réalisée au cours de l'année 2000, dans un vignoble en pleine production, de cépage Tempranillo greffé sur 110-Richter, localisé dans l'Appellation d'Origine Contrôlée «Ribera del Duero» (Nord de l'Espagne). Les ceps ont reçu trois doses différentes d'azote (0, 50 et 200 g N par plante) et trois de potassium (0, 60 et 120 g K_2O par plante) dans un dessin expérimental de blocs complets au hasard avec quatre répétitions. Les parcelles élémentaires étaient constituées de cinq plantes. Les traitements de fertilisation furent appliqués au sol en sillon à 15 centimètres de profondeur, à côté de chaque rang de plantes, utilisant du nitrate d'ammonium (335 g N kg^{-1}) et du sulfate de potasse (500 g·K_2O kg^{-1}). Tous les traitements reçurent, par ailleurs, une dose de 65 g P_2O_5 par cep en forme de super phosphate triple (450 g·P_2O_5 kg^{-1}). À la vendange, les polyphénols furent extraits des pellicules de raisin et la concentration des anthocyanes majoritaires fût déterminée au moyen de la technique HPLC. Postérieurement, les coordonnées CIELAB des extraits de pellicules furent obtenues pour chacun des traitements expérimentaux.
Les doses moyennes de fertilisation azotée augmentèrent le rendement de la vigne de plus de 18% par rapport aux traitements sans fertilisation. Les analyses factorielles de la variance indiquent que les traitements expérimentaux n'ont pas modifié significativement la dimension des baies, mais ont influencé le contenu en polyphénols et en anthocyanes totaux, ainsi que la composition spécifique d'anthocyanes dans les pellicules. Cependant, les contenus de malvidine-3-glucoside et cyanidine-3-glucoside des différents traitements expérimentaux n'ont montré aucune différence significative. Une importante interaction entre azote et potassium a été observée et a provoqué un plus grand contenu en polyphénols des raisins quand les rapports de fertilisation N/K appliqués étaient équilibrés. Les traitements à 200 g N par plante ont augmenté les proportions relatives de monoglucosides de delphinidine et paéonidine par rapport aux témoins non fertilisés, sans que la concentration totale d'anthocyanes ait été altérée, produisant des moûts plus colorés et surtout une augmentation des tonalités rouges et bleues (effets hyperchromique et bathochromique des spectres). Les doses élevées de fertilisation potassique ont diminué les concentrations en monoglucosides de delphinidine, pétunidine et paéonidine dans les pellicules. Ces doses furent associées à une augmentation de la tonalité et du composant jaune de la couleur (effet hypsochromique). D'un autre côté, les effets défavorables de doses élevées de potassium sur la couleur ont été évités quand elles étaient accompagnées par des apports suffisants d'azote. Dans ce cas, la fomentation d'interactions inter et intramoléculaires des anthocyanes des pellicules causées par la grande disponibilité en potassium aurait pu être compensé par une plus grande formation de complexes de copigmentation, induite par de plus fortes doses d'azote.

Key words: CIELAB, colour, copigmentation, polyphenols, *Vitis vinifera*
Mots clés : CIELAB, couleur, copigmentation, phénols, *Vitis vinifera*

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INTRODUCTION

Phenolic composition of grapes determines the colour and astringency of wines (RIBÉREAU-GAYON and GLORIES, 1987). The types and concentrations of polyphenols in grapes are related to the genotype, environmental conditions and cultural practices (KLIEWER, 1977; GONZÁLEZ-SAN JOSÉ and DÍEZ, 1992).

The anthocyanins are the polyphenols responsible for the colour of red grapes. These compounds, found mainly in the solid parts (skin and seeds) of the grapes, are synthesized from veraison onwards, enhancing their concentration in the skin throughout ripening (HRAZDINA, 1986).

Red berries of grapevine contain five main monoglucosides (malvidin-3-monoglucoside, delphinidin-3-monoglucoside, cyanidin-3-monoglucoside, peonidin-3-monoglucoside, petunidin-3-monoglucoside) and their derivatives (WENZEL *et al.*, 1987; ORTEGA *et al.*, 1994). Skin colour is different in function of monoglucosides composition. So hydroxylated monoglucosides increase the blueness tones and metoxylated monoglucosides enhance the red component of the colour (CHEYNIER *et al.*, 1989). The structural forms of anthocyanins can be altered by the pH and, in this way, promote changes in colour (LACOBUCCI and SWEENY, 1983).

Some studies noticed the importance of copigmentation in the stability of coloured forms of anthocyanins (BROUILLARD *et al.*, 1989; MINIATI *et al.*, 1992; BOULTON, 2001). The phenomenon of copigmentation is due to molecular associations between anthocyanins and other (usually noncoloured, like tanins) organic molecules in solution. These associations cause the pigments to exhibit far greater colour than would be expected from their concentration (BOULTON, 2001). The copigmentation of anthocyanins induces a hyperchromic (higher absorbance values) and bathochromic (higher wavelength at which the maximum absorbance is observed in the absorption spectra). The association of anthocyanins with other anthocyanins (self-association) promotes a hyperchromic and hypsochromic (shorter wavelength of the absorbance maximum) shift in the spectra (BROULLIARD *et al.*, 1989).

Fertilization of the soil is one of the viticultural techniques with a great effect on the vineyard yield and the quality of must and wine. The use of fertilizers usually results in yield increases, but excessive or unbalanced applications have negative effects on fruit quality.

Moderate nitrogen application before bloom enhanced the anthocyanin accumulation in the grape (KELLER and HRAZDINA, 1998; DELGADO *et al.*, 2004). Nevertheless excessive nitrogen levels in the soil can delay ripening (KELLER *et al.*, 1999), promote an inadequate vegetative growth (BRAVDO and HEPNER, 1987) and compact excessively the foliage (KLIEWER, 1977). These effects reduce the synthesis of polyphenols in the berry skin and result in poorly coloured fruit (KLIEWER, 1977; SPAYD *et al.*, 1994; DELGADO *et al.*, 2004). On the other hand, anthocyanin composition of the grape berries can be changed by the nitrogen fertilization (HILBERT *et al.*, 2003).

Potassium is also essential for vine yield and quality. Potassium stimulates photosynthesis activity and favours the translocation of sugars to the berries. There is a close relationship between the presence of carbohydrates and the synthesis of polyphenols in the fruit (PIRIE and MULLINS, 1977), and this, directly affect to grape colour (MOHAMMED *et al.*, 1993). Excessive potassium levels in grape berries may decrease free tartaric acid, resulting in an increase in the pH of grape juice, and modify its chromatic features (MPELASOKA *et al.*, 2003).

The aim of this work was to evaluate both the additive and interactive effects of the combined application of different rates of nitrogen and potassium fertilization upon the anthocyanin composition and colour in Tempranillo grapes.

MATERIALS AND METHODS

I - PLANT MATERIAL

The study was carried out in 2000 in a full-production vineyard of Tempranillo cultivar located in La Horra (Burgos), belonging to the 'Ribera del Duero' Apellation d'Origine area (Spain), at an altitude of about 800 m above sea level. The soil is flat, with a sandy texture, pH of 8.4 and 7.6 g kg^{-1} organic matter content. Considering the field conditions in trial, the annual mineralisation of organic matter in the soil could represent from 1 % to 3 % (Urbano, 2001), and could liberate between 15 and 45 Kg of mineral N per hectare and year. The extractable P content of the soil at the start of the investigation was 14.5 g kg^{-1}. The exchangeable K content was 225 mg kg^{-1}. The soil had not received any fertilization treatment in the previous 3 years.

The vineyard was planted in 1990. The vine spacing is 3.0 x 1.5 m (2,222 vines ha^{-1}) and has localized irrigation. Vines are grafted on 110-Richter rootstocks and are trained to a double Cordon Royat system with four spurs of two buds on each arm.

From April to October 2000, the vineyard received (rainfall and irrigation) 312 mm. The summer was long and dry and the average recorded temperature during ripening was 17 ºC.

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II - EXPERIMENTAL DESIGN

Nine experimental treatments were studied consisting of the combination of 3 different N (0, 50 and 200 g N $vine^{-1}$) and K (0, 60 and 120 g K_2O $vine^{-1}$) fertilization doses, which could be previously considered as null, moderate and excessive, respectively. The trial was carried out in a randomized complete block design with 4 replications. Each base plot had 5 vines in the same row. A vine between each treatment as well as between each two rows of treated plants was left untreated. Each row of vines receiving treatment was separated form the next treated row by a row of untreated plants.

Nitrogen treatments were applied as ammonium nitrate (335 g N kg^{-1}). Potassium treatments were applied as potassium sulphate (500 g K_2O kg^{-1}). All treatments included a dose of 65 g P_2O_5 per vine as superphosphate (450 g P_2O_5 kg^{-1}). Only one-third of the nitrogen supply was applied at bloom (22 June), the remainder of the fertilizers having been applied to the soil at the end of March, before budding. Fertilizers were applied in the rows to a depth of 15 cm and to both sides of the vines. Two drip irrigations of 45 l per plant were applied, one after each fertilizer application.

III - AGRONOMIC CONTROLS.

Twenty leaf samples per treatment were taken at bloom (22 June) and at veraison (21 August). The leaves were taken in the opposite first grape cluster area of an intermediate shoot inside each vine branch (OIV, 1996). The nitrogen and potassium contents in blades and petioles were analyzed separately. The nitrogen content was determined by the Kjeldahl method. Potassium was determined by ashing samples at 450 ºC and mineral extraction with 2M HCI, followed by measurement of concentration in an atomic absorption spectrometer (Perkin-Elmer, Mod Analys 100, Norwolk, USA).

The leaf area index (LAI) for the different experimental treatments was measured at the phenological stage of veraison by an automatic meter LAI-2000 Plant Canopy Analyzer (LI-COR inc, Lincoln, NE, USA).

The total production, the number of clusters per vine, the average weight of a cluster and the average weight of 100 berries were recorded for each of the experimental treatments at harvest (3 October).

The vigour of the vines was determined from the average pruning weight.

IV - BERRY SAMPLING AND ANALYSES

At harvest, an average of 100 berries from each base plot were picked and weighed, removing the skins from pulps and seeds. The musts obtained from the pulp and seed fraction allowed us to determine the content of soluble solids (g L^{-1}) and total acidity (g L^{-1} tartaric acid) according to the official method of analysis established in the EEC 2676/90 Regulations (EUROPEAN COMMISSION, 1990).

The skin fraction was subjected to a process of extraction of polyphenolic compounds to determine: total polyphenols (SINGLETON and ROSSI, 1965), total anthocyanins (GARCÍA-BARCELÓ, 1990) and specific anthocyanin composition. The chromatic features of extracts were also measured. The extraction of polyphenolic compounds was carried out as follows: 7.5 ml of distilled water and 10 ml of an acid hydroalcoholic solution (10 % ethanol, 5 g L^{-1} tartaric acid) were added to the skins (collected from 100 berries). The pH was adjusted with sodium hydroxide to 3.6. Next, 32.5 ml of distilled water were added and the mixture was kept at 35 ºC with slugging for 4 hours. The sample was cooled, centrifuged at 2,000·g for 5 min and filtered through glass wool.

The concentration of individual anthocyanins was determined by high-performance liquid chromatography (HPLC). Extracts were filtered through 0.2 µm filters prior to analysis The equipment used was a Hewlett Packard/Agilent 1100 (Palo Alto, CA), coupled with diode array detector and controlled by Agilent ChemStation software. The column was a Hypersil ODS, 5 µm, 200 x 4.6 mm. The following mobile phases were used: formic acid:water (15/85): solvent A; methanol:water:formic acid (45/45/10): solvent B; methanol: solvent C. gradient elution was applied at 0.8 ml/min flow rate, according to the program: linear gradient from 75 % A and 25 % B to 20 % A and 80% B in 25 min, 100 % C for 5 min. Spectra were recorded every second between 250 and 600 nm with a bandwidth of 1.2 nm. Anthocyanins were quantified using the standard malvidín 3-O glusoside solution.

The colour of the extracts was evaluated with a JASCO V-530 UV/VIS spectrophotometer. The absorption spectrum from 380 to 780 nm was obtained and the wavelength for the maximum absorption (^{a}max) was registered. The coordinates L*a*b* were recorded using the D65 Illuminant as reference (CIE, 1986). The values L*, a* and b* describe a uniform three-dimensional colour space where the L* value corresponds to a dark-bright scale (0 black; 100 white), the a* value to a green-red scale (negative value, greenness; positive, redness) and the b* value to a blue-yellow (negative value, blueness; positive yellowness). The hue angle (h) is calculated as arctg (b*/a*), and reflects the degree of brownness. The chroma (C*), calculated as [(a*)2 + (b*)2]1/2, express the colour intensity (BAKKER *et al.*, 1986).

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V - DATA ANALYSIS

The influence of the fertilization treatments on the anthocyanin composition and chromatic parameters of the skins were studied by factorial analysis of variance applying models of fixed effects. The mean separations were determined by the LSD test.

RESULTS AND DISCUSSION

Nitrogen and potassium fertilization of the soil resulted in a significant increase in the contents of these nutrients in the leaf blades at bloom. The plants treated with the highest nitrogen or potassium doses (200 g N per vine or 120 g K_2O per vine) had the highest nitrogen or potassium contents in the leaf (figure 1). However, lower fertilization levels did not significantly increase the nutrient levels in the leaves compared with the control. In all experimental treatments, nitrogen contents at bloom were lower than reference ones calculated by authors for 'Ribera del Duero' Apellation d'Origine area (GONZÁLEZ and MARTÍN, 2005), whereas the values obtained at veraison were included in optimal interval. Potassium contents in petioles and blades, collected at bloom or at veraison, always were included between the reference margins.

Potassium treatments enhanced the nitrogen content in petioles at veraison (figure 2). This fact shows that potassium supplies improve the assimilation of nitrogen (URBANO, 2001). Both nutrients are synergistic.

Although the experimental treatments changed the leaf composition, they did not significantly affect the vigour (pruning weights between 1.10 and 1.30 kg per vine) or the berry size (table I), in line with the results of BELL and ROBSON (1999). However, many authors have showed that excessive nitrogen supply can decrease significantly the berry size (RODRIGUEZ-LOVELLE and GAUDILLÈRE, 2002; HILBERT *et al.*, 2003).

The ANOVA of yield values was not significant ($P<0.05$) for potassium fertilization doses. Regardless of potassium treatments, the yield of vines receiving 50 g

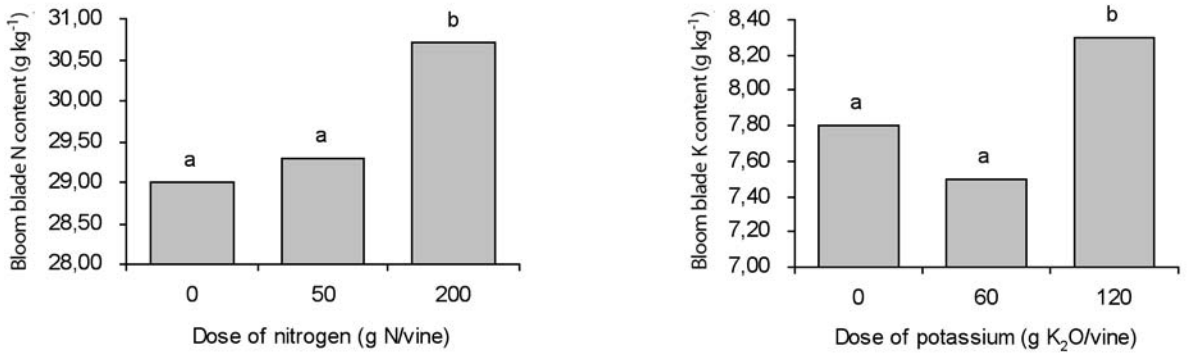


Figure 1 - Nitrogen (A) and potassium (B) contents in leaf blades at bloom for the different nitrogen (mean values of all potassium applications) and potassium (mean values of all nitrogen applications) treatments, respectively.
Values with different letters are significantly different ($P<0.05$, LSD test)

Contenu d'azote (A) et de potassium (B) dans les limbes des feuilles à la floraison pour chacun des traitements de fertilisation azotée (moyenne de tous les traitements de potassium) et potassique (moyenne de tous les traitements d'azote), respectivement.
Les valeurs avec lettres différentes sont significativement différentes ($P< 0.05$, test LSD).

Table I - Vine yield, berry weight and values of must composition parameters for the different nitrogen treatments (average of all potassium fertilization doses).

Values with different letters in the same column are significantly different ($P<0.05$, LSD test)

Rendement du vignoble, poids des baies et paramètres de la composition du moût dans les traitements d'azote (moyenne des doses de fertilisation potassique).

Les valeurs suivies de lettres différentes dans la même colonne sont significativement différentes ($P< 0.05$, test LSD)

Dose of nitrogen (g N per vine)	Yield (Kg.vine^{-1})	Weight of 100 berries (g)	Soluble solids content (g L^{-1})	Total acidity
0	6.20 b	242.5 a	225.5 a	4.45 a
50	7.37 a	235.5 a	228.0 a	4.33 a
200	6.52 ab	238.2 a	213.5 b	4.47 b

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Table II - ANOVA mean squares of the concentration (mg·L-1) of total polyphenols, total anthocyanin, malvidin 3-glucoside (Mv-3-gl); delphinidin 3-glucoside (Dp-3-gl); petunidin 3-glucoside (Pt-3-gl); peonidin 3-glucoside (Pn-3-gl); cyanidin 3-glucoside (Cy-3-gl) in berry skins, according to the nitrogen (N) and potassium (K) fertilization treatments.

Significance levels: P<0.05 (*) ; P<0.01 (**).

Carrés moyens de l'analyse de variance de la concentration (mg·L-1) de polyphénols et anthocyanes totaux, malvidine-3-glucoside (Mv-3-gl); delphinidine 3-glucoside (Dp-3-gl); pétunidine 3-glucoside (Pt-3-gl); paeonidine 3-glucoside (Pn-3-gl) an cyanidine-3-glucoside (Cy-3-gl) dans les pellicules de raisin, en fonction des traitements de fertilisation azotée (N) et potassique (K).

Niveaux de signification : P<0.05 (*) ; P<0.01 (**).

Source of variation	Total polyphenols	Total anthocyanins	Mv-3-gl	Dp-3-gl	Pt-3-gl	Pn-3-gl	Cy-3-gl
N	125895 **	46794 **	3684.69	1047.98 **	570.43 **	22.41 *	1.79
K	33002	68993	7743.21	835.57 **	435.77 **	143.32 **	9.33
N*K	130056 **	73412 **	11302.66	819.67 **	601.09 **	49.55	11.02 *
Block	4149	18601	9647.56	42.68	3.47	35.17	3.02
Error	2166	9880	4505.80	139.75	61.86	21.38	3.11

N per plant was significantly higher than untreated controls, but not significantly differed from the vines receiving 200 g N/plant (table I). There were no differences (P<0.05) between treatments for the number of clusters per vine and the average cluster weight. It is generally accepted that nitrogen fertilization can improve the vineyard yield, especially when soils are deficient in these nutrients (SPAYD *et al.*, 1994). Nitrogen stimulates plant growth and the attainment of a sufficient leaf area. Nevertheless, an excessive invigorating effect of nitrogen may compact the foliage, decreasing the exposure to sunlight, and the photosynthetical capacity of the vines

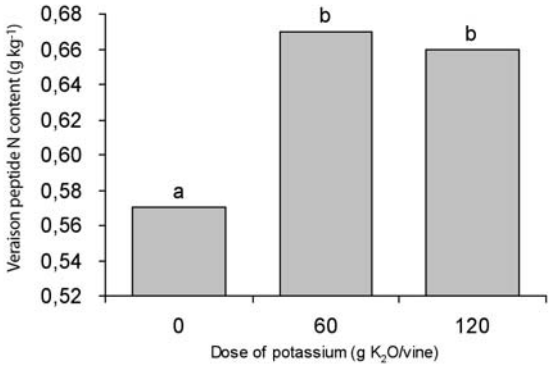


Figure 2 - Nitrogen contents in leaf petioles at veraison for the different potassium treatments (mean values of all nitrogen applications).

Values with different letters are significantly different (P<0.05, LSD test)

Contenu de potassium dans les pétioles des feuilles à la veraison pour chacun des traitements de fertilisation potassique (moyenne de tous les traitements d'azote).

Les valeurs avec lettres différentes sont significativement différentes (P< 0.05, test LSD).

(WINKLER *et al.*, 1974; SMART and ROBINSON, 1991). However, the low yield detected in vines receiving higher nitrogen fertilization levels does not seem to be due to reduced photosynthesis as a result of changes in canopy structure and /or the light microclimate, as no significant differences in the leaf area indices were detected between treatments (values between 0.7 and 1.2, P=0.17).

Regardless of potassium treatments, the fruits from the vines fertilized at the highest nitrogen dose had lower soluble solids content than the control ones (table I). However, the treatments receiving simultaneously 200 g N and 120 g K_2O per plant reached 227 $g \cdot Kg^{-1}$, a value not significantly different from unfertilized controls. Excessive nitrogen delays the sugar accumulation in the grapes during ripening (SPAYD *et al.*, 1994; KELLER *et al.*, 1999). Nevertheless, balanced potassium supplies lessen the adverse effects of excess nitrogen levels on the sugar content in the must (FREGONI, 1980). Potassium nutrition stimulates photosynthetic activity and favors the translocation of sugars to the fruit.

Potassium doses of 60 and 120 g K_2O per vine caused significant reductions in the total acidity of the must compared with the treatments with no fertilizer application (4.09 and 7.08 vs 4.99 g L^{-1} tartaric acid). Loss of acidity is caused by excessive migration of K^+ cations to the fruit, which produces potassium bitartrate from tartaric acid (FREGONI and VERCESI, 1995).

The N and K fertilization treatments did not significantly affect pH values of the must (means between 3.64 and 3.75) and, therefore, they did not produce changes in the molecular structures of the anthocyanins in solution (LACOBUCCI and SWEENY, 1983; CORMIER *et al.*,

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Table III - ANOVA mean squares of the CIELAB coordinates measured in extracts of berry skins, according to the nitrogen (N) and potassium (K) fertilization treatments.

Significance levels: P<0.05 (*) ; P<0.01 (**).

Carrés moyens de l'analyse de variance des coordonnées CIELAB des extraits de pellicules de raisin obtenues pour chacun des traitements de fertilisation azotée (N) et potassique (K).

Niveaux de signification : P<0.05 (*) ; P<0.01 (**).

Source of variation	L*	a*	b*	C*	h*
N	355.44 **	556.44 **	57.89 **	569.47 **	378.96 **
K	87.86	201.31 *	13.37 **	188.58 *	157.19 **
N*K	130.94 *	180.29 *	4.53 **	187.52 *	34.64 **
Block	24.96	36.76	0.31	34.56	4.92
Error	42.81	55.92	0.87	56.38	5.49

1997). So nitrogen and potassium could modify the grape colour by intervening directly in the synthesis of specific anthocyanins and/or other phenols that can influence colour by copigmentation (BOULTON, 2001).

Nitrogen and potassium availability for the vines was an important factor in phenolic ripening. The fertilizer applications significantly modified the anthocyanin composition of the grape skin at harvest (table II), a result similar to those HILBERT *et al.* (2003) reported for nitrogen in cv. Merlot. Table II shows how, apart from the additive effect of the fertilization treatments, there was also a significant N x K interaction on the polyphenol, total anthocyanin, delphinidin-3-glucoside, petunidin-3-glucoside and cyanidin-3-glucoside contents.

The changes observed in the level and specific composition of the anthocyanins led to significant modifications of the chromatic characteristics of the skin extracts. Additive and interactive effects of the nitrogen and potassium treatments were found for all the CIELAB coordinates (table III).

I- TOTAL POLYPHENOLS AND ANTHOCYANIN COMPOSITION

A comparison of the means for total polyphenols in skins highlights the effect of N x K interaction (figure 3A). With average levels of potassium fertilization, the increase of nitrogen dose caused a significant decrease (P<0.05) in the content of total polyphenols, whereas when potassium supply were high, the treatments with maximum nitrogen dose resulted in higher concentrations of these compounds. Excessive nitrogen supply can reduce the synthesis of polyphenols in the berry skin (KLIEWER, 1977; SPAYD *et al.*, 1994). High nitrogen availability for the vines affects the metabolic pathways of athocyanins in different points, and enhances the degradation of polyphenols during the final steps of berry ripening (HILBERT *et al.*, 2003). Adequate potassium nutrition could compensate the adverse effects of nitrogen excess because it stimulates the translocation of sugars to the fruit (see results discussed above). This indirectly benefits the synthesis of phenolic components during ripening, which is closely related to the presence of carbohydrates in the grape (PIRIE and MULLINS, 1977; MOHAMMED *et al.*, 1993).

The N:K ratio in the leaf blades at bloom ranged from 3.1 to 5.1. For the skins with the highest concentrations of polyphenols, the N:K ratios were 3.6-4.3, much higher than those considered by Fregoni (1985) as optimal (1.90-2.40).

The effect of N x K interaction on total anthocyanins detected in ANOVA (table II) produced a mean separation similar to total polyphenols. With null or medium levels of potassium fertilization, the increase of nitrogen doses tended to decrease the content of total anthocyanins in the skin (figure 3B), whereas when potassium supply were high, the concentrations for treatments with maximum nitrogen dose were not different than controls. Regardless of potassium treatments, the intermediate nitrogen dose was associated with produced higher anthocyanins content (600 mg L^{-1}) compared to standard extracts (532 mg L^{-1}, P<0.05). KELLER and HRAZDINA (1998) have observed that moderate nitrogen levels favour the formation of flavonoids in grapes. On the other hand, adequate nitrogen levels stimulate the activity of phenylalanine ammonia-lyase (PAL), which is involved in anthocyanin synthesis (KATAOKA *et al.*, 1983).

When the principal effects of the treatments on the anthocyanin composition of the skins were analysed (table IV), doses over 50 g N/vine were observed to cause decreases of up to 50 % and 38 % (compared with the controls) in the levels of monoglucosides of delphinidin and petunidin respectively. No effect of the nitrogen fer-

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Table IV - Mean concentration (mg·L^{-1}) of anthocyanins, malvidin 3-glucoside (Mv-3-gl); delphinidin 3-glucoside (Dp-3-gl); petunidin 3-glucoside (Pt-3-gl); peonidin 3-glucoside (Pn-3-gl) and cyanidin 3-glucoside (Cy-3-gl), in berry skins, according to the nitrogen (average of all potassium doses) and potassium (average of all nitrogen doses) fertilization treatments.

Values with different letters in the same column are significantly different (P<0.05, LSD test).

Concentration moyenne (mg·L^{-1}) des anthocyanes, malvidine-3-glucoside (Mv-3-gl) ; delphinidine 3-glucoside (Dp-3-gl); pétunidine 3-glucoside (Pt-3-gl); paeonidine 3-glucoside (Pn-3-gl) and cyanidine-3-glucoside (Cy-3-gl), dans les pellicules des baies pour chacun des traitements de fertilisation azotée (moyenne des traitements de potassium) et potassique (moyenne des traitements d'azote).

Les valeurs suivies de lettres différentes dans la même colonne sont significativement différentes (P< 0.05, test LSD).

Treatments	Mv-3-gl	Dp-3-gl	Pt-3-gl	Pn-3-gl	Cy-3-gl
Nitrogen (g N per vine)					
0	216.75 A	56.03 a	50.36 a	20.08 a	9.41 a
50	182.96 A	37.67 b	36.57 b	18.00 a	8.75 a
200	207.96 A	39.88 b	33.40 c	18.09 a	8.38 a
Potassium (g K_2O per vine)					
0	230.26 A	55.56 a	48.64 a	23.51 a	9.99 a
60	196.97 A	49.03 a	44.84 a	18.07 b	8.58 a
120	180.37 A	38.99 b	36.84 b	17.09 b	8.37 a

tilization was detected on the malvidin-3-glucoside content of the skin, which accords with the observations of HILBERT *et al.* (2003). However, other authors report that nitrogen availability does have a definite impact on the levels of these monoglucosides in grapes (KELLER and HRAZDINA, 1998; KELLER *et al.*, 1999).

Applying potassium did not modify the concentration of the total anthocyanins concentration in the skins (table II), which agrees with MORRIS *et al.* (1980) for cv. Concord; but it did modify its specific composition. At maximum dose, potassium significantly reduced the peonidin monoglucoside contents compared with the untreated control (table IV). On the other hand, the effect of potassium doses over monoglucosides of delphinidin and petunidin was dependent of nitrogen doses (N x K interaction showed in table II). With no nitrogen addition or with the intermediate dose, the application of 120 g K2O/vine to the vineyard led to significant reductions in the content of both monoglucosides compared with the rest of the treatments (figure 4A and 4B). When the high potassium doses were balanced with a sufficient nitrogen supply, these reductions were not detected.

II - CHROMATIC FEATURES OF GRAPES

As table V shows, the average nitrogen dose (50 g N/vine) produced a major increase in the hue (h*) and in the yellow component (b*) of the colour of the extracts in relation to the reference values, but did not modify colour density. The moderate supply of nitrogen to the vineyard may have caused a higher level of esterification of the sugars of the anthocyanin molecules, mainly delphinidin and petunidin, with acids such as acetic or p-coumaric (ABRIL and CASP, 1999), thus increasing the total anthocyanin content of the grape skins. This higher degree of esterification may have in turn preconditioned the anthocyanins to establish intramolecular hydrophobic interactions; this would have produced a hypsochromic shift in the absorption spectrum of the extracts corresponding to the 50 g N/vine doses compared to those obtained from the untreated plants, and shifted the maximum toward the yellow area (λ_{max} = 522 vs.λ_{max} = 525, P<0.01). It agrees with what MINIATTI *et al.* (1992) report.

Regardless of the level of potassium fertilization, the application of 200 g N/vine significantly increased the chromaticity and the red and blue shades of the extracts with respect to the control, as well as decreased their luminosity and hue (table V). The high nitrogen uptake may have predisposed the monoglucosides -especially of delphinidin and petunidin- to form more copigmentation complexes, causing a hyperchromic and bathochromic effect in the absorption spectra of the extracts in relation to those of untreated vines (λ_{max} = 526 vs. λ_{max} = 525, P<0.01), which accords with SOMERS (1986). This may have contributed to the decreased anthocyanin content of the skins (table IV), assuming that the vines treated with 200 g N/vine had enough cofactors in the skins to form copigmentation complexes (BOULTON, 2001).

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Table V - Additive effect of the nitrogen fertilization treatments on CIELAB coordinates measured in extracts of berry skins.

Values with different letters in the same column are significantly different (P<0.05, LSD test).

Effet additif des traitements de fertilisation azotée sur les coordonnées CIELAB des extraits de pellicules de raisin.

Les valeurs suivies de lettres différentes dans la même colonne sont significativement différentes (P< 0.05, test LSD).

Dose of nitrogen (g N per vine)	L*	a*	b*	C*	H*
0	77,39 a	24,84 a	-0,87 a	24,92 a	-0,26 a
50	81,97 a	18,56 a	1,68 b	28,70 a	6,45 b
200	71,14 b	32,17 b	-2,69 c	32,45 b	-4,77 c

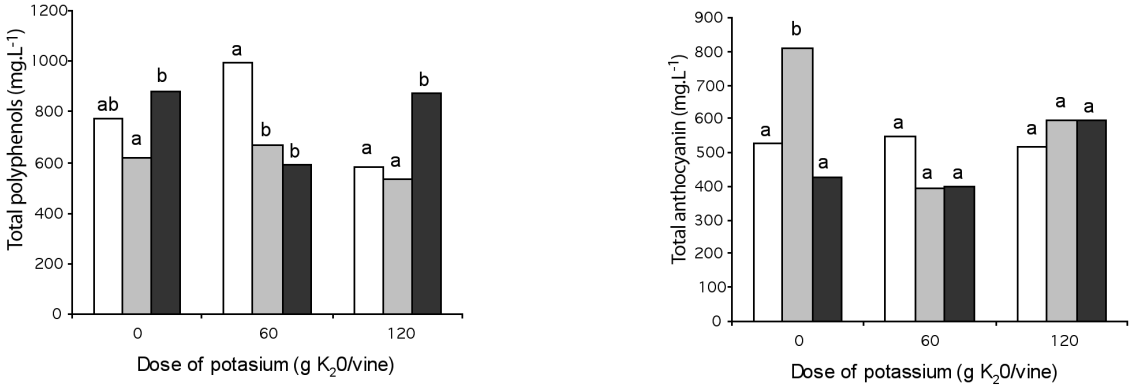


Figure 3 - Combined effects of nitrogen and potassium fertilization treatments on total polyphenol content (A) and total anthocyanin content (B) measured in extracts from berry skins.

Values with different letters are significantly different (P<0.05, LSD test).

Effets combinés des traitements de fertilisation azotée et potassique sur les teneurs en polyphénols (A) et en anthocyanes (B) des extraits des pellicules de raisin.

Les valeurs suivies de lettres différentes sont significativement différentes (P< 0.05, test LSD).

When the chromatic features of the grape skin were analysed exclusively in relation to the treatments with potassium fertilizer, an increase was observed (P<0.01) in the yellow shade of the extracts from vines treated with 120 g K2O (b* = 1.54) compared with the unfertilized ones (b* = 0.56). Furthermore, a hypsochromic effect was recorded in the absorption spectra of the skin extracts of vines treated with 120 g K2O/vine in relation to those untreated (λ_{max} = 524 vs. λ_{max} = 525, P<0.01). These findings support the hypothesis that the highest potassium dose too might have favoured -though to a lesser extent than the intermediate nitrogen dose- the formation of acylated derivatives of the anthocyanins, as well as their hydrophobic molecular interactions in the grape skin, especially those of delphinidin-3-glucoside, petunidin-3-glucoside and peonidin-3-glucoside. It would be interesting to contrast this hypothesis in further studies.

Figure 4 illustrates the influence of N x K interaction on the chromatic characteristics of the grape skins (table III). With no nitrogen addition or with the intermediate dose, the application of 120 g K2O/vine to the vineyard tended to reduce the colour intensity in the extracts (remarkable reductions in the content of monoglucosides of delphinidin and petunidin), while intensifying the yellow component. Nevertheless, these effects did not take place when the high potassium doses were balanced with a sufficient nitrogen supply.

Since the total anthocyanin content was not altered by adding potassium, the high availability of the element, in the absence of enough nitrogen, might be thought to have exerted some kind of action that would promote the inter/intramolecular association of anthocyanins in the grape skins. Conversely, when a high level of nitrogen was available to the vines, the tendency of the potassium to induce inter/intramolecular interactions was possibly neutralised by the greater formation of copigmentation complexes stimulated by the high nitrogen doses.

CONCLUSIONS

The results of this study show that nitrogen and potassium fertilization can modify the total polyphenols content,

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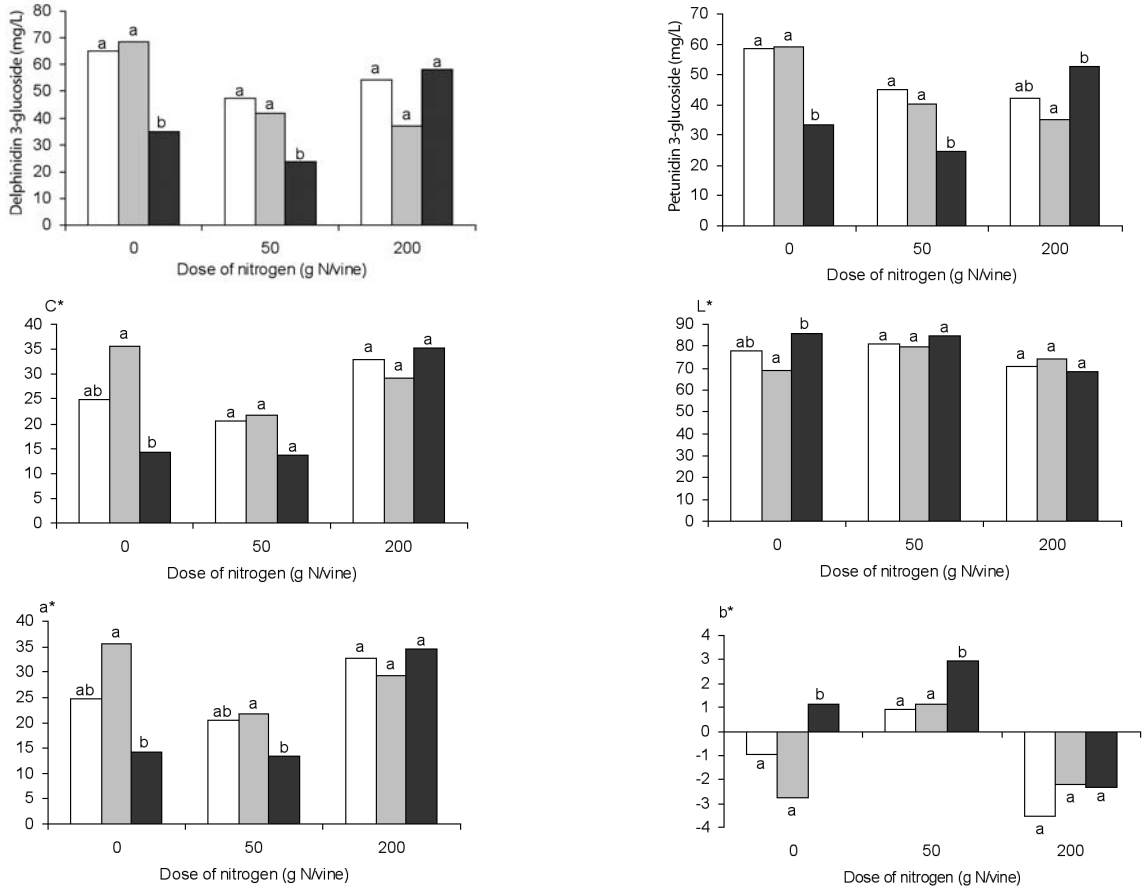


Figure 4 - Combined effects of nitrogen and potassium fertilization treatments on delphinidin 3-glucoside content (A); petunidin 3-glucoside content (B) and CIELAB coordinates (C, D, E and F) measured in extracts from berry skins.

Values with different letters are significantly different (P<0.05, LSD test)

Effets combinés des traitements de fertilisation azotée et potassique sur les contenus en delphinidine 3-glucoside (A); pétunidine 3-glucoside (B) et les coordonnées CIELAB (C, D, E et F) des extraits des pellicules de raisin.

Les valeurs suivies de lettres différentes sont significativement différentes (P< 0.05, test LSD)

anthocyanin composition and the copigmentation of anthocyanins in Tempranillo grapes. There is a strong nitrogen x potassium interaction effect, so optimal N:K fertilization ratios may enhance the polyphenol synthesis and improve the chromatic features of grape berries. For the cultural conditions considered in this work, the optimal N:K ratio in cv Tempranillo blades (at bloom) ranged from 3.6 to 4.3.

Acknowledgements : The present work was supported by grant nº VA-52/00A from Consejería de Educación y Cultura de la Junta de Castilla y León (Spain). The authors are grateful to Grandes Bodegas S.A. for their collaboration to carry out our research.

REFERENCES

ABRIL I. and CASP A., 1999. Los compuestos fenólicos del vino. Alimentación. *Equipos y Tecnología*, **12**, 97-103.

BAKKER J., BRIDLE P. and TIMBERLAKE C.F., 1986. Triestimulus measurements (CIELAB76) of port wine colour. *Vitis*, **25**, 67-78.

BELL S.J. and ROBSON A., 1999. Effect of nitrogen fertilization on growth, canopy density, and yield of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.*, **50** (3), 351-358.

BOULTON R., 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.*, **52** (2), 67-87.

BRAVDO B. and HEPNER Y., 1987. Irrigation management and fertigation to optimise grape composition and vine performance (Review). *Acta Horticulturae*, **206**, 49-67.

BROUILLARD R., MAZZA G., SAAD Z., ALBRECHTGARY A.M. and CHEMINAT A., 1989. The copigmentation reaction of anthocyanins: a microprobe for the structural study of aqueous solutions. *J. Am. Chem. Soc.*, **111**, 2604-2610.

CHEYNIER V., BASIRE M. and RIGAUD J., 1989. Mechanism of trans-caffeoyltartaric acid and catechin oxidation in modal

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solutions containing grape polyphenoloxidase. *J. Agric. Food Chem.*, **37**, 1069-1071.

CIE. 1986. *Colorimetry* (2nd edition). Ed. C.I.E., Viena.

CORMIER F., COUTURE R., DO C.B., PHAM T.Q. and TOUNG V.H., 1997. Properties of anthocyanins from grape cell culture. *J. Food Sci.*, **62** (2), 246-248.

DELGADO R., MARTIN P., DEL ALAMO M. and GONZALEZ M.R., 2004. Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilization rates. *J. Sci. Food Agric.*, **84**, 623-630.

EUROPEAN COMMISION, 1990. Regulation (EEC) Nº 2676/90 of 17 September 1990. Community methods for the analysis of wines, 0001-0192. *In Official Journal of the European Communities L272 (3 October 1990)*, Ed by European Commision, Brussels.

FREGONI M., 1980. *Nutrizione y fertilizzacione della vite*. Ed. Edagricole, Bologna.

FREGONI M,, 1985. Exigences d'élements nutritifs en viticulture. *Bull. OIV*, **58** (650-651), 416-434.

FREGONI M. and VERCESI A., 1995. Relationships between mineral status of Pinot noir grapevines and must acidity. *Acta Horticulturae*, **383**, 229-232.

GARCÍA-BARCELÓ J., 1990. Compuestos fenólicos, 8.3-8.33. *In: Técnicas Analíticas para Vinos*. Ed. G.A.B., Moja-Olérdola.

GONZÁLEZ, M.R. and MARTÍN, P., 2005. Valores de referencia para el diagnóstico foliar del viñedo cv. Tempranillo en la Ribera del Duero. *La semana vitivinícola*, **3073**, 2198-2202.

GONZÁLEZ-SAN JOSÉ, M.L. and DÍEZ, C., 1992. Relationship between anthocyanins and sugars during the ripening of grape berries. *Food Chemistry*, **43**, 193-197.

HILBERT G., SOYER J.P., MOLOT C., GIRAUDON J., MILIN S., and GAUDILLERE, J.P., 2003. Effect of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis*, **42**(2), 69-76.

HRAZDINA G., 1986. Glucosylation of flavonoid compounds in Hippeastrum. *Bull. Liaison Groupe Polyphenols*, **13**, 27-32.

KATAOKA, I., KUBO, Y., SUGIURA, A. and TOMANA, T., 1983. Changes in L-phenylalanine ammonia-lyase activity and anthocyanin synthesis during berry development of three grape cultivars. *J. Japanese Society Hortic. Sci.*, **52**, 273-279.

KELLER M. and HRAZDINA G., 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol. Vitic.*, **49** (3), 341-349.

KELLER M., POOL R.M. and HENICK-KLING T., 1999. Excessive nitrogen supply and shoot trimming can impair colour development in Pinot noir grapes and wine. *Austral. J. Grape Wine Res.*, 5, 45-55.

KLIEWER W.M., 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *Am. J. Enol. Vitic.*, **28**, 96-103.

LACOBUCCI G.A. and SWEENY J.G., 1983. The chemistry of anthocyanins, anthocyanidins and related flavylium salts. *Tetrahedron*, **39**, 3005-3038.

MOHAMMED S., SINGH D. and AHLAWAT V.P., 1993. Growth, yield and quality of grapes as affected by pruning and basal application of potassium. *J. Hort. Sci.*, **22**, 179-182.

MINIATTI E., DAMIANI P. and MAZZA, G., 1992. Copigmentation and self-association of anthocyanins in food models system. *Ital. J. Food Sci.*, **4**, 109-116.

MORRIS J.R., CAWTHON D.L. and FLEMING J.W., 1980. Effects of high rates of potassium fertilization on raw product quality and changes in pH and acidity during storage of Concord grape juice. *Am. J. Enol. Vitic.*, **31** (4), 323-328.

MPELASOKA B.S., SCHACHTMAN D.P., TREEBY M.T. and THOMAS M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. *Austral. J. Grape Wine Res.*, **9**, 154-158.

OIV, 1996. Résolution VITI 4/95. Diagnostic foliare: une métode harmoniseé. *Bull OIV*, **69**, 779-780.

ORTEGA M.D., RIVAS J.C., VICENTE J.L. and SANTOS C., 1994. Diferenciación de variedades de uvas tintas por su composición antociánica. *Revista Española de Ciencia y Tecnología de los Alimentos*, **34** (4), 409-426.

PIRIE A.J.G. and MULLINS M.G., 1977. Interrelationships of sugars, anthocyanins, total polyphenols and dry weight in the skin of grapes berries during ripening. *Am. J. Enol. Vitic.*, **31**, 34-36.

RIBÉREAU-GAYON P. and GLORIES Y., 1987. Phenolics in grapes and wines, 247-256. *In: proceedings of Six Australian Wine Industry Technical Conference*. Lee, T. ed. Australian Industrial Publishers. Adelaide.

RODRIGUEZ-LOVELLE B. and GAUDILLÈRE J.P., 2002. Carbon and nitrogen partitioning in either fruiting or non-fruiting grapevines: effects of nitrogen limitation before and after veraison. *Aust. J. Grape Wine Res.*, **8**, 86-94.

SINGLETON V.L. and ROSSI, J.A., 1965. Colorimetry of total phenolics with phosphomolibdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, **16** (3), 144-158.

SMART, R. and ROBINSON, M., 1991. *Sunlight into wine*. Adelaide, Australia: Winetitles.

SOMERS T.C., 1986. Assessment of phenolic components in viticulture and enology, 257-260. *In: Proceedings of the Sixth Australian Wine*. ed Ind. Tech. Conf. Adelaide.

SPAYD S.E., WAMPLE R.L., EVANS R.G., STEVENS R.G., SEYMOUR B.J. and NAGEL C.W., 1994. Nitrogen fertilization of White Riesling grapes in Washington. Must and wine composition. *Am. J. Enol. Vitic.*, **45**, 34-42.

URBANO P., 2001. *Tratado de Fitotecnia general*. Ed. Mundi-Prensa, Madrid.

WENZEL K., DITTRICH H.H. and HEIMFARTH M., 1987. Die Zusammensetzung der Anthocyane in den Beeren verschiedener Rebsorten. *Vitis*, **26**, 65-78.

WINKLER A.J., COOK J.A., KLIEWER W.M. and LIDER L.A., 1974. *General Viticulture*. University of California Press, Berkeley, California.

Manuscrit reçu le 30 novembre 2005 ; accepté pour publication, après modifications le 24 juillet 2006

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